SAR Studies on Trisubstituted Benzimidazoles as Inhibitors of *Mtb* FtsZ for the Development of Novel Antitubercular Agents

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Abstract

FtsZ, an essential protein for bacterial cell division, is a highly promising therapeutic target, especially for the discovery and development of new-generation anti-TB agents. Following up the identification of two lead 2,5,6-trisubstituted benzimidazoles, **1** and **2**, targeting *Mtb*-FtsZ in our previous study, an extensive SAR study for optimization of these lead compounds was performed through systematic modification of the 5 and 6 positions. This study has successfully led to the discovery of a highly potent advanced lead **5f** (MIC 0.06 μg/mL) and several other compounds with comparable potencies. These advanced lead compounds possess a dimethylamino group at the 6 position. The functional groups at the 5 position exhibit substantial effects on the antibacterial activity as well. *In vitro* experiments such as the FtsZ polymerization inhibitory assay and TEM analysis of *Mtb*-FtsZ treated with **5f** and others indicate that *Mtb*-FtsZ is the molecular target for their antibacterial activity.

Keywords

Mycobacterium tuberculosis; Antibacterial; Benzimidazoles; FtsZ; Structure-activity relationship

Introduction

Tuberculosis (TB) was responsible for 1.4 million deaths in 2011, which was second only to HIV/AIDS related fatalities among all infectious diseases.[1, 2] TB is caused by *Mycobacterium tuberculosis* (*Mtb*) and is highly contagious when airborne.[3] It has been estimated that 90% of humans who are exposed to or infected with the pathogen have latent-TB, which has a 10% chance of progressing to an active-TB diseased state during their lifetime.[4, 5] Recent statistics from the World Health Organization (WHO) indicate an estimated 8.7 million new TB cases globally in 2011.[1, 2] Among these cases, 13% were HIV-positive individuals with 430,000 deaths, which demonstrates the ability of the bacteria to target immunocompromised patients.[1, 2] It has been reported that MDR-TB is the causative agent of 3.7% of the new cases and 20% of the previously reported cases worldwide.[1, 2] MDR-TB resistant to at least one of the three injectable "second-line" antibiotics and fluoroquinolones is classified as extensively drug resistant-TB (XDR-TB).

About 9% of MDR-TB cases have been classified as XDR-TB by WHO and 84 countries have reported at least one case of XDR-TB.[1, 2]

The widespread resistance to existing therapeutics has become a key hurdle for effective treatment of the disease. Therefore, in order to counterattack the drug resistance, there is a dire need for the identification of novel therapeutic targets. In this context, FtsZ, an essential bacterial cytokinesis protein, is a highly promising therapeutic target.[6–9] In the presence of GTP, FtsZ polymerizes bi-directionally at the center of the cell on the inner membrane to form a highly dynamic helical structure known as the "Z-ring".[10–14] The recruitment of several other cell division proteins leads to Z-ring contraction, resulting in septum formation and eventually cell division.[10–14] Accordingly, the inhibition of proper FtsZ assembly would block the septum formation and then cell division, which should lead to bacterial growth inhibition and cell death.[6, 8, 9, 15–17]

Recently, FtsZ inhibitors have been actively investigated for pathogen specific as well as broadspectrum antibacterial drug discovery against a variety of pathogens.[6, 18] Several classes of compounds have been identified as promising leads for antibacterial drug development, including OTBA,[19] 2-alkoxycarbonylaminopyridines,[20] 2-carbamoylpteridine,[21] taxanes,[15] benzimidazoles,[22] GTP analogs,[23, 24] benzo[c]phenathridines,[25, 26] isoquinolines,[27–29] PC190723,[30, 31] Zantrins,[7] and chrysophaentins[32]. However, against *Mtb* FtsZ, only taxanes[15] and benzimidazoles[22] have emerged as promising leads in the last several years after the pioneering work by the Southern Research Institute group.[20, 21, 27, 33]

We have previously reported the design and synthesis of libraries of 2,5,6- and 2,5,7-trisubstituted benzimidazoles, which gave a good number of hit compounds through screening against *Mtb* H37Rv strain at 5 μg/mL concentration.[22] Moreover, selected lead compounds from these hits were found to exhibit excellent MIC values in the range of 0.39–6.1 μg/mL against drug sensitive (H37Rv) as well as drug resistant strains (Figure 1).[22] These lead compounds inhibited *Mtb* FtsZ assembly in a dose dependent manner, while enhancing the GTPase activity.[22]

Subsequently, benzimidazoles **1** and **2** were found to be bactericidal and also active against non-replicating *Mtb* grown under low oxygen conditions.[34] The latter result is particularly exciting since it indicates that these compounds have potential to be effective against latent TB infection. Furthermore, **1** exhibited good efficacy *in vivo* in the standard acute infection model using immune incompetent GKO mice.[35] These promising results will be published elsewhere shortly.

Since these two lead compounds have shown very promising antibacterial activities *in vitro* and *in vivo*, we set out for the SAR study and optimization of these leads through systematic structural modifications at the 5 and 6 positions, keeping the cyclohexyl group at the 2 position intact as shown in Figure 2. We describe here our SAR study and successful optimization of the early lead compounds, which has led to the identification of a highly potent lead compound **5f** (MIC 0.06 μg/mL) and others with comparable potencies.

Chemical Synthesis

Synthesis of compounds for the optimization library of 2,5,6-trisubstituted benzimidazoles (63 compounds in total) is outlined in Scheme 1, which in most part follows the protocol previously published by our laboratory.[22] The aromatic nucleophilic substitution of commercially available 2,4-dinitro-5-fluoroaniline with various amines afforded 5-dialkylaminodinitroanilines **2a–g** in 94–98 % yields. The acylation of **2a–g** with cyclohexanecarbonyl chloride gave the corresponding *N*-acylanilines **3a–g** in 75–95%

yields. One-pot reduction and the subsequent cyclization in the presence of stannous chloride dihydrate and 4 M hydrochloric acid gave 5-aminobenzimidazoles **4a–g** in 65–79% yields. The derivatization of **4a–g** was carried out using five different methods depending on the functional group to be introduced, and analytically pure compounds **5, 6** and **7** were obtained in 42–97% yields after chromatographic purification. Compounds **5, 6** and **7** bear a carbamate, urea and amide group, respectively, at the 5 position.

Evaluation of antibacterial activities and SAR study

The 2,5,6-trisubstituted benzimidazoles, **5, 6** and **7** (63 compounds) were evaluated for their activity against *Mtb* H37Rv using the "Microplate Alamar Blue Assay (MABA)".[36] The antibacterial activities of the compounds are indicated by MIC values.

Since the early lead compounds, **1** (MIC 0.63 μg/mL) and **2** (MIC 0.39 μg/mL), possess a carbamate group at the 5 position, we examined the effect of different carbamate groups at the 5-position as well as different dialkylamino groups at the 6 position on the potency against *Mtb* (H37Rv). Results are summarized in Table 1.

As Table 1 shows, the substituents at both 5 and 6 positions have substantial effects on antibacterial activity, but it is very clear that bulky dialkylamino groups at the 6 position are detrimental to the potency of compounds (**5b, 5c, 5d, 5e**). On the contrary, a smaller dialkyl group, i.e., dimethylamino group, at the 6 position exerts a remarkable enhancement in potency (**5f, 5h, 5l, 5p**), leading to the discovery of a highly active lead compound, **5f** (MIC 0.06 μg/mL), which is 11 times more potent than **1**. The nature of carbamate groups at 5 position exhibits moderate effects on the potency. An analog of **1, 5g** bearing a branched alkyl carbamate group at the 5-position showed substantially lower potency with an MIC value >50 μg/mL, but another analog **5h** with a benzyl carbamate group showed enhanced potency. The introduction of ethyleneglycol moiety into the carbamate group should increase solubility in aqueous media. 2-(Methoxy)ethyl and 2-(ethoxy)ethyl carbamate groups are well tolerated and **5l** bearing 5-dimethylamino moiety possesses high potency. However, the use of the MeO(CH2CH2O)2-carbamate group resulted in a substantial decrease in potency (**5m**). A polar and lipophilic 2,2,2-trifluoroethyl carbamate group is well tolerated (**5n, 5o, 5p**).

Next, we examined the effect of urea moieties at the 5 position on the antibacterial activity. As Table 2 shows, the introduction of a urea group to the 5 position in place of a carbamate group is detrimental to the potency (**6a–f**). Nevertheless, compound **6g** with a 5-dimethylamino group retains good potency.

As exemplified in Figure 1, we identified several lead compounds showing good antibacterial activities (MIC 3.1 μg/mL), which bear a benzamide group at the 5 position. In a manner similar to compound **1** and **2**, 2-cyclohexyl-6-*N,N*-diethylamino-5-(4-methoxybenzamido)-1*H*–benzo[*d*]imidazole (**4**) is equally active against drug-sensitive (H37Rv) and drug-resistant clinical isolates of *Mtb*.[22] Since the amide linkage may have better metabolic stability *in vivo*, we synthesized 40 compounds for the SAR study and evaluated their activity against *Mtb* H37Rv. Results are summarized in Table 3. Compounds **7a-1~15** bear a 6-diethylamino group, **7b-1~7** bear a 6-pyrrolidino group, **7c-1~17** a 6-dimethylamino group and **7d** a 6-dipropylamino group.

As Table 3 shows, the bulkiness of the 6-dialkylamino group exerts a dramatic influence on the antibacterial activity of these compounds, and it is very clear that the 6-dimethylamino group is by far the best substituent in this library of 2,5,6-trisubstituted benzimidazoles. As

anticipated, 5-benzamido-6-dipropylamino-benzimidazole (**7d**) does not show appreciable activity.

In the 6-diethylamino series of compounds (**7a-1∼15**), the potency is very sensitive to the substitution pattern of the 5-benzamide moiety. Thus, the compounds with 4-difluoromethoxy-, 4-trifluoromethoxy-, 4-ethoxy-, 4-propyl-, 4-bromo-, 4-fluoro-, 2,4-difluoro- and 3-bromobenzamide moieties at the 5 position do not show appreciable antibacterial activity (MIC >100 μg/mL). 4-Methyl-, 4-ethyl-, 4-dimethylaminobenzamide moieties as well as pyrazin-2-ylcarbonylamino and pentanamide moieties are tolerated. 4-Ethylbenzamide analog of compound **4, 7a-5**, shows better potency than the parent compound (MIC 1.56 μg/mL). 4-Dimethylamino- and 3-vinylbenzamide moieties as well as ethoxyacetamide moiety show only modest activities.

In the 6-pyrrolidinylamino series of compounds (**7b-1∼7**), the potency of the compounds is much less sensitive to the substitution pattern of the 5-amido moiety. Nevertheless, 3-phenylpropanamide and ethoxyacetamide moieties decrease potency. Two compounds bearing 4-methylbenzamide and 4-bromobenzamide moieties, **7b-3** (MIC 3.13 μg/mL) and **7b-4** (MIC 1.56 μg/mL) possess better activity than compound **4**.

In the 6-dimethylamino series of compounds (**7c-1∼17**), all compounds examined possess good to excellent antibacterial activities, wherein 9 compounds have MIC less than 1.0 μg/mL. The results clearly indicate that the introduction of the dimethylamino group to the 6 position is a breakthrough in this SAR study. Also, the 4-substituents on the 5-benzamide moiety are found to be beneficial to increase the potency. The most potent compounds in this series, at present, are **7c-4** and **7c-6** (MIC 0.16 μg/mL). Thus, **7c-4** and **7c-6** have emerged as advanced lead compounds together with **5f** for further preclinical drug development. Table 4 lists 9 most potent compounds found based on this SAR study.

These advanced lead compounds are also highly active against clinical isolates of *Mtb*, possessing different resistance profiles. Table 5 shows the antibacterial activity of two representative advanced lead compounds, **5f** and **7c-6**, against four *Mtb* strains; laboratory strain H37Rv (drug sensitive), clinical strain NHN382 (isoniazide-resistant, Kat G S315t mutation)[37], clinical strain TN587 (isoniazide-resistant, Kat G 3315T mutation)[37] and clinical strain W210 (drug-sensitive).[37] Both **5f** and **7c-6** possess substantially higher potency than compound **1** for all strains and more importantly there is no difference in their potencies against drug-resistant *Mtb* strains. The unbiased ability of these compounds to kill drug-resistant strains of *Mtb* demonstrates the significance of FtsZ as the drug target to develop new generation anti-TB drugs.

Mtb-FtsZ Polymerization Inhibitory Assay

To confirm that this series of compounds actually target *Mtb* FtsZ, the most potent compound **5f** was selected for the light scattering assay.[22, 38] In addition, compound **5p** and **7c-6** with MIC of 0.63 μg/mL and 0.16 μg/mL, respectively, were also tested. As Figure 3 shows, **5f, 5p** and **7c-6** inhibit the assembly of *Mtb* FtsZ in a dose dependent manner. Since the extent of light scattering correlates to the extent of FtsZ polymerization/aggregation, reduction in light scattering in presence of the compounds demonstrates their inhibitory effect on *Mtb* FtsZ assembly.

Transmission Electron Microscopy (TEM) Analysis

TEM imaging of *Mtb* FtsZ is also useful to validate the molecular target of lead compounds.[22] *Mtb* FtsZ (5 μM) was incubated with 40 μM or 80 μM of **5f** followed by addition of GTP (25 μM) to initiate polymerization. The TEM images of *Mtb* FtsZ treated

with 40 μM of **5f** clearly show reduction in the density and population of FtsZ polymers, protofilaments and aggregates when compared to the untreated protein polymers (Figure 4A, B). The effect is more apparent at 80 μM treatment (Figure 4C). In addition to inhibiting the assembly of *Mtb* FtsZ in the presence of GTP, **5f** was also capable of disrupting the formed FtsZ polymers and aggregates as shown by images D and E. *Mtb* FtsZ (5 μM) was polymerized by adding GTP (25 μM) and then treated with 40 μM or 80 μM of **5f**. The polymerized protein was depolymerized/disassembled after the addition of **5f**. These results clearly indicate that **5f** interacts with *Mtb* FtsZ and deters its normal GTP initiated polymerization.

Conclusions

The extensive SAR study on 2-cyclohexyl-5,6-disubstituted benzimidazoles for their antibacterial activities against *Mtb* H37Rv strain was performed, building upon the identification of promising early lead compounds, **1** and **2**, in our previous study. The purpose of this SAR study was to optimize the nitrogen substituents at the 5 and 6 positions of these two lead compounds through systematic modifications. It has been found that the nature of the 6-amino group exerts remarkable effects on the antibacterial activity. For example, long or bulky 6-alkylamino groups are detrimental to the activity. On the contrary, a small dimethylamino group at this position dramatically increases the potency. This breakthrough finding in this SAR study has led to the discovery of **5f** with exceptional potency (MIC 0.06 μg/mL), which bears a butoxycarbonylamino group at the 5 position and a dimethylamino group at the 6 position. Also, 12other compounds were found to possess comparable potencies (MIC <0.63 μg/mL), including **7c-6** (MIC 0.16 μg/mL), bearing a 4-ethylbenzamido group at the 5 position and a dimethylamino group at the 6 position. These advanced lead compounds do not show appreciable cytotoxicity against Vero cells (IC50 >100 μg/mL). The advanced leads, **5f** and **7c-6**, exhibit the same potencies against drug-resistant *Mtb* clinical isolates, as anticipated. Other important findings in this SAR study include the fact that urea groups at the 5 position are detrimental to the antibacterial activity of this series of benzimidazoles, while carbamate and amide groups are well tolerated although alkylamides and heteroaromatic amides are not favorable. The light scattering assays for FtsZ polymerization inhibition by selected advanced lead compounds, **5f, 5p** and **7c-6**, show effective dose-dependent inhibition, which validates that *Mtb* FtsZ is the molecular target of these compounds. The TEM analysis also supports the mechanism of action for these compounds. Furthermore, the TEM analysis has revealed that the pre-formed FtsZ polymers and their aggregates are effectively disassembled by **5f**. These observations strongly suggest that the advanced lead benzimidazoles have a novel mechanism of action on the inhibition of *Mtb* FtsZ assembly and Z-ring formation. Extensive preclinical evaluations for the pharmacological properties of these advanced lead compounds as well as *in vivo* efficacy evaluations are actively underway, and the results will be reported in due course.

