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Structure–activity relationship studies on 2,5,6-trisubstituted benzimidazoles targeting *Mtb*-FtsZ as antitubercular agents†

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Filamenting temperature sensitive protein Z (FtsZ) is an essential bacterial cell division protein and a promising target for the development of new antibacterial therapeutics. As a part of our ongoing SAR studies on 2,5,6-trisubstituted benzimidazoles as antitubercular agents targeting *Mtb*-FtsZ, a new library of compounds with modifications at the 2 position was designed, synthesized and evaluated for their activity against *Mtb*-H37Rv. This new library of trisubstituted benzimidazoles exhibited MIC values in the range of 0.004–50 μg mL^{-1}. Compounds **6b**, **6c**, **20f** and **20g** showed excellent growth inhibitory activities ranging from 0.004–0.08 μg mL^{-1}. This SAR study has led to the discovery of a remarkably potent compound **20g** (MIC 0.0039 μg mL^{-1}; normalized MIC 0.015 μg mL^{-1}). Our 3DQSAR model predicted **20g** as the most potent compound in the library.

Introduction

Tuberculosis (TB) an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*), was one of the top 10 killers claiming 1.6 million lives in 2017 alone.[1,2] Globally, an estimated 10 million new TB cases were reported in 2017.[1] TB being the most common opportunistic infection in HIV-positive patients, resulted in 0.3 million deaths in 2017.[1,3] Nearly one fourth of the world's population is afflicted with latent TB.[4] Once an individual is infected with TB, there is a 10% probability of developing an active infection.[1]

With the increase in emergence of drug resistant strains of TB, there is an urgent need for newer drugs with novel mechanisms of action.[5] In the past decade, 3 new drugs bedaquiline, delamanid and pretomanid have been approved to treat resistant forms of TB.[6–8] Although recently there has been progress in antitubercular drug discovery, the newer drugs are associated with safety concerns. Bedaquiline and delamanid are known to cause QT prolongation, which if left unmonitored can lead to cardiac arrest.[9,10] It is imperative that patients receiving either of these two medications need to monitored very closely and for the same reason, their combined use has not yet been recommended by WHO.[10] Pretomanid is associated with side effects such as nerve damage, hypoglycemia and lower respiratory tract infections.[11] Pretomanid has been approved by FDA only in cases of pulmonary XDR-TB and treatment intolerant or non-responsive MDR-TB in combination with other anti-TB drugs.[12]

With the increase in the number of cases of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) and new drugs displaying life threatening side effects, there is a dire need for the development of new anti-tubercular agents with novel targets, that would not only make it difficult for the bacteria to develop resistance, but are also safe. In this context, filamentous temperature sensitive protein Z (FtsZ), a bacterial cell division protein seems to be a promising target.[13,14] FtsZ, a guanosine triphosphate (GTP) dependent prokaryotic cell division protein, is a homolog of eukaryotic tubulin.[15] In the presence of GTP, FtsZ first polymerizes into protofilaments which then assemble to form a highly dynamic Z-ring at the center of the bacterial cell.[16,17] With the aid of other cell division proteins the Z-ring then forms a septum, which eventually results in cytokinesis.[18]

From a drug discovery perspective, targeting FtsZ offers several advantages. First, FtsZ is highly conserved among prokaryotes.[19] Thus, developing agents that target FtsZ could

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potentially lead to broad spectrum antibiotics. Second, FtsZ and tubulin share only ~7% sequence identity but high structural similarity.[20,21] This would help design inhibitors that specifically target FtsZ and reduce the chances of toxicity to mammalian cells. Lastly, since there are no FtsZ inhibitors as drugs in the market, there are less chances of cross resistance within the drug resistant bacterial species that already exist.[22]

Libraries of 2,5,6- and 2,5,7-trisubstituted benzimidazoles have been designed and synthesized as *Mtb*-FtsZ inhibitors in our laboratories, and evaluated for their antitubercular (anti-TB) activities against *Mtb*-H37Rv strain.[13,14,23] In general, 2,5,6-trisubstituted benzimidazoles exhibited better anti-TB activities than the 2,5,7-trisubstituted counterparts.[14] Some of the lead 2,5,6-trisubstituted benzimidazoles, including SB-P17G-C2, SB-P17G-A20, SB-P17G-A38 and SB-P17G-A42 (Fig. 1), exhibited excellent MIC values (0.06–0.31 μg mL^{-1}) against *Mtb* H37Rv.[13,24] Selected lead compounds were found to inhibit the assembly of *Mtb* FtsZ in a dose dependent manner by increasing the GTPase activity.[13,14] When evaluated their efficacies *in vivo* against immune compromised GKO mice, SB-P17G-A38 and SB-P17G-A42 reduced the bacterial load in the lung by 5.7–6.3 log_{10} CFU and in the spleen by 3.9–5.0 log_{10} CFU, respectively.[24]

Our previous structure activity relationship (SAR) studies on these 2,5,6-trisubstituted benzimidazoles for optimization of potency and metabolic properties were conducted on the 5 and 6 positions, wherein a cyclohexyl group was kept at the 2 position intact. In our present SAR study, we fixed the 6 position as a dimethylamino group and investigated the effects of various substituents at the 2 and 5 positions on the potency and pharmacological properties (Fig. 2).