Experimental Section

Methods

^{1}H and ^{13}C NMR spectra were measured on a Brucker or Varian 300, 400 or 500 MHz NMR spectrometer. Melting points were measured on a Thomas Hoover Capillary melting point apparatus and are uncorrected. TLC was performed on silica coated aluminum sheets (thickness 200 μm) or alumina coated (thickness 200 μm) aluminum sheets supplied by Sorbent Technologies and column chromatography was carried out on Siliaflash® P60 silica gel, 40–63 μm (230–400 mesh) supplied by Silicycle. Aluminum oxide, activated, neutral, Brockmann Grade I, 58 Å, was supplied by Alfa Aesar. High-resolution mass spectra were

obtained from the Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL. Purity of synthesized compounds was determined by a Shimadzu LC-2010A HT series HPLC assembly or Agilent 1100 series HPLC assembly. Four analytical conditions were used and noted as a part of the characterization data for synthesized compounds. HPLC (1): Adsorbosphere Silica 5 μ, 250 × 4.6 mm column, isopropanol and hexanes, flow rate of 1 mL/min, t = 0–40 min, gradient of 5–50% isopropanol. HPLC (2): Adsorbosphere Silica 5 μ, 250 × 4.6 mm column, isopropanol and hexanes, flow rate of 1.0 mL/min, t = 0–20 min, gradient of 5–50% isopropanol. HPLC (3): Kinetex PFP, 2.6 μm, 4.6 mm × 100 mm column, methanol and water, flow rate of 0.3 mL/min, t = 0–30 min, gradient of 40–95% MeOH. HPLC (4): Kinetex PFP, 2.6 μm, 4.6 mm × 100 mm column, solvent A of water/acetonitrile 95:5 (25 mM ammonium acetate, pH 6.5), solvent B of water/acetonitrile, 5:95 (25mM ammonium acetate, pH 6.5), temperature of 25 °C, flow rate of 1.0 mL/min, t = 0–15 min, gradient of 20–95% solvent B. Measurements were made at 254 nm and 303 nm.

Light scattering assays were performed using a PTI-QM4 spectrofluorimeter. FEI Tecnai12 BioTwinG transmission electron microscope with an AMT XR-60 CCD digital camera system was used to acquire transmission electron microscopy images.

Materials

BL21(DE3) cells, His-Bind protein purification resin and the buffers were purchased from Novagen. Bradford kit for protein concentration determination was purchased from Sigma. Buffer salts (reagent grade or better), solvents (HPLC grade or better), and all the other chemicals were purchased from Fisher Scientific Co. (Pittsburgh, PA). The chemicals were purchased from Aldrich Co., Synquest Inc. and Sigma and purified before use by standard methods. Tetrahydrofuran (THF) was freshly distilled from sodium metal and benzophenone. Dichloromethane was also distilled immediately prior to use under nitrogen from calcium hydride. Compounds, **2a, 2b, 2e-2g, 3a, 3b, 3e-3g, 4a, 4b**, and **4e–g** were prepared using the procedures previously published by us.[22]

2,4-Dinitro-5-*N,N*-di-*n*-propylaminoaniline (2d)

To a solution of 5-fluoro-2,4-dinitroaniline (1.01 g, 4.97 mmol) and diisopropylethylamine (DIPEA) (1.04 mL, 5.96 mmol) in THF (50 mL) was added di-*n*-propylamine (818 μL, 5.96 mmol). The reaction mixture was stirred at 50 °C overnight. After completion of the reaction, solvent was evaporated and water was added to give a yellow precipitate. The solution was extracted with dichloromethane (30 mL × 3). The organic layer was collected, dried over anhydrous magnesium sulfate and evaporated to give **2d** as a yellow solid (1.25 g, 89% yield); mp 68–70 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.86 (t, 6 H, J = 7.4 Hz), 1.60 (m, 4 H), 3.12 (t, 4 H, J = 7.28 Hz), 6.09 (s, 1 H), 6.40 (br. s, NH), 8.75 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 11.28, 20.45, 53.51, 102.9, 123.4, 128.3, 131.7, 147.6, 150.1; MS (ESI) *m/z* 283.1 (M+1)+.

Same procedure was used for the synthesis of **2c**.

5-*N,N*-Dimethylamino-2,4-dinitroaniline (2c)

Yellow solid (98% yield); mp 164–165 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.94 (br. s, 6 H), 6.01 (br. s, 1 H), 6.41 (br. s, 2H), 8.8 (br. s, 1 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 42.5 100.7, 123.6, 128.8, 130.5, 147.9, 150.7; HRMS (ESI) *m/z* calcd for C$_8$H$_{10}$N$_4$O$_4$ H$^+$: 227.0775, Found: 227.0778 (Δ = 1.4 ppm).

1-Cyclohexylamido-2,4-dinitro-5-*N,N*-di-*n*-propylaminobenzene (3d)

A solution of **2d** (1.25 g, 4.40 mmol) and cyclohexanecarbonyl chloride (0.721 mL, 5.34 mmol) in pyridine (10 mL) was refluxed overnight. After completion of the reaction, pyridine was removed *in vacuo* to give a brown solid. This crude mixture was washed with methanol and collected by filteration to afford **3d** as a yellow solid which was used directly in the following step. MS (ESI) *m/z* 393.2 (M+1)⁺.

Same procedure was used for the synthesis of **3c**.

1-Cyclohexylamido-5-*N,N*-dimethylamino-2,4-dinitrobenzene (3c)

Yellow solid (90% yield); mp 148–149 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.21–1.43 (m, 3 H), 1.67–1.79 (m, 1 H), 1.80–1.94 (m, 2 H), 1.97–2.09 (m, 2 H), 2.32–2.45 (m, 1 H), 3.06 (s, 6 H), 8.60 (s, 1 H), 8.85 (s, 1 H), 11.00 (s, 1 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 25.5, 25.6, 29.4, 42.5, 47.3, 106.1, 125.0, 127.4, 131.3, 138.7, 150.1, 175.9; HRMS (ESI) *m/z* calcd for C$_{15}$H$_{20}$N$_4$O$_5$H⁺: 337.1507, Found: 337.1511 (Δ = 1.3 ppm).

5-Amino-2-cyclohexyl-6-*N,N*-di-*n*-propylamino-1*H*-benzo[*d*]imidazole (4d)

To a solution of **3d** from previous step (1.75 g, 4.45 mmol) in ethanol (50 mL) was added solid stannous chloride dihydrate (7.03 g, 31.1 mmol). Concentrated hydrochloric acid (15 mL) was added to the reaction mixture such that the final concentration of HCl is 4 M in the reaction flask. The reaction mixture was refluxed for 4 h. Upon completion, the reaction mixture was basified with 30% sodium hydroxide solution. Excess stannous chloride formed a soluble salt in excess base. The desired product was extracted with dichloromethane and purified by flash chromatography on silica gel using ethyl acetate as eluent to afford **4d** as a pale green solid (721 mg, 55% yield in two steps); mp 76–78 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.78 (t, 6 H, *J* = 7.4 Hz), 1.20–1.23 (m, 3 H), 1.35–1.40 (m, 4 H), 1.59–1.63 (m, 5 H), 2.01 (d, 2 H, *J* = 7.28 Hz), 2.77 (m, 5 H), 6.80 (s, 1 H), 7.25 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 11.60, 20.45, 25.64, 25.91, 31.84, 38.43, 57.04, 98.19, 110.0, 132.9, 134.3, 135.0, 140.0, 158.0; HRMS (ESI) *m/z* calcd for C$_{19}$H$_{30}$N$_4$H⁺: 315.2543, Found: 315.2549 (Δ = 1.9 ppm).

Same procedure was used for the synthesis of **4c**.

5-Amino-2-cyclohexyl-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (4c)

Light brown solid (69% yield); mp 135–137 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.14–1.46 (m, 4 H), 1.54–1.86 (m, 5 H), 1.99–2.17 (m, 2 H), 2.64 (s, 6 H), 2.73–2.87 (m, 1 H), 6.81 (s, 5 H), 7.26 (s, 1 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 26.0, 26.2, 32.2, 38.8, 44.8, 98.9, 106.7, 133.1, 134.5, 138.0, 138.3, 158.2; HRMS (ESI) *m/z* calcd for C$_{15}$H$_{22}$N$_4$H⁺: 259.1917, Found: 259.1914 (Δ = −1.2 ppm).

2-Cyclohexyl-5-(3-propylurea)-6-pyrrolidinyl-1*H*-benzo[*d*]imidazole (6f)

To a solution of **4b** (100 mg, 0.35 mmol) in dichloromethane (DCM) (3 mL), was added *n*-propylisocyanate (32.9 mg, 0.39 mmol) and triethylamine (0.39 mmol). The reaction was refluxed for 14 h and then diluted with ethyl acetate, transferred to a separatory funnel and washed with water, sat. sodium bicarbonate solution and brine. The organic layer was dried over magnesium sulfate and concentrated using rotary evaporator. The crude product was purified by flash chromatography (column was packed with DCM, 2% methanol was used steadily increasing to 5% methanol) to obtain the product as a white solid (80% yield); mp 231–232 °C; ^{1}H NMR (400 MHz, Methanol-d$_4$) δ 0.95 (t, *J* = 7.2 Hz, 3 H), 1.26–1.69 (m, 8 H), 1.75–1.78 (m, 1 H), 1.84–1.88 (m, 2 H), 1.92–2.11 (m, 6 H), 2.83 (m, 1 H), 3.04 (s, 4 H), 3.16 (t, *J* = 7.03 Hz, 2 H), 7.24 (s, 1 H), 7.92 (s, 1 H); ^{13}C NMR (126 MHz, Methanol-

d$_4$) δ 11.9, 24.6, 25.5, 27.1, 27.4, 33.0, 40.0, 42.9, 53.9, 106.3, 107.1, 130.9, 134.5, 136.5, 139.2, 159.2, 160.5; HRMS (FAB) *m/z* calcd for C$_{21}$H$_{31}$N$_5$OH$^+$: 370.2601, Found: 370.2611 (Δ = 2.6 ppm); HPLC (1): t =17.7 min, purity > 99%.

Same procedure was followed for the synthesis of **6a-6g**

5-(4-Butylurea)-2-cyclohexyl-6-*N,N*-diethylamino-1*H*-benzo[*d*]imidazole (6a)

White solid (70% yield); mp 197–198 °C; ^{1}H NMR (500 MHz, Methanol-d$_4$) δ 0.95–1.10 (m, 9 H), 1.36–1.66 (m, 7 H), 1.67–1.79 (m, 2 H), 1.80–1.90 (m, 1 H), 1.90–2.01 (m, 2 H), 2.14 (d, *J* = 13.73 Hz, 2 H), 2.93 (m, 1 H), 3.06 (quin, *J* = 7.20 Hz, 4 H), 3.29 (q, *J* = 7.20 Hz, 2 H), 7.42 (d, *J* = 7.02 Hz, 1 H), 8.27 (d, *J* = 7.63 Hz, 2 H); ^{13}C NMR (126 MHz, Methanol-d$_4$) δ 13.0, 13.01 14.3, 21.3, 27.1, 27.4, 33.0, 33.5, 40.0, 40.7, 51.7, 103.2, 111.3, 135.3, 135.9, 136.2, 158.7, 160.7; HRMS (FAB) *m/z* calcd for C$_{22}$H$_{35}$N$_5$OH$^+$: 386.2914, Found: 386.2911 (Δ = –0.9 ppm); HPLC (1): t =12.7 min, purity > 99%.

5-(Benzylurea)-2-cyclohexyl-6-*N,N*-diethylamino-1*H*-benzo[*d*]imidazole (6b)

White solid (94% yield); mp 198–199 °C; ^{1}H NMR (400 MHz, Methanol-d$_4$) δ 0.90 (t, *J* = 7.15 Hz, 6 H), 1.23–1.52 (m, 4 H), 1.57–1.70 (m, 2 H), 1.77 (d, *J* = 14.31 Hz, 1 H), 1.86 (d, *J* = 13.05 Hz, 2 H), 1.98–2.12 (m, 2 H), 2.84–2.87 (m, 1 H), 2.95 (q, *J* = 7.00 Hz, 4 H), 4.34–4.45 (m, 2 H), 7.19–7.26 (m, 1 H), 7.28–7.39 (m, 5 H), 8.22 (s, 1 H); ^{13}C NMR (101 MHz, Methanol-d$_4$) δ 13.0, 27.1, 27.3, 33.0, 40.0, 44.8, 51.6, 103.4, 111.3, 128.2, 128.4, 129.7, 135.1, 136.0, 136.3, 141.3, 158.5, 160.8; HRMS (FAB) *m/z* calcd for C$_{25}$H$_{33}$N$_5$OH$^+$: 420.2758, Found: 420.2761 (Δ = 0.7 ppm); HPLC (1): t =13.4 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(3-propylurea)-1*H*-benzo[*d*]imidazole (6c)

White solid (87% yield); mp 216–217 °C; ^{1}H NMR (400 MHz, Methanol-d$_4$) δ 0.87–1.01 (m, 9 H), 1.23–1.50 (m, 4 H), 1.51–1.70 (m, 4 H), 1.71–1.79 (m, 1 H), 1.85 (dt, *J* = 13.18, 3.33 Hz, 2 H), 2.05 (d, *J* = 13.40 Hz, 2 H), 2.84–2.88 (m, 1 H), 2.96 (q, *J* = 7.2 Hz, 4 H), 3.16 (t, *J* = 7.20 Hz, 2 H), 7.33 (s, 1 H), 8.20 (s, 1 H); ^{13}C NMR (101 MHz, Methanol-d$_4$) δ 11.9, 13.0, 24.5, 27.1, 27.4, 33.0, 40.0, 42.8, 51.6, 103.2, 111.3, 135.3, 136.0, 136.2, 158.7, 160.7; HRMS (FAB) *m/z* calcd for C$_{21}$H$_{33}$N$_5$OH$^+$: 372.2758, Found: 372.2754 (Δ = − 1.0 ppm); HPLC (1): t =12.6 min, purity > 99%.

5-(4-Butylurea)-2-cyclohexyl-6-pyrrolidinyl-1*H*-benzo[*d*]imidazole (6d)

White solid (96% yield); mp 206–207 °C; ^{1}H NMR (400 MHz, Methanol-d$_4$) δ 0.95 (t, *J* = 7.2 Hz, 3 H), 1.26–1.55 (m, 7 H), 1.56–1.70 (m, 2 H), 1.71–1.82 (m, 1 H), 1.82–1.91 (m, 2 H), 1.91–2.11 (m, 6 H), 2.79–2.87 (m, 1 H), 3.03 (br. s, 4 H), 3.20 (t, *J* = 7.2 Hz, 2 H), 7.24 (s, 1 H), 7.94 (s, 1 H); ^{13}C NMR (101 MHz, Methanol-d$_4$) δ 14.3, 21.2, 27.12, 25.5, 27.3, 33.0, 33.5, 39.9, 40.8, 53.9, 106.3, 107.1, 131.0, 134.4, 136.4, 139.3, 159.2, 160.4; HRMS (FAB) *m/z* calcd for C$_{22}$H$_{33}$N$_5$OH$^+$: 384.2758, Found: 384.2755 (Δ = −0.8 ppm); HPLC (1): t =14.2 min, purity > 99%.

5-(4-Benzylurea)-2-cyclohexyl-6-pyrrolidinyl-1*H*-benzo[*d*]imidazole (6e)

White solid (91% yield); mp 225–226 °C; ^{1}H NMR (500 MHz, Methanol-d$_4$) δ 1.26–1.49 (m, 3 H), 1.56–1.68 (m, 2 H), 1.71–1.79 (m, 1 H), 1.80–1.89 (m, 2 H), 1.92 (br. s, 4 H), 1.98–2.10 (m, 2 H), 2.80–2.85 (m, 1 H), 3.01 (br. s, 4 H), 4.39 (s, 2 H), 7.18–7.26 (m, 2 H), 7.26–7.37 (m, 4 H), 7.96 (s, 1 H); ^{13}C NMR (126 MHz, Methanol-d$_4$) δ 25.5, 27.1, 27.3, 33.0, 39.9, 44.8, 53.8, 106.2, 107.4, 128.2, 128.4, 129.7, 130.7, 134.4, 136.5, 139.4, 141.3, 159.1, 160.5; HRMS (FAB) *m/z* calcd for C$_{25}$H$_{31}$N$_5$OH$^+$: 418.2601, Found: 418.2604 (Δ = 0.6 ppm); HPLC (1): t =14.5 min, purity > 99%.

5-(4-Butylurea)-2-cyclohexyl-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (6g)

White solid (74% yield); mp 185–186 °C; ^{1}H NMR (400 MHz, Methanol-d_4) δ 1.71 (t, *J* = 7.28 Hz, 3 H), 1.98–2.27 (m, 7 H), 2.30–2.44 (m, 2 H), 2.49 (d, *J* = 12.30 Hz, 1 H), 2.54–2.65 (m, 2 H), 2.73–2.85 (m, 2 H), 3.38 (s, 6 H), 3.57 (m, 1 H), 3.89 (q, *J* = 6.53 Hz, 2 H), 4.15 (br. s, 1 H), 7.80 (br. s, 1 H), 8.05 (br. s, 1 H), 8.83 (br. s, 1 H), 8.97 (s, 1 H), 12.54 (br. s, 1 H); ^{13}C NMR (126 MHz, Methanol-d_4) δ 14.3, 21.2, 27.0, 27.3, 32.9, 33.5, 39.7, 40.7, 45.8, 104.3, 107.7, 132.3, 134.3, 135.0, 141.4, 158.8, 160.1; HRMS (FAB) *m/z* calcd for $C_{20}H_{31}N_5OH^+$: 358.2601, Found: 358.2609 (Δ = 2.1 ppm); HPLC (1): t =15.3 min, purity > 99%.

5-Butoxycarbonylamino-2-cyclohexyl-6-*N,N*-dipropylamino-1*H*-benzo[*d*]imidazole (5a)

To a solution of **4d** (124 mg, 0.39 mmol) in dichloromethane (5 mL) was added a solution of *N*-butoxycarbonyloxysuccinimide (80 mg, 0.39 mmol) in dichloromethane (10 mL) dropwise at room temperature. The reaction mixture was stirred at room temperature overnight and after completion of the reaction, the reaction mixture was concentrated. The crude was purified via flash chromatography on silica gel (gradient 20–40% EtOAc/hexanes) to give **5a** as white solid (72 mg, 33% yield): mp 128–130 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.83 (t, 6 H, *J* = 7.2 Hz), 0.94 (t, 3 H, *J* = 7.5 Hz), 1.33 (m, 9 H), 1.65–1.69 (m, 5 H), 1.80–1.82 (m, 2 H, *J* = 10 Hz), 2.05–2.07 (m, 2 H, *J* = 10 Hz), 2.79–2.81 (m, 5 H), 4.17 (t, 2 H, *J* = 6.8 Hz), 7.47 (s, 1 H), 8.22 (s, 1 H), 8.54 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 11.70, 13.74, 19.09, 20.61, 25.78, 26.00, 31.04, 31.76, 38.42, 58.59, 64.82, 99.39, 112.6, 131.9, 135.4, 154.1, 158.8, 173.3; HRMS (ESI) *m/z* calcd for $C_{24}H_{38}N_4O_2 H^+$: 415.3068, Found: 415.3073 (Δ = 1.3 ppm); HPLC (1): t =6.1 min, purity > 97%.

Same procedure was used for the synthesis of **5b**.

5-Benzyloxycarbonylamino-2-cyclohexyl-6-*N,N*-dipropylamino-1*H*-benzo[*d*]imidazole (5b)

White solid (51% yield): mp 138–140 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.80 (t, 6 H, *J* = 7.2 Hz), 1.32–1.34 (m, 7 H), 1.59–1.62 (m, 3 H), 1.77–1.79 (m, 2 H, *J* = 10 Hz), 2.04–2.06 (m, 2 H, *J* = 10 Hz), 2.77–2.80 (m, 5 H), 5.23 (s, 2 H), 7.37 (m, 5 H), 7.49 (s, 1 H), 8.24 (s, 1 H), 8.67 (s, 1 H), 9.84 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 11.70, 20.57, 25.77, 25.96, 31.76, 38.42, 58.66, 66.57, 98.87, 113.3, 127.9, 128.1, 128.5, 131.7, 135.3, 136.4, 153.6, 158.9; HRMS (ESI) *m/z* calcd for $C_{27}H_{36}N_4O_2H^+$: 449.2911, Found: 449.2909 (Δ = −0.4 ppm); HPLC (1): t =6.5 min, purity > 99%.

5-Butyloxycarbonylamino-2-cyclohexyl-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (5f)

A solution of **4c** (100 mg, 0.39 mmol) and 1,1'-carbonyldiimidazole (69 mg, 0.43 mmol) in dichloromethane (2 mL) was stirred under reflux conditions for 4 hours. After all the starting material had disappeared, *n*-butanol (71 μL, 0.77 mmol) was added and the reaction was refluxed for additional 12 hours. After the completion of the reaction, the reaction mixture was concentrated. The crude was purified by flash chromatography on silica gel (gradient: 30–70% ethyl acetate /hexanes) to give **5f** as a white solid (111 mg, 79% yield); mp 182–183 °C; 1H NMR (300 MHz, CDCl$_3$) δ 0.97 (t, *J* = 7.28 Hz, 3 H), 1.23–1.51 (m, 6 H), 1.55–1.78 (m, 5 H), 1.79–1.91 (m, 2 H), 2.10 (d, *J* = 12.91 Hz, 2 H), 2.64 (s, 6 H), 2.76–2.92 (m, 1 H), 4.20 (t, *J* = 6.73 Hz, 2 H), 7.55 (br. s, 1 H), 8.20 (br. s, 2 H), 9.20 (br. s, 1 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 14.0, 19.3, 26.0, 26.2, 31.3, 32.0, 38.6, 45.8, 65.2, 100.9, 109.5, 129.8, 132.1, 136.6, 139.1, 154.4, 159.0; HRMS (FAB) *m/z* calcd for $C_{20}H_{30}N_4O_2H^+$: 359.2442, Found: 359.2443 (Δ = 0.4 ppm); HPLC (1): t =8.9 min, purity > 99%.

Same procedure was used for the synthesis of **5c-5p**.

5-Butyloxycarbonylamino-2-cyclohexyl-6-*N,N*-dimethyl-*N*-ethylethylenediamino-1*H*-benzo[*d*]imidazole (5c)

White solid (44% yield): mp 141–143 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.86 (t, 3 H, *J* = 7.2 Hz), 0.94 (t, 3 H, *J* = 7.2 Hz), 1.23–1.27 (m, 3 H), 1.41–1.43 (m, 2 H), 1.67–1.75 (m, 6 H), 2.00 (m, 2 H, *J* = 10 Hz), 2.19 (s, 6 H), 2.74–2.75 (m, 1 H), 2.92–2.93 (m, 4 H), 4.16 (t, 2 H, *J* = 7.2 Hz), 7.47 (s, 1 H), 8.24 (s, 1 H), 9.88 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.08, 13.73, 19.12, 25.77, 26.01, 31.12, 31.70, 38.48, 45.03, 50.99, 55.03, 57.04, 64.51, 100.2, 113.8, 131.8, 132.9, 135.0, 138.8, 154.9, 159.2; HRMS (ESI) *m/z* calcd for C$_{24}$H$_{39}$N$_5$O$_2$H+: 430.3176, Found: 430.3179 (Δ = 0.7 ppm); HPLC (3): t =23.8 min, purity > 98%.

5-Butyloxycarbonylamino-2-cyclohexyl-6-*N,N,N*-trimethylethylenediamino-1*H*-benzo[*d*]imidazole (5d)

White solid (45% yield); mp 116–118 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.95 (t, 3 H, *J* = 7.2 Hz), 1.24–1.45 (m, 6 H), 1.60–1.68 (m, 5 H), 1.79–1.80 (m, 2 H), 2.03–2.04 (m, 2 H), 2.24 (s, 6 H), 2.31 (t, 2 H, *J* = 7.2 Hz), 2.68 (s, 3 H), 2.84–2.85 (m, 3 H), 4.17 (t, 2 H, *J* = 6.8 Hz), 7.49 (s, 1 h), 8.26 (s, 1 H), 9.80 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.77, 19.15, 25.81, 26.01, 31.16, 31.77, 38.45, 43.65, 45.41, 57.02, 57.23, 64.55, 100.0, 112.3, 131.2, 137.8, 154.8, 158.8; HRMS (ESI) *m/z* calcd for C$_{23}$H$_{37}$N$_5$O$_2$H$^+$: 416.3020, Found: 416.3031 (Δ = 2.6 ppm); HPLC (1): t =6.5, purity > 98%.

5-Butyloxycarbonylamino-2-cyclohexyl-6-*N,N,N*-triethylethylenediamino-1*H*-benzo[*d*]imidazole (5e)

White solid (60% yield); mp 150–151 °C; 1H NMR (400 MHz, CDCl$_3$) δ 0.95 (t, 3 H, *J* = 7.2 Hz), 0.97–1.00 (m, 9 H), 1.24–1.48 (m, 6 H), 1.57–1.70 (m, 6 H), 1.78–1.79 (m, 2 H), 2.02–2.05 (m, 2 H), 2.37 (t, 2 H, *J* = 7.2 Hz), 2.50–2.55 (m, 4 H, *J* = 7.2 Hz), 2.76–2.79 (m, 1 H), 2.93–2.95 (m, 4 H), 4.17 (t, 2 H, *J* = 6.9 Hz), 7.52 (s, 1 H), 8.23 (s, 1 H), 9.84 (s, 1 H), 10.5 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 11.10, 12.96, 13.73, 19.12, 25.79, 26.03, 31.30, 31.70, 38.48, 46.81, 50.41, 50.67, 55.34, 64.56, 100.5, 113.6, 131.6, 132.7, 135.4, 138.9, 154.9, 159.1; HRMS (ESI) *m/z* calcd for C$_{26}$H$_{43}$N$_5$O$_2$H$^+$: 458.3489, Found: 458.3489 (Δ = 0.0 ppm); HPLC (1): t =25.5 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(1-methylbutoxycarbonylamino)-1*H*-benzo[*d*]imidazole (5g)

White solid (50% yield): mp 88–90 °C; 1H NMR (400 MHz, CDCl$_3$) δ 0.88–0.92 (m, 9 H), 1.37–1.63 (m, 16 H), 1.79–1.81 (m, 2 H, *J* = 10 Hz), 2.07–2.10 (m, 2 H, *J* = 10 Hz), 2.83–2.93 (m, 5 H), 4.90–4.93 (m, 1 H), 7.45 (s, 1 H), 8.22 (s, 1 H), 8.41 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 12.68, 13.96, 18.72, 20.34, 25.76, 25.97, 29.67, 31.74, 38.35, 50.41, 71.46, 99.98, 112.2, 132.6, 134.5, 153.9, 158.6; HRMS (ESI) *m/z* calcd for C$_{23}$H$_{36}$N$_4$O$_2$H$^+$: 401.2911, Found: 401.2917 (Δ = 1.5 ppm); HPLC (1): t =6.8 min, purity > 97%.

5-Benzyloxycarbonylamino-2-cyclohexyl-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (5h)

White solid (82% yield); mp 145–146 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.15–1.40 (m, 3 H), 1.45–1.87 (m, 5 H), 2.03 (d, *J* = 11.73 Hz, 2 H), 2.60 (s, 6 H), 2.74–2.92 (m, 1 H), 5.23 (s, 2 H), 7.28–7.60 (m, 6 H), 8.14–8.42 (m, 2 H), 10.23 (br. s, 1 H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 21.8, 25.9, 26.1, 31.80, 31.84, 38.3, 38.3, 45.8, 67.1, 100.9, 109.5, 128.5, 128.8, 129.9, 131.6, 136.0 136.4, 139.4, 153.9, 158.9, 175.9; HRMS (FAB) *m/z* calcd for C$_{23}$H$_{28}$N$_4$O$_2$H$^+$: 393.2285, Found: 393.2290 (Δ = 1.3 ppm); HPLC (1): t =9.0 min, purity > 99%.

2-Cyclohexyl-5-methoxyethoxycarbonylamino-6-pyrrolidinyl-1*H*-benzo[*d*]imidazole (5i)

White solid (55% yield): mp 75–77 °C; ^{1}H NMR (400 MHz, CDCl3) δ 1.25–1.43 (m, 3 H), 1.57–1.93 (m, 6 H), 1.88 (br. s, 4 H), 2.00–2.02 (m, 2 H, J = 10 Hz), 2.76–2.77 (m, 1 H), 2.89 (br. s, 4 H), 3.39 (s, 3 H), 3.64 (t, 2 H, J = 6.5 Hz), 4.32 (t, 2 H, J = 6.5 Hz), 7.37 (s, 1 H), 8.09 (br. s, 1 H), 8.16 (br. s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 24.22, 25.66, 25.88, 31.70, 38.37, 53.55, 58.87, 63.89, 70.81, 101.6, 108.7, 129.4, 132.3, 135.7, 136.9, 153.8, 159.1; HRMS (ESI) *m/z* calcd for C$_{21}$H$_{30}$N$_4$O3H$^+$: 387.2390, Found: 387.2400 (Δ = 2.6 ppm); HPLC (1): t =14.2 min, purity > 97%.

2-Cyclohexyl-5-ethoxyethoxycarbonylamino-6-*N,N*-diethylamino-1*H*-benzo[*d*]imidazole (5j)

White solid (42% yield); mp 158–160 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.84 (t, 6 H, J = 7.2 Hz), 1.19–1.22 (m, 5 H), 1.57–1.60 (m, 3 H), 1.74–1.77 (m, 2 H), 2.02–2.05 (m, 2 H, J = 10 Hz), 2.78–2.88 (m, 5 H), 3.52–3.57 (m, 2 H, J = 7 Hz), 3.68–3.69 (m, 2 H), 4.33 (t, 2 H, J = 6.5 Hz), 7.40 (s, 1 H), 8.23 (br. s, 1 H), 8.58 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 12.49, 15.03, 25.72, 25.94, 31.74, 38.44, 50.34, 64.10, 66.61, 68.73, 99.27, 112.9, 131.9, 134.2, 138.0, 153.7, 159.1; HRMS (ESI) *m/z* calcd for C$_{22}$H$_{34}$N$_4$O$_3$H$^+$: 403.2703, Found: 403.2711 (Δ = 1.9 ppm); HPLC (1): t =9.1 min, purity > 97%.

2-Cyclohexyl-5-ethoxyethoxycarbonylamino-6-pyrrolidinyl-1*H*-benzo[*d*]imidazole (5k)

White solid (56% yield); mp 72–73 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.19–1.22 (m, 6 H), 1.54–1.57 (m, 3 H), 1.72–1.75 (m, 2 H), 1.89 (br. s, 4 H), 1.99–2.02 (m, 2 H, J = 10 Hz), 2.76–2.78 (m, 1 H), 2.90 (br. s, 4 H), 3.51–3.56 (m, 2 H, J = 7.2 Hz), 3.68 (t, 2 H, J = 6.5 Hz), 4.32 (t, 2 H, J = 6.5 Hz), 7.38 (s, 1 H), 8.08 (bs, 1 H), 8.16 (bs, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 15.01, 24.25, 25.69, 25.91, 31.72, 38.38, 53.57, 64.14, 66.59, 68.73, 101.5, 108.8, 129.5, 132.1, 135.7, 136.8, 153.8, 159.0; HRMS (ESI) *m/z* calcd for C$_{22}$H$_{32}$N$_4$O$_3$H$^+$: 401.2547, Found: 401.2550 (Δ = 0.7 ppm); HPLC (1): t =11.8 min, purity > 99%.

2-Cyclohexyl-5-ethoxyethoxycarbonylamino-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (5l)

White solid (86% yield); mp 158–159 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.20–1.44 (m, 7 H), 1.62 (qd, J = 12.31, 3.05 Hz, 2 H), 1.72 (d, J = 12.82 Hz, 1 H), 1.83 (d, J = 13.12 Hz, 2 H), 2.09 (d, J = 12.51 Hz, 2 H), 2.61 (s, 6 H), 2.77–2.93 (m, 1 H), 3.58 (q, J = 5.00 Hz, 2 H), 3.71 (t, J = 5.00 Hz, 2 H), 4.36 (t, J = 5.00 Hz, 2 H), 7.51 (br. s, 1 H), 8.07–8.48 (m, 2 H), 9.40 (br. s, 1 H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 15.3, 26.0, 26.2, 29.9, 32.0, 38.6, 45.9, 64.4, 66.9, 69.0, 99.5, 111.1, 129.7, 139.0, 154.0, 159.0; HRMS (FAB) *m/z* calcd for C$_{20}$H$_{30}$N$_4$O$_3$H$^+$: 375.2391, Found: 375.2410 (Δ = 5.1 ppm); HPLC (1): t =12.7 min, purity > 99%.