Results and discussion

Chemical synthesis

Synthesis of 2,5,6-trisubstituted benzimidazoles with modifications at the 2 position are outlined in Schemes 1–3. Compounds shown in Scheme 1 were synthesized by the method previously described.[13,14] The 5-aminobenzimidazoles **4** were further derivatized with 35

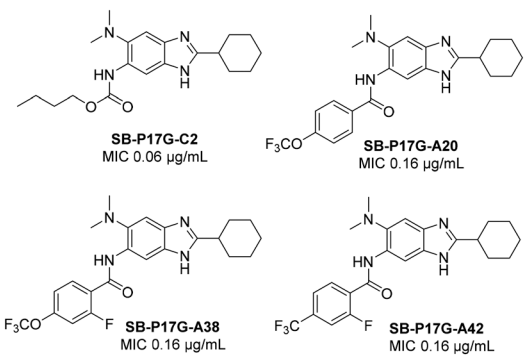


Fig. 1 Lead compounds arising from 2,5,6-trisubstituted benzimidazole libraries previously studied.[13,24]

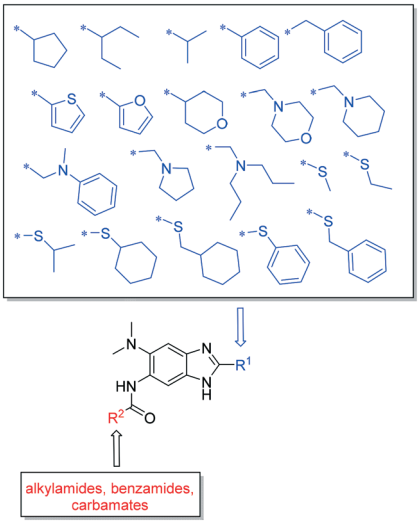
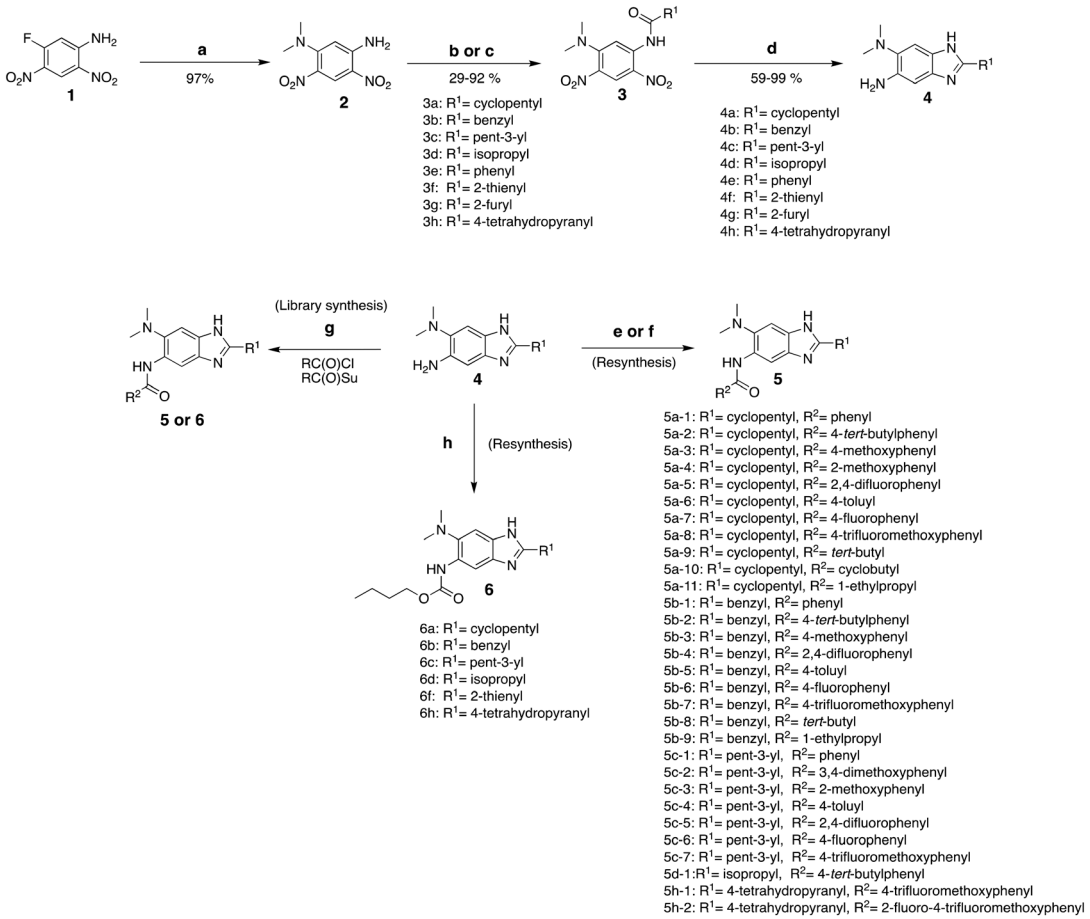