2-Cyclohexyl-6-*N, N*-diethylamino-5-(2-methoxyethoxyethoxycarbonylamino)-1*H*-benzo[*d*]imidazole (5m)

White solid (52% yield); mp 109–110 °C; ^{1}HNMR (400 MHz, CDCl$_3$) δ 0.87 (t, 6 H, J = 7.2 Hz), 1.25–1.32 (m, 3 H), 1.58–1.63 (m, 3 H), 1.70–1.78 (m, 2 H), 2.05–2.08 (m, 2 H, J = 10 Hz), 2.87–2.88 (m, 5 H), 3.37 (s, 3 H), 3.57 (bs, 2 H), 3.67 (t, 2 H, J = 7 Hz), 3.76 (t, 2 H, J = 7 Hz), 4.34 (t, 2 H, J = 6.5 Hz), 7.42 (s, 1 H), 8.22 (bs, 1 H), 8.56 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 12.53, 25.75, 25.95, 31.74, 38.42, 50.34, 58.98, 63.92, 69.58, 70.43, 71.84, 99.81, 112.5, 132.0, 134.2, 153.6, 159.0; HRMS (ESI) *m/z* calcd for C$_{23}$H$_{36}$N$_4$O$_4$H$^+$: 433.2809, Found: 433.2815 (Δ = 1.4 ppm); HPLC (1): t =16.9 min, purity > 97%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-trifluoroethoxycarbonylamino-1*H*-benzo[*d*]imidazole (5n)

White solid (42% yield); mp 203–204 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.90 (t, 6 H, *J* = 6.9 Hz), 1.25–1.37 (m, 4 H), 1.61–1.65 (m, 4 H), 1.81–1.84 (m, 2 H), 2.09–2.10 (m, 2 H), 2.85–2.94 (m, 5H), 4.53–4.57 (m, 2 H, *J* = 8.5 Hz), 7.51 (s, 1 H), 8.13 (s, 1 H), 8.78 (s, 1 H), 9.76 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 12.69, 25.81, 25.99, 31.83, 38.49, 50.59, 98.99, 113.5, 119.0, 121.7, 124.5, 127.3, 131.1, 131.4, 139.0, 151.5, 159.2; HRMS (ESI) *m/z* calcd for C$_{20}$H$_{27}$F$_3$N$_4$O$_2$H$^+$: 413.2159, Found: 413.2175 (Δ = 3.8 ppm); HPLC (1): t =7.3 min, purity > 99%.

2-Cyclohexyl-5-trifluoroethoxycarbonylamino-6-prrolidinyl-1*H*-benzo[*d*]imidazole (5o)

White solid (69% yield); mp 123–125 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.16–1.31 (m, 3 H), 1.59–1.62 (m, 3 H), 1.75–1.78 (m, 2 H), 1.92 (bs, 4 H), 2.03–2.07 (m, 2H), 2.79–2.82 (m, 1 H), 2.92–2.93 (m, 4 H), 4.52–4.59 (q, 2 H, *J* = 7.4 Hz), 7.41 (s, 1 H), 8.12 (s, 1 H), 8.21 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 24.30, 25.70, 25.92, 31.82, 38.48, 53.65, 102.0, 108.8, 118.9, 121.7, 124.4, 127.2, 128.7, 135.9, 151.7, 159.4; HRMS (ESI) *m/z* calcd for C$_{20}$H$_{25}$F$_3$N$_4$O$_2$H$^+$: 411.2002, Found: 411.2007 (Δ = 1.2 ppm); HPLC (1): t =7.1 min, purity > 97%.

2-Cyclohexyl-5-trifluoroethoxycarbonylamino-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (5p)

White solid (70% yield); mp 190–191°C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.05–1.43 (m, 4 H), 1.50–1.91 (m, 5 H), 1.96–2.21 (m, 2 H), 2.61 (s, 6 H), 2.83–2.89 (m, 1 H), 4.56 (q, *J* = 8.53 Hz, 2 H), 7.45 (s, 1 H), 8.19 (s, 1 H), 8.41 (s, 1 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 14.3, 22.8, 25.9, 26.2, 32.1, 38.8, 45.8, 60.4, 60.7, 61.1, 61.5, 101.8, 109.7, 119.2, 121.9, 124.7, 127.5, 128.6, 139.1, 151.8, 159.8; HRMS (FAB) *m/z* calcd for C$_{18}$H$_{23}$F$_3$N$_4$O$_2$H$^+$: 385.1846, Found: 385.1851 (Δ = 1.3 ppm); HPLC (1): t =9.1 min, purity > 99%.

5-Benzamido-2-cyclohexyl-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-1)

To a solution of **4c** (70 mg, 0.27 mmoles) in 2 mL of dichloromethane was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (57.5 mg, 0.3 mmol) (EDC) and benzoic acid (36.4 mg, 0.30 mmol). 4-Dimethylaminopyridine (DMAP) (36.4 mg, 0.30 mmol) was added to the above reaction mixture and the reaction was refluxed overnight. After completion of the reaction as per TLC, the reaction mixture was diluted with dichloromethane, transferred to a separatory funnel and the organic layer was washed with water, sodium bicarbonate and finally brine. The organic layer was dried over magnesium sulfate and concentrated using rotary evaporator. The crude product was purified by flash chromatography (column was packed with hexane, 30% ethyl acetate was used with gradual increase to 50% ethyl acetate) to obtain the product as a white solid (83 mg, 85% yield). mp 158–159°C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.07–1.19 (m, 3 H), 1.54–1.71 (m, 5 H), 1.97–2.03 (m, 2 H, *J* = 12.9 Hz), 2.74 (s, 6 H), 2.85 (m, 1 H), 7.58 (m, 3 H), 7.67 (s, 1 H), 7.95 (d , 1H, *J* = 6.6 Hz), 8.91 (s, 1 H), 9.88 (s, 1 H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 25.9, 26.1, 29.9, 32.0, 38.6, 46.2, 101.8, 110.9, 127.2, 129.0, 129.2, 131.4, 132.0, 135.8, 139.4, 139.9, 160.1, 165.7; HRMS (FAB) *m/z* calcd for C$_{22}$H$_{26}$N$_4$OH$^+$: 363.2179, Found: 363.2190 (Δ = 2.9ppm); HPLC (1): t =11.0 min, purity > 99%.

Same procedure was followed for the synthesis of **7d, 7a-1∼3, 7a-5, 7a-7∼10, 7a-15, 7b-1, 7b-4, 7b-7, 7c-3∼6, 7c-11∼12**, and **7c-16**.

5-Benzamido-2-cyclohexyl-6-*N,N*-di-*n*-propylamino-1*H*-benzo[*d*]imidazole (7d)

White solid (52% yield): mp 212–213 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.78 (t, 6 H, *J* = 7.2 Hz), 1.05–1.08 (m, 3 H), 1.36–1.39 (m, 4 H), 1.54–1.63 (m, 5 H), 1.90–1.95 (m, 2 H, *J* = 10 Hz), 2.65–2.67 (m, 1 H), 2.88–2.89 (m, 4 H, *J* = 7.5 Hz), 7.56–7.60 (m, 3 H), 7.99 (d, 2 H, *J* = 6.9 Hz), 9.00 (s, 1 H), 10.40 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 11.72, 20.69, 20.81, 25.57, 25.87, 31.71, 38.40, 59.10, 100.8, 113.3, 126.8, 128.9, 131.2, 131.6, 131.7, 135.4, 135.8, 139.7, 160.1, 165.2; HRMS (ESI) *m/z* calcd for C$_{26}$H$_{34}$N$_4$OH$^+$: 419.2805, Found: 419.2813 (Δ = 1.9 ppm); HPLC (1): t =6.3 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(4-difluoromethoxybenzamido)-1*H*-benzo[*d*]imidazole (7a-1)

White solid (66% yield); mp 194–196 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.95 (t, 6 H, *J* = 7.2 Hz), 1.07–1.10 (m, 3 H), 1.54–1.64 (m, 5 H), 1.96–1.98 (m, 2 H, *J* = 10 Hz), 2.69–2.70 (m, 1 H), 2.99 (q, 4 H, *J* = s Hz), 6.61 (t, 1 H (CHF2), *J* = 73 Hz), 7.28 (d, 2 H, *J* = 8.5 Hz), 7.57 (s, 1 H), 8.00 (d, 2 H, *J* = 8.6 Hz), 8.92 (s, 1 H), 10.40 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.07, 25.55, 25.88, 31.70, 38.48, 50.79, 100.8, 112.7, 113.2, 115.3, 117.9, 119.5, 128.7, 131.5, 131.8, 132.3, 139.7, 153.5, 160.0, 163.8; HRMS (ESI) *m/z* calcd for C$_{25}$H$_{30}$F$_2$N$_4$O$_2$H$^+$: 457.2409, Found: 457.2411 (Δ = 0.4 ppm); HPLC (1): t =8.9 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(4-trifluoromethoxybenzamido)-1*H*-benzo[*d*]imidazole (7a-2)

White solid (71% yield); mp 207–208 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.96 (t, 6 H, *J* = 7.2 Hz), 1.09–1.11 (m, 3 H), 1.55–1.66 (m, 5 H), 1.96–1.98 (m, 2 H, *J* = 10 Hz), 2.68–2.70 (m, 1 H), 3.00 (q, 4 H, *J* = 7.2 Hz), 7.25 (d, 2 H, *J* = 8.5 Hz), 7.58 (s, 1 H), 8.02 (d, 2 H, *J* = 8.6 Hz), 8.92 (s, 1 H), 10.43 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.10, 25.59, 25.88, 31.72, 38.49, 50.84, 100.8, 113.5, 119.0, 121.0, 121.6, 128.7, 131.5, 133.8, 135.0, 151.6, 159.9, 163.5; HRMS (ESI) *m/z* calcd for C$_{25}$H$_{29}$F$_3$N$_4$O$_2$H$^+$: 475.2315, Found: 475.2323 (Δ = 1.7 ppm); HPLC (1): t =7.5 min, purity > 99%.

2-Cyclohexyl-5-(4-ethoxybenzamido)-6-*N,N*-diethylamino-1*H*-benzo[*d*]imidazole (7a-3)

White solid (41% yield); mp 191–193 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.96 (t, 6 H, *J* = 7.2 Hz), 1.07–1.10 (m, 3 H), 1.45 (t, 6 H, *J* = 7.2 Hz), 1.55–1.66 (m, 5 H), 1.94–1.97 (m, 2 H, *J* = 10 Hz), 2.68–2.70 (m, 1 H), 3.00 (q, 4 H, *J* = 7.2 Hz), 4.13 (q, 2 H, *J* = 7 Hz) 7.03 (d, 2 H, *J* = 8.8 Hz), 7.55 (s, 1 H), 7.94 (d, 2 H, *J* = 8.8 Hz), 8.95 (s, 1 H), 10.32 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.10, 14.68, 25.60, 25.93, 31.71, 38.43, 50.77, 63.74, 100.7, 113.2, 114.6, 127.4, 128.7, 131.8, 134.9, 139.3, 159.8, 161.8, 164.7; HRMS (ESI) *m/z* calcd for C$_{26}$H$_{34}$N$_4$O$_2$H$^+$: 435.2754, Found: 435.2754 (Δ = 0.0 ppm); HPLC (1): t =8.6 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(4-ethylbenzamido)-1*H*-benzo[*d*]imidazole (7a-5)

White solid (75% yield); mp 175–177 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.94–1.03 (m, 9 H), 1.26 (t, 3 H, *J* = 7.6 Hz), 1.46–1.56 (m, 5 H), 1.91–1.94 (m, 2 H, *J* = 10 Hz), 2.68–2.75 (m, 3 H), 2.99 (q, 4 H, *J* = 7.2 Hz), 7.36 (d, 2 H, *J* = 8.5 Hz), 7.56 (s, 1 H), 7.93 (d, 2 H, *J* = 8.6 Hz), 9.03 (s, 1 H), 10.41 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.02, 15.26, 25.50, 25.82, 28.76, 31.68, 38.37, 50.73, 100.9, 113.1, 126.9, 128.4, 131.6, 131.9, 132.8, 134.8, 139.6, 148.3, 160.0, 165.1; HRMS (ESI) *m/z* calcd for C$_{26}$H$_{34}$N$_4$OH$^+$: 419.2805, Found: 419.2809 (Δ = 0.9 ppm); HPLC (1): t =7.2 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(4-*N,N*-dimethylbenzamido)-1*H*-benzo[*d*]imidazole (7a-7)

White solid (65% yield); mp 189–191 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.96 (t, 6 H, *J* = 7.6 Hz), 1.08–1.11 (m, 3 H), 1.53–1.64 (m, 5 H), 1.90–1.94 (m, 2 H, *J* = 10 Hz), 2.69 (m, 1 H), 2.99 (q, 4 H, *J* = 7.2 Hz), 3.06 (s, 6 H), 6.79 (d, 2 H, *J* = 8.5 Hz), 7.54 (s, 1 H), 7.92 (d, 2 H, *J* = 8.6 Hz), 8.98 (s, 1 H), 10.26 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.08, 25.58, 25.91, 31.70, 38.37, 40.09, 50.71, 100.6, 111.35, 113.0, 122.0, 128.5, 132.0, 133.0, 134.7, 139.2, 152.5, 159.7, 165.2; HRMS (ESI) *m/z* calcd for C$_{26}$H$_{35}$N$_5$OH$^+$: 434.2914, Found: 434.2914 (Δ = 0.0 ppm); HPLC (1): t =4.6 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(2-pyrazinamido)-1*H*-benzo[*d*]imidazole (7a-8)

White solid (64% yield); mp > 230°C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.96 (t, 6 H, *J* = 7.6 Hz), 1.14–1.16 (m, 3 H), 1.53–1.64 (m, 5 H), 2.01–2.02 (m, 2 H), 2.76–2.8 (m, 1 H), 2.99 (q, 4H, *J* = 7.2 Hz), 7.55 (s, 1 H), 8.65 (d, 2 H, *J* = 8.6 Hz), 8.77 (d, 2 H, *J* = 8.6 Hz), 8.92 (s, 1 H), 9.53 (s, 1 H), 10.91 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.05, 25.66, 25.94, 31.82, 38.64, 50.54, 101.1, 113.6, 131.1, 136.3, 139.9, 142.9, 144.4, 145.6, 147.0, 160.1, 160.6; HRMS (ESI) *m/z* calcd for C$_{22}$H$_{28}$N$_6$OH$^+$: 393.2397, Found: 393.2412 (Δ = 3.8 ppm); HPLC (1): t =10.9 min, purity > 99%.

5-(4-Bromobenzamido)-2-cyclohexyl-6-*N,N*-diethylamino-1*H*-benzo[*d*]imidazole (7a-9)

White solid (61% yield); mp >230 °C; Mixture of rotamers, ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.89 (t, 6 H, *J* = 7.2 Hz), 1.27–1.40 (m, 3 H), 1.57–1.67 (m, 3 H), 1.76–1.78 (m, 2 H), 1.96–1.98 (m, 2 H, *J* = 10 Hz), 2.80–2.81 (m, 1 H), 2.95–3.01 (m, 4 H, *J* = 7 Hz), 7.52 (m, 1 H), 7.81–7.82 (m, 4 H), 8.51 (s, 1 H), 10.1 (m, 2 H), 12.0 (m, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 12.82, 25.47, 25.54, 31.22, 37.68, 49.92, 100.1, 112.9, 125.4, 128.6, 130.9, 131.5, 132.0, 133.9, 134.4, 139.4, 159.2, 162.4; HRMS (ESI) *m/z* calcd for C$_{24}$H$_{29}$BrN$_4$OH$^+$: 469.1597, Found: 469.1604 (Δ= 1.5 ppm); HPLC (1): t =7.6 min, purity > 99%.

5-(3-Bromobenzamido)-2-cyclohexyl-6-*N,N*-diethylamino-1*H*-benzo[*d*]imidazole (7a-10)

White solid (71% yield); mp 218–220 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.95 (t, 6 H, *J* = 7.2 Hz), 1.09–1.10 (m, 3 H), 1.57–1.66 (m, 5 H), 1.94–1.96 (m, 2 H, *J* = 10 Hz), 2.69–2.70 (m, 1 H), 2.99–3.00 (m, 4 H), 7.41 (t, 1 H, *J* = 7.8 Hz), 7.58 (s, 1 H), 7.69 (d, 1 H, *J* = 7.7 Hz), 7.84 (d, 1 H, *J* = 7.7 Hz), 8.14 (s, 1 H), 8.93 (s, 1 H), 10.42 (s, 1 H), 11.39 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.07, 25.57, 25.84, 31.69, 38.43, 50.69, 100.8, 113.3, 123.2, 124.9, 130.4, 130.5, 131.2, 131.8, 134.6, 135.0, 137.4, 139.8, 160.1, 163.4; HRMS (ESI) *m/z* calcd for C$_{24}$H$_{29}$N$_4$OH$^+$: 469.1597, Found: 469.1604 (Δ = 1.5 ppm); HPLC (1): t =8.0 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(2-propoxyacetamido)-1*H*-benzo[*d*]imidazole (7a-15)

White solid (64% yield); mp 148–150 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.94 (t, 6 H, *J* = 7.2 Hz), 1.01 (t, 3 H, *J* = 7.5 Hz), 1.23–1.46 (m, 3 H), 1.57–1.72 (m, 5 H), 1.85–1.88 (m, 2 H, *J* = 10 Hz), 2.12–2.15 (m, 2 H, *J* = 10 Hz), 2.83–2.85 (m, 1 H), 2.94 (q, 4 H, *J* = 7.2 Hz), 3.59 (t, 2 H, *J* = 6.5 Hz), 4.15 (s, 2 H), 7.55 (s, 1 H), 8.77 (s, 1 H), 10.44 (s, 1 H); NMR (100 MHz, CDCl3) δ 10.40, 12.75, 22.98, 25.87, 26.11, 31.74, 38.66, 50.42, 70.98, 73.46, 100.7, 113.4, 131.0, 131.6, 135.2, 139.6, 159.6, 167.7; HRMS (ESI) *m/z* calcd for C$_{22}$H$_{34}$N$_4$O$_2$H$^+$: 387.2754, Found: 387.2769 (Δ = 3.9 ppm); HPLC (1): t =8.4 min, purity > 99%.

5-Benzamido-2-cyclohexyl-6-pyrrolinyl-1*H*-benzo[*d*]imidazole (7b-1)

White solid (76% yield); mp 197–198 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.04–1.35 (m, 4 H), 1.51–1.68 (m, 3 H), 1.74 (dt, *J* = 13.20, 3.32 Hz, 2 H), 1.92–2.11 (m, 6 H), 2.75 (m, 1

H), 3.07 (br. s, 4 H), 7.46–7.71 (m, 4 H), 7.95 (d, J = 8.80 Hz, 2 H), 8.86 (s, 1 H), 9.91 (br. s, 1 H), 10.45 (br. s, 1 H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 24.6, 25.9, 26.2, 32.0, 38.7, 54.2, 101.8, 111.1, 127.1, 129.2, 129.9, 131.9, 135.7, 136.0, 159.8, 165.3; HRMS (FAB) m/z calcd for C$_{24}$H$_{28}$N$_4$OH$^+$: 389.2336, Found: 389.2349 (Δ = 3.4 ppm); HPLC (1): t =8.1 min, purity > 99%.

5-(4-Bromobenzamido)-2-cyclohexyl-6-pyrrolidinyl-1*H*-benzo[*d*]imidazole (7b-4)

White solid (76% yield); mp 215–216 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ 1.19–1.45 (m, 3 H), 1.58 (qd, J = 12.21, 3.05 Hz, 2 H), 1.64–1.74 (m, 1 H), 1.79 (dt, J = 12.89, 3.17 Hz, 2 H), 1.88 (br. s, 4 H), 2.00 (d, J = 10.68 Hz, 2 H), 2.72–2.86 (m, 1 H), 3.06 (br. s, 4 H), 7.16 (br. s, 1 H), 7.68–7.86 (m, 3 H), 7.91 (d, J = 8.24 Hz, 2 H), 9.82 (s, 4 H), 11.90 (br. s, 1 H); ^{13}C NMR (101 MHz, DMSO-d6 at 55 °C) δ 24.1, 25.2, 25.4, 31.0, 37.5, 51.9, 124.9, 125.8, 128.9, 131.4, 134.0, 138.3, 158.6, 163.4; HRMS (FAB) m/z calcd for C$_{24}$H$_{27}$BrN$_4$OH$^+$: 467.1441, Found: 467.1443 (Δ = 0.4 ppm); HPLC (2): t =9.6 min, purity > 98%.

2-Cyclohexyl-5-(2-propoxyacetamido)-6-(pyrrolidin-1-yl)-1*H*-benzo[*d*]imidazole (7b-7)

White solid (78% yield); mp 179–181 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.01 (t, 3 H, J = 7.5 Hz), 1.35–1.38 (m, 3 H), 1.70–1.72 (m, 5 H), 1.80–1.82 (m, 2 H), 1.94 (br. s, 4 H), 2.09–2.12 (m, 2 H), 2.81–2.87 (m, 1 H), 2.99 (br. s, 4 H), 3.56 (t, 2 H, J = 6.5 Hz), 4.12 (s, 2 H), 7.54 (s, 1 H), 8.69 (s, 1 H), 9.93 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 10.48, 22.96, 24.27, 25.84, 26.07, 31.76, 38.63, 53.46, 70.90, 73.46, 101.5, 110.2, 128.6, 130.8, 135.8, 139.7, 159.5, 167.6; HRMS (ESI) m/z calcd for C$_{22}$H$_{32}$N$_4$O$_2$H$^+$: 385.2598, Found: 385.2594 (Δ = −1.0 ppm); HPLC (1): t =9.7 min, purity > 99%.

2-Cyclohexyl-5-(4-difluoromethoxybenzamido)-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-3)

White solid (65% yield); mp 183–185 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.02–1.33 (m, 5 H), 1.51–1.79 (m, 6 H), 2.00 (d, J = 13.55 Hz, 2 H), 2.72 (s, 7 H), 6.61 (d, J = 73.53 Hz, 1 H), 7.25–7.31 (m, 2 H), 7.59 (s, 1 H), 7.97 (d, J = 9.03 Hz, 2 H), 8.82 (s, 1 H), 9.87 (s, 1 H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 14.3, 25.9, 26.1, 31.8, 32.0, 34.9, 38.7, 46.1, 101.8, 110.9, 113.5, 115.6, 117.7, 119.7, 128.9, 129.1, 132.7, 139.5, 153.8, 160.1, 164.4; HRMS (FAB) m/z calcd for C$_{23}$H$_{26}$F$_2$N$_4$O$_2$H$^+$: 429.2097, Found: 429.2100 (Δ = 0.8 ppm); HPLC (1): t =12.8 min, purity > 99%.

2-Cyclohexyl-5-(4-trifluoromethoxybenzamido)-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-4)

White solid (70% yield); mp 210–211 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.08–1.31 (m, 3 H), 1.53–1.70 (m, 3 H), 1.70–1.81 (m, 2 H), 1.99–2.09 (m, 2 H), 2.69–2.83 (m, 7 H), 7.35–7.42 (m, 2 H), 7.60 (s, 1 H), 7.97–8.04 (m, 2 H), 8.81 (s, 1 H), 9.90 (s, 1 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 25.68, 25.93, 31.78, 38.47, 45.95, 119.08, 121.00, 121.01, 128.85, 128.91, 133.95, 139.43, 151.64, 159.68, 163.91; HRMS (FAB) m/z calcd for C$_{23}$H$_{25}$F$_3$N$_4$O$_2$H$^+$: 447.2002, Found: 447.2007 (Δ = 1.0 ppm); HPLC (1): t =10.3 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-dimethylamino-5-(4-methylbenzamido)-1*H*-benzo[*d*]imidazole (7c-5)

White solid (76% yield); mp 179–180 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.05–1.27 (m, 3 H), 1.50–1.77 (m, 5 H), 1.99 (d, J = 12.51 Hz, 2 H), 2.46 (s, 3 H), 2.66–2.78 (m, 7 H), 7.35 (d, J = 8.24 Hz, 2 H), 7.61 (s, 1 H), 7.87 (d, J = 8.24 Hz, 2 H), 8.89 (s, 1 H), 9.90 (s, 1 H), 10.65 (br. s, 1 H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 21.7, 25.9, 26.2, 32.0, 38.6, 46.1, 101.7, 110.9, 127.2, 129.2, 129.8, 131.3, 133.0, 139.4, 139.8, 142.5, 159.9, 165.7; HRMS (FAB)

m/z calcd for $C_{23}H_{28}N_4OH^+$: 377.2336, Found: 377.2349 (Δ = 3.5 ppm); HPLC (1): t =10.6 min, purity > 99%.

2-Cyclohexyl-5-(4-ethylbenzamido)-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-6)

White solid (72% yield); mp 177–178 °C; ^{1}H NMR (400 MHz, CDCl₃) δ 1.03–1.23 (m, 3 H), 1.30 (t, *J* = 7.65 Hz, 3 H), 1.50–1.63 (m, 3 H), 1.68 (d, *J* = 7.28 Hz, 2 H), 1.97 (d, *J* = 11.80 Hz, 2 H), 2.60–2.87 (m, 9 H), 7.38 (d, *J* = 8.03 Hz, 2 H), 7.61 (br. s, 1 H), 7.90 (d, *J* = 8.03 Hz, 2 H), 8.93 (d, *J* = 5.02 Hz, 1 H), 9.93 (br. s, 3 H); ^{13}C NMR (101 MHz, CDCl₃) δ 15.6, 25.9, 26.1, 29.1, 32.0, 38.6, 46.1, 101.8, 110.8, 127.3, 128.7, 129.1, 129.2, 131.5, 133.3, 139.3, 139.8, 148.7, 160.0, 160.1, 165.8; HRMS (FAB) m/z calcd for $C_{24}H_{30}N_4OH^+$: 391.2492, Found: 391.2500 (Δ = 1.9 ppm); HPLC (1): t =9.9 min, purity > 99%.

5-(4-Bromobenzamido)-2-cyclohexyl-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-11)

Yellow solid (78% yield); mp 207–208 °C; ^{1}H NMR (500 MHz, Methanol-d₄) δ 0.80–1.02 (m, 1 H), 1.16–2.21 (m, 13 H), 2.54–3.00 (m, 7 H), 7.44 (s, 1 H), 7.60–7.98 (m, 4 H), 8.46 (s, 1 H); ^{13}C NMR (126 MHz, Methanol-d₄) δ 27.1, 27.3, 31.9, 33.0, 40.0, 46.1, 65.6, 106.9, 108.2, 127.6, 129.9, 130.0, 133.4, 135.5, 142.3, 161.6, 166.2; HRMS (FAB) m/z calcd for $C_{22}H_{25}BrN_4OH^+$: 441.1285, Found: 441.1287 (Δ = 0.6 ppm); HPLC (1): t =9.9 min, purity > 99%.

5-(3-Bromobenzamido)-2-cyclohexyl-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-12)

Yellow solid (59% yield); mp 148–149 °C; ^{1}H NMR (500 MHz, Methanol-d₄) δ 1.28–1.38 (m, 1 H), 1.40–1.52 (m, 2 H), 1.57–1.93 (m, 5 H), 2.06 (d, *J* = 11.60 Hz, 2 H), 2.65–2.80 (m, 6 H), 2.80–2.93 (m, 1 H), 7.38–7.49 (m, 2 H), 7.66–7.76 (m, 1 H), 7.80–7.91 (m, 1 H), 8.02–8.13 (m, 1 H), 8.43 (s, 1 H); ^{13}C NMR (126 MHz, Methanol-d₄) δ 27.10, 27.31, 32.9, 39.95, 46.06, 106.96, 108.03, 124.08, 126.62, 129.75, 131.56, 131.91, 135.21, 135.95, 137.36, 138.51, 138.53 , 142.33, 142.36, 161.53, 161.55, 165.48, 165.54; HRMS (FAB) m/z calcd for $C_{22}H_{25}BrN_4OH^+$: 441.1285, Found: 441.1288 (Δ = 0.8 ppm); HPLC (1): t =10.5 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-dimethylamino-5-(2-propoxyacetamido)-1*H*-benzo[*d*]imidazole (7c-16)

White solid (61% yield); mp 146–148 °C; ^{1}H NMR (400 MHz, CDCl₃) δ 1.03 (t, 3 H, *J* = 7.2 Hz), 1.23–1.33 (m, 3 H), 1.60–1.72 (m, 5 H), 1.81–1.84 (m, 2 H, *J* = 10 Hz), 2.09–2.12 (m, 2 H, *J* = 10 Hz), 2.82 (s, 6 H), 2.83–2.85 (m, 1 H), 3.58 (t, 2 H, *J* = 6.5 Hz), 4.14 (s, 2 H), 7.53 (s, 1 H), 8.71 (s, 1 H), 10.07 (s, 1 H); ^{13}C NMR (100 MHz, CDCl₃) δ 10.42, 23.01, 25.89, 26.13, 31.83, 38.71, 45.68, 70.95, 73.53, 100.7, 113.4, 128.1, 131.6, 135.2, 139.2, 159.8, 167.9; HRMS (ESI) m/z calcd for $C_{20}H_{30}N_4O_2H^+$: 359.2441, Found: 359.2450(Δ = 2.5 ppm); HPLC (1): t =10.2 min, purity > 99%.

2-Cyclohexyl-5-(4-methoxybenzamido)-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-2)

To a solution of **4c** (100 mg, 0.39 mmol) in dichloromethane (2 mL) was added triethylamine (60 μL, 0.43 mmol). To this, 4-methoxybenzoyl chloride (53.3 μL, 0.39 mmol) was added dropwise at 0 °C and reacted at room temperature. After completion of the reaction, the reaction mixture was diluted with ethyl acetate and transferred to a separatory funnel. The organic layer was washed with saturated sodium bicarbonate solution, followed by water and finally brine. The organic layer was dried over magnesium sulfate. The crude product was purified by flash chromatography on silica gel (gradient: 30–70% ethyl acetate / hexanes) to obtain the product which was then treated with activated charcoal to yield the product as a white solid (130 mg, 86%); mp 201-202 °C; ^{1}H NMR (300 MHz, CDCl₃) δ 1.06–1.15 (m, 3 H), 1.54–1.68 (m, 5 H), 1.94–1.98 (m, 2 H, *J* = 12.9 Hz), 2.67–2.71 (m, 7 H), 3.90 (s, 3 H), 7.04 (d, 1 H, *J* = 9 Hz), 7.60 (s, 1H), 7.93 (d, 2 H, *J* = 9 Hz), 8.86 (s, 1 H),

9.82 (s, 1 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 25.8, 26.1, 32.0, 38.7, 46.1, 55.7, 101.9, 110.6, 114.3, 127.9, 129.0, 129.0, 131.6, 139.3, 160.1, 162.6, 165.2; HRMS (FAB) *m/z* calcd for C$_{23}$H$_{28}$N$_4$O$_2$H$^+$: 393.2285, Found: 393.2285 (Δ = 0.0 ppm); HPLC (1): t =13.2 min, purity > 99%.

Same Procedure was used for the synthesis of **7a-4, 7a-6, 7a-11~14, 7b-2, 7b-3, 7b-5, 7b-6, 7c-2, 7c-7~10, 7c-13~15**, and **7c-17**.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(4-methylbenzamido)-1*H*-benzo[*d*]imidazole (7a-4)

White solid (65% yield); mp 183–184 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.97 (t, 6 H, *J* = 6 Hz), 1.20–1.30 (m, 3 H), 1.58–1.78 (m, 5 H), 2.06–2.08 (m, 2 H), 2.45 (s, 3 H), 2.76–2.77 (m, 1 H), 3.03 (q, 4 H, *J* = 7.2 Hz), 7.34 (d, 2 H, *J* = 8.4 Hz) 7.59 (s, 1 H), 7.87 (d, 2 H, *J* = 8.1 Hz), 8.91 (s, 1 H), 10.31 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.09, 21.48, 25.63, 25.92, 31.72, 38.40, 50.75, 100.9, 113.1, 126.8, 129.6, 131.8, 132.7, 135.0, 139.4, 142.1, 159.8, 165.1; HRMS (FAB) *m/z* calcd for C$_{25}$H$_{32}$N$_4$OH$^+$: 405.2649, Found: 405.2654 (Δ = 1.2 ppm); HPLC (4): t =10.1 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(4-propylbenzamido)-1*H*-benzo[*d*]imidazole (7a-6)

White solid (56% yield); mp 169–171 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.98–1.10 (m, 12 H), 1.56–1.69 (m, 7 H), 1.93–1.95 (m, 2 H, *J* = 10 Hz), 2.67–2.71 (m, 3 H), 3.01 (q, 4 H, *J* = 7.2 Hz), 7.36 (d, 2 H, *J* = 8.5 Hz), 7.57 (s, 1 H), 7.92 (d, 2 H, *J* = 8.6 Hz), 9.00 (s, 1 H), 10.40 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.08, 13.73, 24.29, 25.59, 25.89, 31.72, 37.90, 38.40, 50.78, 100.8, 113.2, 126.9, 129.0, 131.8, 132.8, 134.9, 146.9, 159.9, 165.1; HRMS (ESI) *m/z* calcd for C$_{27}$H$_{36}$N$_4$OH$^+$: 433.2962, Found: 433.2961 (Δ = − 0.2 ppm); HPLC (1): t =7.5 min, purity > 99%..

2-Cyclohexyl-6-*N,N*-dimethylamino-5-(4-propylbenzamido)-1*H*-benzo[*d*]imidazole (7c-7)

White solid (75% yield); mp 190–191°C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.98 (t, *J* = 7.40 Hz, 3 H), 1.03–1.36 (m, 4 H), 1.48–1.77 (m, 8 H), 1.94 (d, *J* = 13.30 Hz, 2 H), 2.62–2.80 (m, 9 H), 7.36 (d, *J* = 8.28 Hz, 2 H), 7.61 (s, 1 H), 7.91 (d, *J* = 8.03 Hz, 2 H), 8.94 (s, 1 H), 9.95 (s, 1 H), 11.15 (br. s, 1 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 14.0, 24.5, 25.8, 26.1, 32.0, 38.1, 38.6, 46.1, 101.8, 110.8, 127.2, 129.0, 129.3, 131.5, 133.2, 139.3, 139.8, 147.2, 160.2, 165.8; HRMS (FAB) *m/z* calcd for C$_{25}$H$_{32}$N$_4$OH$^+$: 405.2649, Found: 405.2651 (Δ = 0.5 ppm); HPLC (1): t =8.0 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(4-fluorobenzamido)-1*H*-benzo[*d*]imidazole (7a-11)

White solid (71% yield); mp 213–214 °C; ^{1}H NMR (400 MHz, Methanol-d$_4$) δ 0.98 (t, 6 H, *J* = 7.2 Hz), 1.09–1.46 (m, 3 H), 1.66–170 (m, 3 H), 1.86–1.89 (m, 2 H), 2.08–2.12 (m, 2 H, *J* = 10 Hz), 2.89–2.92 (m, 1 H), 3.04–3.10 (m, 4 H, *J* = 7.16 Hz), 7.31 (t, 1 H, *J* = 7.8 Hz), 7.49 (s, 1 H), 7.97–7.99 (m, 1 H), 8.66 (s, 1 H); ^{13}C NMR (100 MHz, Methanol-d$_4$) δ13.55, 27.11, 27.33, 32.98, 40.03, 51.89, 117.0, 117.2, 130.5, 130.6, 132.8, 133.3, 137.3, 161.7, 165.2, 165.4, 167.7; HRMS (ESI) *m/z* calcd for C$_{24}$H$_{29}$FN$_4$OH$^+$: 409.2398, Found: 409.2401 (Δ = 0.7 ppm); HPLC (1): t =8.3 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(2,4-difluorobenzamido)-1*H*-benzo[*d*]imidazole (7a-12)

White solid (79% yield); mp 204–206 °C; ^{1}H NMR (400 MHz, Methanol-d$_4$) δ 0.94 (t, 6 H, *J* = 7.2 Hz), 1.32–1.47 (m, 3 H), 1.64–1.78 (m, 5 H), 1.84–1.86 (m, 2 H, *J* = 10 Hz), 2.05–2.09 (m, 2 H, *J* = 10 Hz), 2.77–2.79 (m, 1 H), 2.84–2.89 (m, 4 H), 7.16–7.18 (m, 2 H), 7.46 (s, 1 H), 8.13–8.19 (m, 1 H), 8.72 (s, 1 H); ^{13}C NMR (100 MHz, Methanol-d$_4$) δ 13.15, 27.11, 27.33, 32.97, 40.02, 51.70, 105.0, 105.7, 111.5, 113.7, 119.8, 134.7, 135.7, 137.3,

160.9, 161.4, 161.7, 163.5, 165.4, 167.9; HRMS (ESI) *m/z* calcd for $C_{24}H_{28}F_2N_4OH^+$: 427.2304, Found: 427.2309 (Δ = 1.1 ppm); HPLC (1): t =7.1 min, purity > 99%.

5-(3-Allylbenzamido)-2-cyclohexyl-6-*N,N*-diethylamino-1*H*-benzo[*d*]imidazole (7a-13)

White solid (69% yield); mp 89–90 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.96 (t, 6 H, *J* = 7.2 Hz), 1.09–1.10 (m, 3 H), 1.55–1.58 (m, 5 H), 1.91–1.95 (m, 2 H, *J* = 10 Hz), 2.67 (m, 1 H), 3.00 (q, 4 H, *J* = 7.2 Hz), 5.36 (d, 1 H, *J* = 11.1 Hz), 5.85 (d, 1 H, *J* = 17.5 Hz), 6.75–6.80 (m, 1 H), 7.50 (t, 1 H, *J* = 7.7 Hz), 7.55 (s, 1 H), 7.61 (d, 1 H, *J* = 7.7 Hz), 8.82 (d, 1 H, *J* = 7.8 Hz), 8.04 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.06, 25.49, 25.78, 31.64, 38.39, 50.66, 101.2, 112.9, 115.3, 124.9, 125.7, 129.0, 129.3, 131.4, 135.0, 135.7, 138.3, 160.0, 164.9; HRMS (ESI) *m/z* calcd for $C_{26}H_{32}N_4OH^+$: 417.2649, Found: 417.2648 (Δ = − 0.2 ppm); HPLC (1): t =7.3 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-diethylamino-5-(hexanamido)-1*H*-benzo[*d*]imidazole (7a-14)

White solid; 56% yield; mp 183–184 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.91–1.01 (m, 9 H), 1.23–1.39 (m, 7 H), 1.63–1.83 (m, 7 H), 2.10–2.13 (m, 2 H), 2.47 (t, 2 H, *J* = 7.2 Hz), 2.86–2.88 (m, 1 H), 2.94 (q, 4 H, *J* = 7.2 Hz), 7.50 (s, 1 H), 8.74 (s, 1 H), 9.46 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 12.89, 13.81, 22.39, 25.88, 26.11, 31.43, 31.55, 38.59, 38.72, 50.60, 101.0, 112.6, 131.6, 132.0, 134.4, 139.0, 159.7, 171.1; HRMS (ESI) *m/z* calcd for $C_{23}H_{36}N_4O$: 385.2962, Found:385.2967 (Δ = 1.3 ppm); HPLC (2): t =6.6 min, purity > 98%.

2-Cyclohexyl-5-(4-methoxybenzamido)-6-pyrrolydinyl-1*H*-benzo[*d*]imidazole (7b-2)

White solid (56% yield); mp 195–196 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.08–1.35 (m, 5 H), 1.52–1.69 (m, 3 H), 1.70–1.80 (m, 2 H), 1.96–2.10 (m, 6 H), 2.76 (m, 1 H), 3.06 (br. s, 4 H), 3.90 (s, 3 H), 7.03 (d, *J* = 8.8 Hz, 2 H), 7.57 (br. s, 1 H), 7.91 (d, *J* = 8.8 Hz, 2 H), 8.80 (s, 1 H), 9.77 (br. s, 1 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 24.6, 25.9, 26.2, 32.0, 38.7, 54.1, 55.7, 100.2, 114.4, 128.0, 128.9, 130.1, 136.0, 159.7, 162.6, 164.9; HRMS (FAB) *m/z* calcd for $C_{25}H_{30}N_4O_2H^+$: 419.2442, Found: 419.2450 (Δ = 2.0 ppm); HPLC (2): t =12.8 min, purity > 98%.

2-Cyclohexyl-5-(4-methylbenzamido)-6-pyrrolodinyl-1*H*-benzo[*d*]imidazole (7b-3)

White solid (40% yield); mp 179–180 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.00–1.37 (m, 4 H), 1.49–1.65 (m, 3 H), 1.71 (dt, *J* = 13.05, 3.40 Hz, 2 H), 1.92–2.09 (m, 6 H), 2.45 (s, 3 H), 2.73 (m, 1 H), 3.05 (br. s, 4 H), 7.34 (d, *J* = 8.24 Hz, 2 H), 7.58 (br. s, 1 H), 7.85 (d, *J* = 8.20 Hz, 2 H), 8.86 (s, 1 H), 9.85 (br. s, 1 H), 10.70 (br. s, 1 H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 21.7, 24.6, 25.9, 26.2, 32.0, 38.7, 54.1, 101.9, 110.9, 127.1, 129.8, 129.9, 130.1, 131.3, 132.9, 136.0, 139.9, 142.4, 159.9, 165.4; HRMS (FAB) *m/z* calcd for $C_{25}H_{30}N_4OH^+$: 403.2492, Found: 403.2496 (Δ = 0.9 ppm); HPLC (2): t =9.1 min, purity > 98%.

2-Cyclohexyl-5-(3-phenylpropanamido)-6-pyrrolidinyl-1*H*-benzo[*d*]imidazole (7b-5)

White solid (97% yield); mp 146–147 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.16–1.40 (m, 4 H), 1.57–1.73 (m, 3 H), 1.80 (dt, *J* = 13.05, 3.40 Hz, 2 H), 1.84–1.94 (m, 4 H), 2.09 (d, *J* = 12.90 Hz, 2 H), 2.76 (t, *J* = 7.40 Hz, 2 H), 2.80–2.92 (m, 5 H), 3.11 (t, *J* = 7.40 Hz, 2 H), 7.15–7.34 (m, 5 H), 7.47 (s, 1 H), 8.58 (s, 1 H), 8.75 (br. s, 1 H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 24.5, 26.0, 26.2, 29.9, 32.0, 32.2, 38.7, 40.5, 53.7, 103.2, 109.3, 126.6, 128.5, 128.8, 129.4, 131.6, 136.0, 138.5, 140.6, 159.6, 170.4; HRMS (FAB) *m/z* calcd for $C_{26}H_{32}N_4OH^+$: 417.2649, Found: 417.2654 (Δ = 1.2 ppm); HPLC (2): t =11.3 min, purity > 98%.

2-Cyclohexyl-5-hexanamido-6-pyrrolidinyl-1*H*-benzo[*d*]imidazole (7b-6)

White solid; 61% yield; mp 127–129 °C; ^{1}H NMR (400 MHz, CDCl₃) δ 0.91 (t, 3 H, *J* = 7.2 Hz), 1.20–1.43 (m, 7 H), 1.63–1.85 (m, 7 H), 1.97 (br. s, 4 H), 2.05–2.07 (m, 2 H), 2.47 (t, 2 H, *J* = 7.2 Hz), 2.83–2.85 (m, 1 H), 2.99 (br. s, 4 H), 7.52 (s, 1 H), 8.59 (s, 1 H), 8.91 (s, 1 H); ^{13}C NMR (100 MHz, CDCl₃) δ 13.86, 22.43, 24.33, 25.84, 26.09, 31.43, 31.78, 38.57, 53.72, 102.3, 109.7, 129.4, 131.4, 135.5, 138.5, 159.4, 171.2; HRMS (ESI) *m/z* calcd for $C_{23}H_{34}N_4O$: 383.2805, Found: 383.2811 (Δ = 1.5 ppm); HPLC (2): t =8.8 min, purity > 98%.

5-(4-*tert*-Butylbenzamido)-2-cyclohexyl-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-8)

White solid (74% yield); mp 222–223 °C; ^{1}H NMR (500 MHz, CDCl₃) δ 0.96–1.20 (m, 4 H), 1.39 (s, 9 H), 1.49–1.72 (m, 5 H), 1.96 (d, *J* = 12.21 Hz, 2 H), 2.74 (s, 7 H), 7.58 (d, *J* = 8.24 Hz, 3 H), 7.93 (d, *J* = 8.54 Hz, 2 H), 8.97 (s, 1 H), 9.98 (s, 1 H), 11.00 (s, 1 H); ^{13}C NMR (126 MHz, CDCl₃) δ 25.8, 26.1, 31.4, 32.0, 35.2, 38.6, 46.2, 101.7, 110.9, 126.2, 127.1, 129.2, 133.0, 139.3, 139.8, 155.5, 160.1, 165.8; HRMS (FAB) *m/z* calcd for $C_{26}H_{34}N_4OH^+$: 419.2805, Found: 419.2809 (Δ = 0.9 ppm); HPLC (1): t =9.0 min, purity > 99%.

2-Cyclohexyl-6-*N,N*-dimethylamino-5-(thiophene-2-carboxamido)-1*H*-benzo[*d*]imidazole (7c-9)

White solid (75% yield); mp 139–140 °C; ^{1}H NMR (500 MHz, CDCl₃) δ 1.00–1.08 (m, 1 H), 1.11–1.24 (m, 2 H), 1.51–1.61 (m, 3 H), 1.63–1.69 (m, 2 H), 1.97 (d, *J* = 11.90 Hz, 2 H), 2.12 (s, 1 H), 2.69 (s, 6 H), 2.77–2.84 (m, 1 H), 7.16 (dd, *J* = 4.88, 3.66 Hz, 1 H), 7.56 (d, *J* = 1.00 Hz, 2 H), 7.67 (dd, *J* = 3.66, 0.61 Hz, 1 H), 8.72 (s, 1 H), 9.75 (s, 1 H), 10.20 (br. s, 1 H); ^{13}C NMR (126 MHz, CDCl₃) δ 22.2, 25.7, 26.1, 31.8, 38.5, 45.9, 102.6, 109.7, 128.2, 128.4, 128.7, 130.8, 131.7, 137.9, 139.5, 140.1, 160.1, 160.1, 175.3; HRMS (FAB) *m/z* calcd for $C_{20}H_{23}N_4OSH^+$: 369.1744, Found: 369.1742 (Δ = −0.4 ppm); HPLC (1): t =11.8 min, purity > 98%.

2-Cyclohexyl-5-(furan-2-carboxamido)-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-10)

White solid (71% yield); mp 214–215 °C; ^{1}H NMR (400 MHz, CDCl₃) δ 1.10–1.37 (m, 3 H), 1.55–1.84 (m, 5 H), 2.06 (d, *J* = 14.81 Hz, 2 H), 2.67–2.88 (m, 7 H), 6.56–6.66 (m, *J* = 3.60, 1.90 Hz, 1 H), 7.22–7.32 (m, 1 H), 7.52–7.68 (m, 2 H), 8.79 (s, 1 H), 9.98 (s, 1 H), 10.59 (s, 1 H); ^{13}C NMR (101 MHz, CDCl₃) δ 25.9, 26.2, 32.0, 38.7, 46.0, 101.8, 110.8, 112.6, 114.9, 128.6, 131.2, 139.6, 139.9, 144.7, 148.8, 156.4, 159.9; HRMS (FAB) *m/z* calcd for $C_{20}H_{24}N_4O_2H^+$: 353.1972, Found: 353.1982 (Δ = 2.8 ppm); HPLC (1): t =11.6 min, purity > 99%.

2-Cyclohexyl-5-(4-fluorobenzamido)-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-13)

White solid (70% yield); mp 196–197 °C; ^{1}H NMR (500 MHz, Methanol-d₄) δ 2.00–2.26 (m, 3 H), 2.34–2.45 (m, 2 H), 2.46–2.55 (m, 1 H), 2.60 (dt, *J* = 12.97, 3.28 Hz, 2 H), 2.81 (d, *J* = 13.12 Hz, 2 H), 3.48 (s, 6 H), 3.58–3.67 (m, 1 H), 8.20 (t, *J* = 8.85 Hz, 2 H), 8.82 (dd, *J* = 8.85, 5.49 Hz, 2 H), 9.07 (br. s, 1 H), 10.50 (br. s, 1 H), 12.80 (s, 1 H); ^{13}C NMR (126 MHz, Methanol-d₄) δ 35.1, 35.2, 40.9, 47.3, 54.9, 111.6, 112.1, 119.5, 121.1, 125.3, 125.5, 136.7, 137.5, 139.2, 139.3, 140.3, 141.1, 141.1, 149.3, 150.7, 168.8, 172.7, 174.7; HRMS (FAB) *m/z* calcd for $C_{22}H_{25}FN_4OH^+$: 381.2085, Found: 381.2088 (Δ = 0.7 ppm); HPLC (1): t =11.4 min, purity > 99%.

2-Cyclohexyl-5-(2,4-difluorobenzamido)-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-14)

White solid (70% yield); mp 212–213 °C; ^{1}H NMR (400 MHz, METHANOL-d$_4$) δ 1.29–1.55 (m, 3 H), 1.58–1.94 (m, 5 H), 2.07 (d, *J* = 12.05 Hz, 2 H), 2.72 (s, 6 H), 2.81–2.95 (m, 1 H), 7.10–7.24 (m, 2 H), 7.45 (s, 1 H), 8.06–8.21 (m, 1 H), 8.63 (s, 1 H); ^{13}C NMR (101 MHz, Methanol-d$_4$) δ 27.12, 27.34, 30.81, 32.98, 40.00 46.16 , 105.42, 105.69, 105.71, 105.97, 106.17, 108.39, 113.54, 113.57, 113.76, 113.78, 119.94, 119.97, 120.05, 120.09, 130.57, 134.62, 134.65, 134.72, 134.76, 141.75, 161.04, 161.16, 161.54, 161.90, 161.94, 163.53, 163.65, 165.26, 165.39, 167.79; HRMS (FAB) *m/z* calcd for $C_{22}H_{24}F_2N_4OH^+$: 399.1991, Found: 399.1988 (Δ = −0.7 ppm); HPLC (1): t =8.9 min, purity > 99%.

2-Cyclohexyl-5-(hexanamido)-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-15)

White solid (63% yield); mp 114–117 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.90 (t, 3 H, *J* = 7.2 Hz), 1.30–1.39 (m, 7 H), 1.63–1.83 (m, 7 H), 2.06–2.10 (m, 2 H), 2.46 (t, 2 H, *J* = 7.2 Hz), 2.65 (s, 6 H), 2.82–2.84 (m, 1 H), 7.51 (s, 1 H), 8.62 (s, 1 H), 8.97 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.89, 22.41, 25.80, 26.07, 31.43, 31.77, 38.56, 45.69, 101.8, 110.1, 128.6, 138.7, 159.6, 171.4; HRMS (ESI) *m/z* calcd for $C_{21}H_{32}N_4OH^+$: 357.2649, Found: 357.2650 (Δ = 0.2 ppm); HPLC (2): t =8.9 min, purity > 99%.

2-Cyclohexyl-5-[2-(4-methoxyphenyl)acetamido]-6-*N,N*-dimethylamino-1*H*-benzo[*d*]imidazole (7c-17)

White solid (88% yield); mp 167–168 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.17–1.42 (m, 3 H), 1.55–1.77 (m, 3 H), 1.83 (d, *J* = 12.80 Hz, 2 H), 2.12 (d, *J* = 12.05 Hz, 2 H), 2.34 (s, 6 H), 2.79–3.00 (m, 1 H), 3.78 (s, 2 H), 3.83 (s, 3 H), 6.95 (d, *J* = 8.28 Hz, 6 H), 7.28 (s, 2 H), 7.44 (s, 1 H), 8.63 (s, 1 H), 9.00 (s, 1 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 26.0, 26.2, 31.9, 38.7, 44.8, 45.5, 55.6, 101.8, 109.9, 114.0, 114.7, 126.7, 129.3, 130.5, 131.0, 139.5, 159.4, 159.5, 169.9; HRMS (FAB) *m/z* calcd for $C_{24}H_{30}N_4O_2H^+$: 407.2442, Found: 407.2246 (Δ = 1.1 ppm); HPLC (1): t =13.4 min, purity > 99%.

Bacterial Strains and Growth.[22, 37]

H37Rv, the drug sensitive laboratory strain of *Mtb*, and clinical *Mtb* strains W210, NHN382, and TN587 exhibiting different resistant profiles to isoniazid were used. For evaluation of drug sensitivity all strains were grown in Difco™ 7H9 Middlebrook liquid media (BD Biosciences, 271310) supplemented with 10% Middlebrook OADC Enrichment (VWR, 9000-614), 0.05% Tween (G-Biosciences, 786-519), and 0.2% Glycerol at 37 °C.

Antibacterial Activity.[22, 36]

M. tuberculosis H37Rv strain and clinical isolates were used in the antibacterial studies, which were grown at 37 °C in Difco™ 7H9 Middlebrook liquid media supplemented with 10% Middlebrook OADC Enrichment, 0.05% Tween, and 0.2% Glycerol. The minimum inhibitory concentration (MIC) of the compounds was determined by the microplate Alamar Blue assay (MABA) in triplicates. The compounds were prepared in DMSO and were serially diluted 2-fold in 96-well microtiter plates. Bacteria were added to the appropriate wells in the plate and the plates were incubated for 6 days at 37 °C. AlamarBlue was added to the plates, and the plates were incubated for an additional 24 h at 37 °C. The MIC was the lowest concentration (μg/mL) of compound that inhibited bacterial growth and prevented a color change.

Cytotoxicity Assay

The cytotoxicity of the compounds was tested against a cell line that originated from the epithelial cells from the kidneys of *Cercopithecus aethiops* (Vero). Vero cells were grown without CO_2 in L15 media supplemented with antibiotics and heat inactivated calf serum. Serial 2-fold dilutions of the drugs were prepared in the 96-well microplates. The Vero cells, in media containing 2X Alamar Blue, were added to the wells to a final concentration of 1.3×10^4 cells per well. The plates were incubated for 3 days at 37 °C. The IC_{50} was calculated according to manufacturer directions.

Mtb FtsZ Protein Preparation.[22]

E.coli expression plasmid encoding the *ftsz* gene (pET 15b vector) was transformed into 100 µL of BL21(DE3) cells. The transformed cells were plated onto LB plates, containing 100 µg/mL ampicilin. The antibiotic concentration was kept the same for the following steps. The plates were incubated overnight at 37 °C. The colonies were picked and grown in 10 mL of LB media at 37 °C at 250 rpm shake rate. The inoculum was transferred to 1 L of LB media in a 4 L flask and grown to an OD of 0.6 at A600. Then, 1 mM IPTG was added to induce protein expression overnight at 25 °C at 250 rpm shake rate. Next day the cells were harvested at 4,225×*g* for 15 min and re-suspended in approximately 20–30 mL binding buffer (500 mM NaCl, 20 mM sodium phosphate, pH 7.8). The re-suspended cells were lysed using cell disruptor. The lysate was centrifuged in an ultracentrifuge at 126,603×*g*, 4 °C for 90 min. The supernatant was filtered and loaded onto Ni2+-NTA column washed with 50 mL of binding buffer and eluted using a gradient of binding buffer with 30–500 mM imidazole. The eluted protein was first dialyzed against the polymerization buffer (50 mM MES, 5mM $MgCl_2$, 50 mM KCl, pH 6.5) and then polymerization buffer containing 10% v/v glycerol. Alternatively, the eluted protein was dialyzed against buffer containing 50 mM Tris, 5mM $MgCl_2$, 50 mM KCl, pH 7.2 followed by buffer containing 10% v/v glycerol. The protein after dialysis was concentrated and stored at −80 °C for further use. Since the number of aromatic residues in *Mtb* FtsZ protein are low (Tyr: 1, Trp: 0), it is not reliable to follow concentration of protein by scanning at A280. The concentration of protein was therefore ascertained using the Bradford kit from Sigma.

Polymerization Assay.[22, 38]

FtsZ (15 µM) was equilibrated in polymerization buffer (50 mM MES, 100 mM KCl, 5 mM $MgCl_2$, pH 6.5) at 25 °C. Polymerization was measured by light scattering using a PTI-QM4 spectrofluorimeter set at 400nm excitation/emission, 2 nm slit width, 860 V). Polymerization was initiated with 50 µM GTP and monitored for up to 30 minutes. Compound stock solutions were prepared in DMSO and incubated with *Mtb* FtsZ prior to initiation of polymerization with GTP. The percentage of DMSO was maintained at 2% for all experiments.

Transmission Electron Microscopy (TEM) Analysis.[22]

Stock solution of compound **5f** was prepared in ethanol. *Mtb* FtsZ (5 µM) was incubated with 40 or 80 µM of compound **5f** in the polymerization buffer (50 mM MES, 5mM $MgCl_2$, 100 mM KCl, pH 6.5) for 15 min on ice. To each solution was added GTP to the final concentration of 25 µM. The resulting solution was incubated at 37 °C for 30 min. The incubated solution was diluted 2 times with the polymerization buffer and immediately transferred to carbon coated 300 mesh formvar copper grid and negatively stained with 1% uranyl acetate. To visualize the effect of compound **5f** on preformed polymers, *Mtb* FtsZ (5 µM) was allowed to polymerize at 37 °C in the presence of GTP (25 µM) for 30 mins. Compound **5f** (40 µM or 80 µM) was added to the polymerized protein and incubated for additional 5 min. The resulting solution was diluted 2 times with the polymerization buffer

and immediately transferred to carbon coated 300 mesh formvar copper grid and negatively stained with 1% uranyl acetate. The samples were viewed with a FEI Tecnai12 BioTwinG transmission electron microscope at 80 kV. Digital images were acquired with an AMT XR-60 CCD digital camera system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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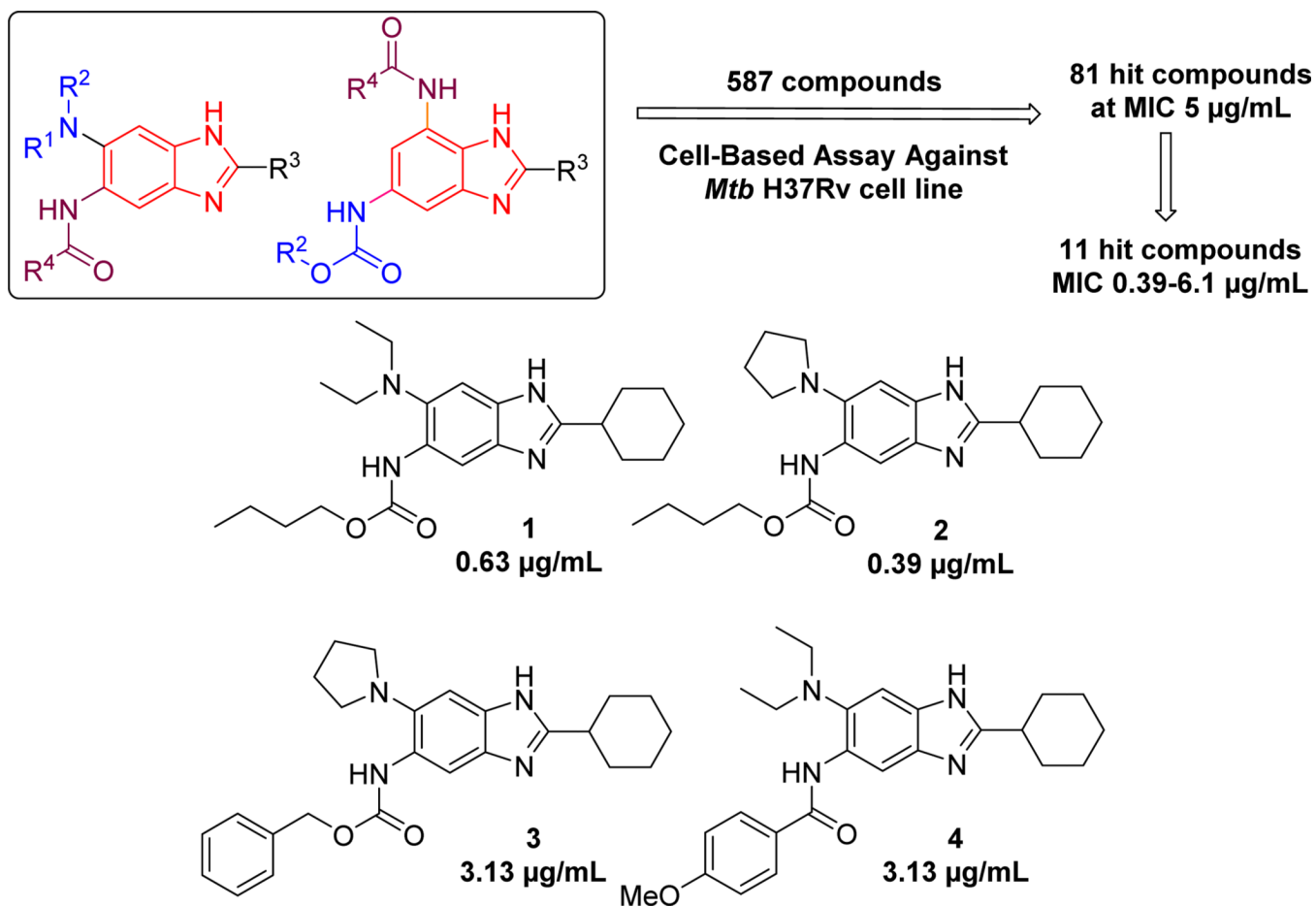
Figure 1. Early Lead Compounds from Hit Benzimidazoles[22]
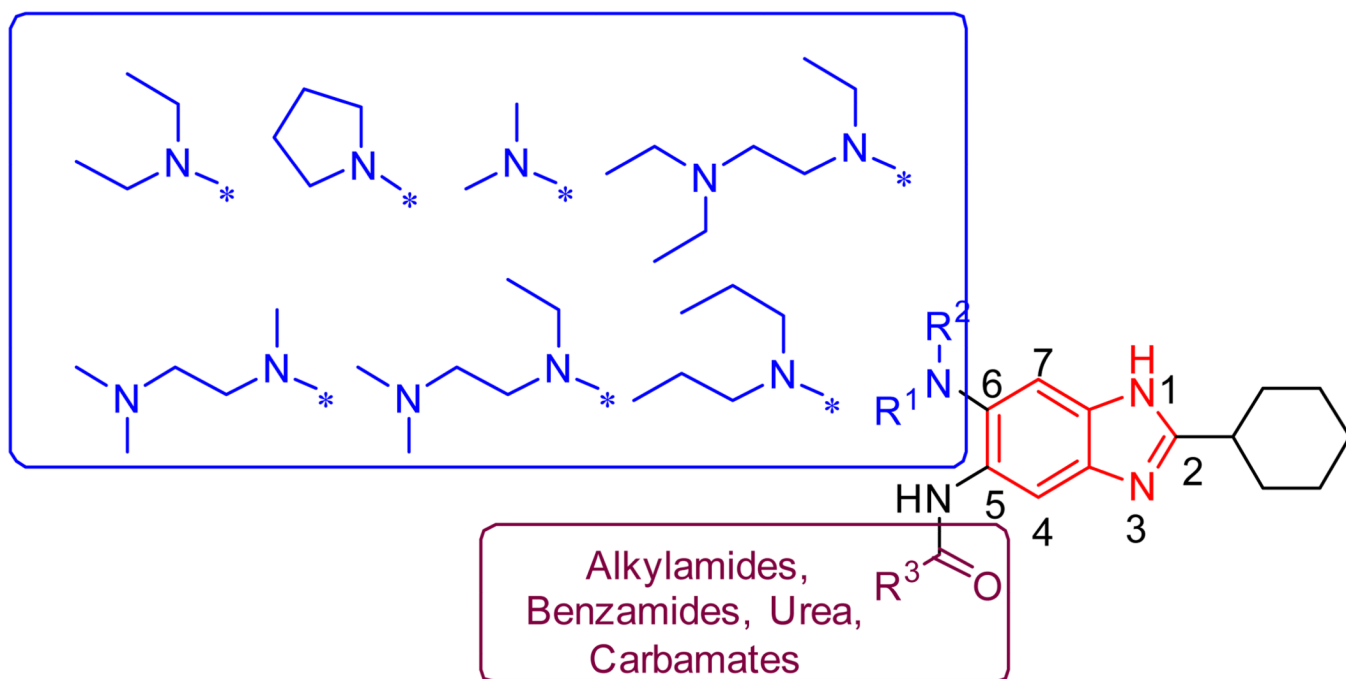
Figure 2. Optimization of 2,5,6-Trisubstituted Benzimidazoles
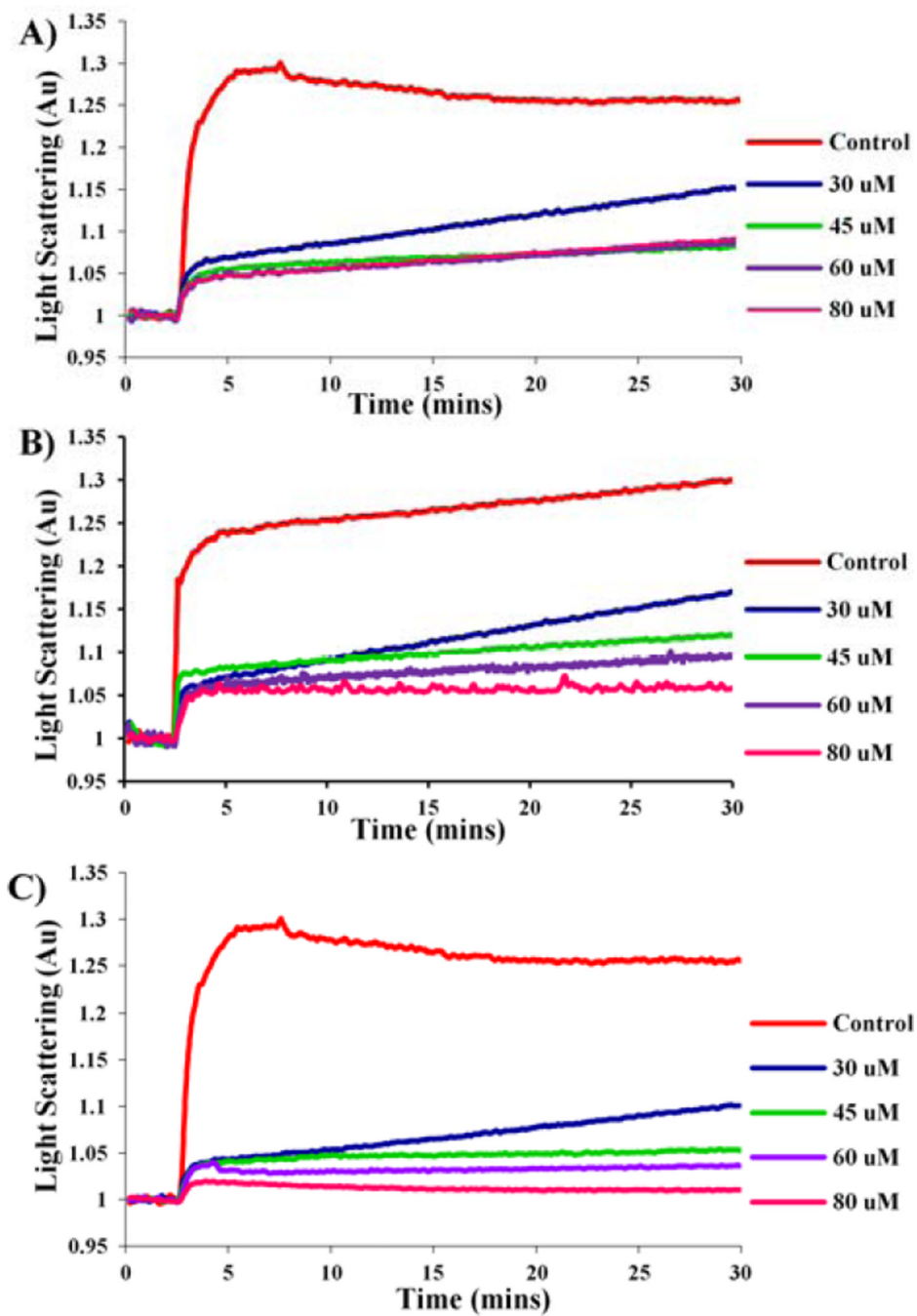
Figure 3. Dose Dependent Inhibition of *Mtb* FtsZ Polymerization by 5f (A), 5p (B) and 7c-6 (C)
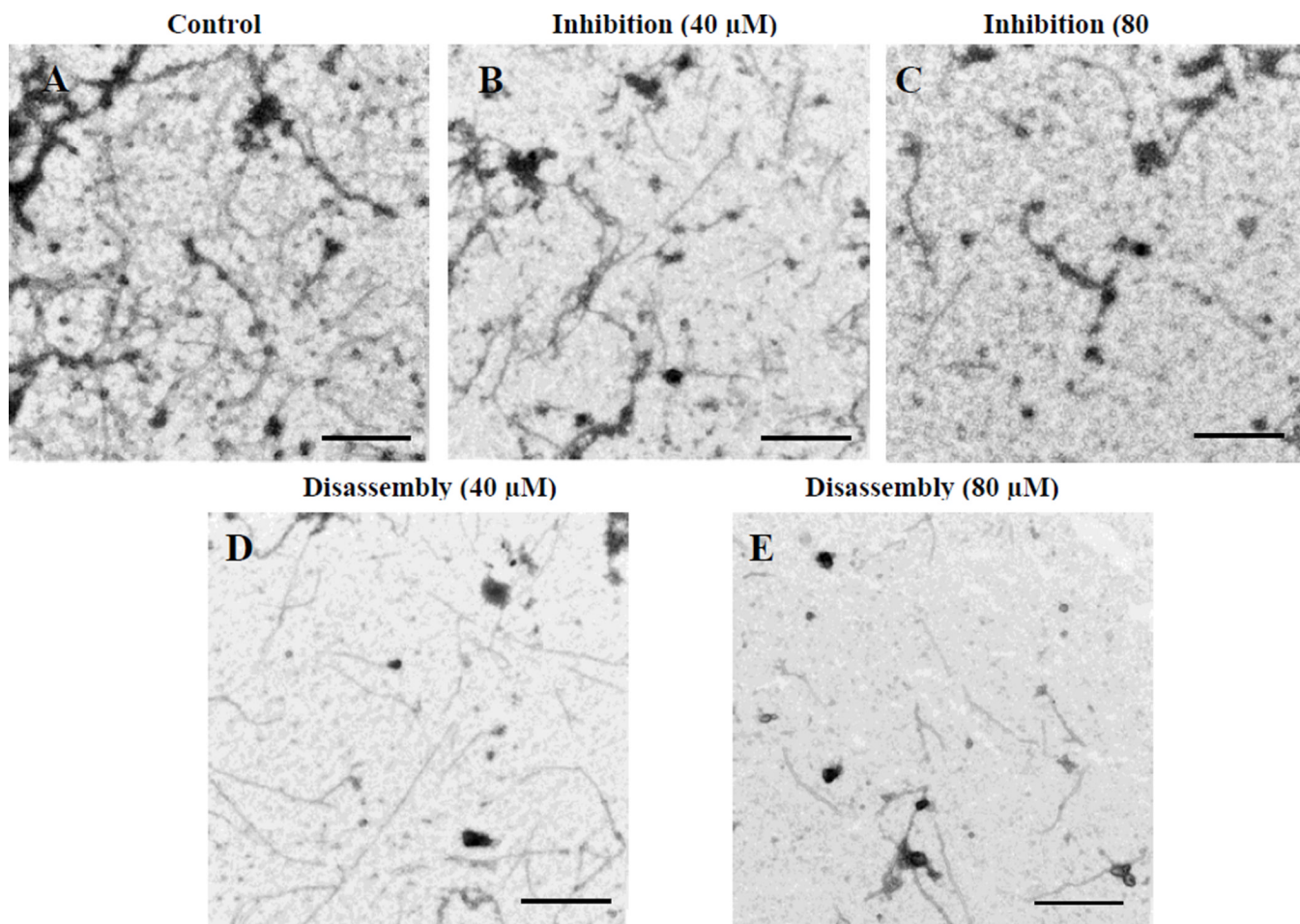
Figure 4. TEM Images of FtsZ

FtsZ (5 µM) was polymerized by GTP (25 µM) in the absence (A) and presence of **5f** at 40 µM (B) and 80 µM (C). Images D and E display the effect of the compound on preformed polymers, wherein FtsZ polymers formed after addition of GTP were treated with 40 µM (D) or 80 µM (E) of **5f**. Images are at 49,000x magnification (scale bar 500 nm).
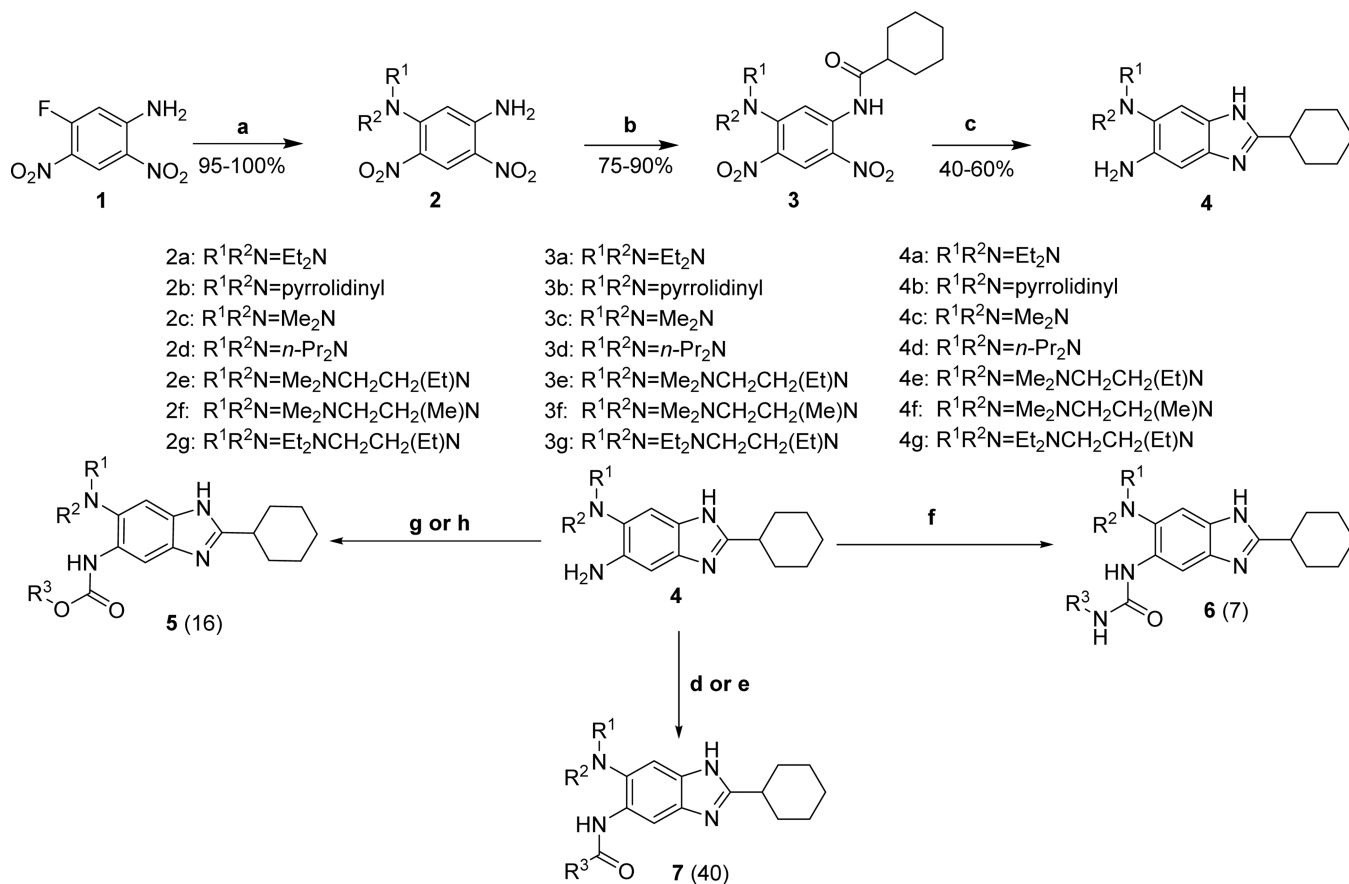
Scheme 1. Synthesis of 2,5,6-Trisubstituted Benzimidazoles a[22]
a Reagents and conditions: (a) R^1R^2NH, DIPEA, THF, 2 h, room temperature (RT)/ 12 h, 50 °C; (b) cyclohexanecarbonyl chloride, pyridine, reflux, overnight; (c) $SnCl_2 \cdot 2H_2O$, 4 M HCl, EtOH, reflux, 4 h; (d) R^3COCl, Et_3N, 0 °C-RT, overnight; (e) R^3COOH, EDC.HCl, DMAP, CH_2Cl_2, reflux, overnight; (f) R^3NCO, Et_3N, reflux, overnight; (g) (i) 1,1'-carbonyldiimidazole, CH_2Cl_2, reflux, 4 h; (ii) R^3OH, CH_2Cl_2, reflux, overnight; (h) R^3OSu ester, CH_2Cl_2, 0 °C-RT, overnight.

Table 1

Antibacterial Activity of Benzimidazoles **5** Against *Mtb* H37Rv Strain (MIC, µg/mL) and Their Cytotoxicity (IC$_{50}$, µg/mL)

Compound	R^{1}R^{2}N	R^{3}O	*Mtb*H37Rv MIC	Vero Cytotoxicity
1			0.63	>100
5a			6.25	>100
5b			>100	>100
5c			100	>100
5d			>100	>100
5e			50.0	>50
5f			0.06	>100
5g			>50	>100
5h			0.16	>100
5i			3.13	>100
5j			1.56	>100
5k			1.56	>100

Compound	R¹R²N	R³0	*Mtb*H37Rv MIC	Vero Cytotoxicity
5l			0.31	>100
5m			12.5	>100
5n			1.56	>100
5o			0.31	>100
5p			0.63	>100

Table 2

Antibacterial Activity of Benzimidazoles **6** Against *Mtb* H37Rv Strain (MIC, µg/mL) and Their Cytotoxicity (IC$_{50}$, µg/mL)
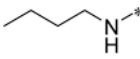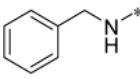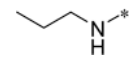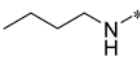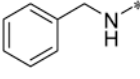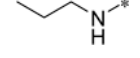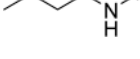
Compound	R^{1}R^{2}N	R^{3}NH	*Mtb* H37Rv MIC	Vera Cytotoxicity
6a			>100	>100
6b			>100	>100
6c			>100	>100
6d			25.0	>100
6e			25.0	>100
6f			100	>100
6g			6.25	>100

Table 3

Antibacterial Activity of Benzimidazoles 7 Against *Mtb* H37Rv Strain (MIC, μg/mL) and Their Cytotoxicity (IC$_{50}$, μg/mL)

Compound	R¹R²N	R³	*Mtb* H37Rv	Vero Cytotoxicity	Compound	R¹R²N	R³	*Mtb* H37Rv MIC	Vero Cytotoxicity
7a-1	diethylamino	F$_2$HCO-phenyl	>100	>100	7b-6	pyrrolidinyl	pentyl	6.25	>100
7a-2	diethylamino	F$_3$CO-phenyl	>100	>100	7b-7	pyrrolidinyl	propoxy	12.5	>100
7a-3	diethylamino	EtO-phenyl	>100	>100	7c-1	dimethylamino	phenyl	1.56	>100
7a-4	diethylamino	methylphenyl	12.5	>100	7c-2	dimethylamino	MeO-phenyl	0.31	>100
7a-5	diethylamino	ethylphenyl	1.56	>100	7c-3	dimethylamino	F$_2$HCO-phenyl	0.31	>100
7a-6	diethylamino	propylphenyl	>100	n.d*	7c-4	dimethylamino	F$_3$CO-phenyl	0.16	>100
7a-7	diethylamino	Me$_2$N-phenyl	12.5	n.d*	7c-5	dimethylamino	methylphenyl	0.31	>100
7a-8	diethylamino	pyridinyl	10.0	>100	7c-6	dimethylamino	ethylphenyl	0.16	>100
7a-9	diethylamino	Br-phenyl	100	>100	7c-7	dimethylamino	propylphenyl	0.63	>100
7a-10	diethylamino	Br-phenyl	>100	>100	7c-8	dimethylamino	tert-butylphenyl	1.56	>100

Compound	R¹R²N	R³	Mtb H37Rv	Vero Cytotoxicity
7a-11	(diethylamino)	4-fluorophenyl	>100	>100
7a-12	(diethylamino)	2,4-difluorophenyl	>100	>100
7a-13	(diethylamino)	3-vinylphenyl	25.0	>100
7a-14	(diethylamino)	pentyl	12.5	n.d*
7a-15	(diethylamino)	propoxymethyl	25.0	>100
7b-1	(pyrrolidinyl)	phenyl	6.25	>100
7b-2	(pyrrolidinyl)	4-methoxyphenyl	6.25	>100
7b-3	(pyrrolidinyl)	4-methylphenyl	3.13	>100
7b-4	(pyrrolidinyl)	4-bromophenyl	1.56	>100
7b-5	(pyrrolidinyl)	phenethyl	12.5	>100

Compound	R¹R²N	R³	Mtb H37Rv MIC	Vero Cytotoxicity
7c-9	(dimethylamino)	thiophen-2-yl	5.0	>100
7c-10	(dimethylamino)	furan-2-yl	6.25	>100
7c-11	(dimethylamino)	4-bromophenyl	0.31	>100
7c-12	(dimethylamino)	3-bromophenyl	3.13	>100
7c-13	(dimethylamino)	4-fluorophenyl	0.63	>100
7c-14	(dimethylamino)	2,4-difluorophenyl	0.31	>100
7c-15	(dimethylamino)	pentyl	1.25	>100
7c-16	(dimethylamino)	propoxymethyl	6.25	<50
7c-17	(dimethylamino)	4-methoxybenzyl	>100	>100
7d	(di-n-propylamino)	phenyl	>100	>100

*
nd. = not determined

Table 4

Nine Most Potent 6-Dimethylaminobenzimidazoles (*Mtb* H37Rv, µg/mL)
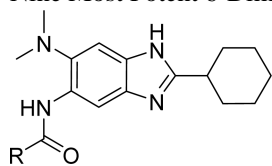
Compound	R	MIC
5f		0.06
5h		0.16
7c-6		0.16
7c-4		0.16
7c-5		0.31
7c-14		0.31
7c-11		0.31
5l		0.31
5p		0.63

Table 5

Antibacterial Activity (MIC μg/mL) of **5f** and **7c-6** Against Clinical Isolates of *Mtb*

| Compound | *Mtb* strains | | | |
	H37Rv	NHN382	TN587	W210
INH	0.013	0.25	0.25	0.01
SB-P3G2	0.63	1.25	1.25	1.25
5f	0.06	0.06	0.13	0.06
7c-6	0.16	0.16	0.16	0.16