Fig. 2 New 2,5,6-trisusbstituted benzimidazoles in the present SAR study.

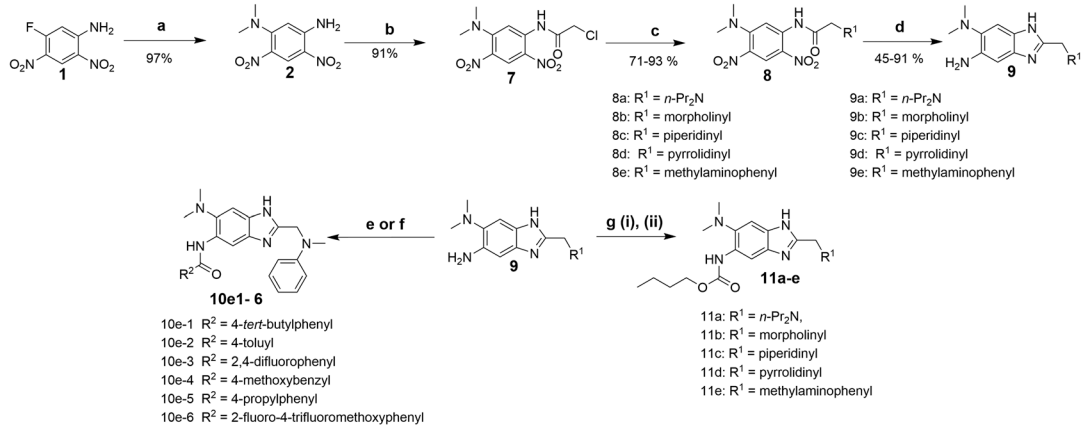
different acyl chlorides, hydroxysuccinimide esters and chloroformates (1.1 eq.) in dichloromethane in 96 well plates. The plates were gently shaken at room temperature for 24 h, followed by treatment with aminomethylated polystyrene resin EHL/2% DVB (200–400 mesh) (10 equiv.) in order to scavenge any unreacted acyl chlorides, succinate esters or chloroformates. Then, the reaction mixture was filtered to give 245 novel 2,5,6-trisubstituted benzimidazoles, **5** and **6**. In the case of trisubstituted benzimidazoles with *N*,*N*-dialkylaminomethyl substitution at the 2-position (Scheme 2), commercially available 2,4-dinitro-5-fluoroaniline (**1**) was subjected to nucleophilic aromatic substitution with dimethylamine to give 5-dimethylaminodinitroaniline (**2**) in 97% yield. Acylation of **2** with chloroacetyl chloride afforded 1-chloromethylcarboxamido-5-*N*,*N*-dimethylamino-2,4-dinitrobenzene (**7**) in 91% yield. Nucleophilic substitution of **7** with a variety of dialkylamines afforded compounds **8a–e** in 71–93% yields. This was followed by one-pot reduction and cyclization using stannous chloride dihydrate and 4 M hydrochloric acid to give the key intermediates **9a–e**. Then, 5-aminobenzimidazoles **9a–e** were converted to either benzamides **10** or carbamates **11**. For the synthesis of trisubstituted benzimidazoles with 2-sulfanyl substitution (Scheme 3), commercially available 1,2-diamino-4-fluorobenzene **12** was protected, using *p*-toluenesulfonyl chloride to yield **13** in 77% yield, and the subsequent nitration of **13** afforded **14** in 52% yield. Aromatic nucleophilic substitution of **14** with dimethylamine gave **15**. Deprotection of the tosyl groups in **15** afforded 1,2-diamino-4-dimethylamino-5-nitrobenzene (**16**) in 69% yield for two steps. The reaction of **16** with carbon disulfide to give 6-dimethylamino-5-nitro-2-thio-1*H*-benzo[*d*]imidazole (**17**) in 76% yield. Compound **17** was alkylated, using a variety of alkyl or aromatic halides, to afford **18a–g** in 31–84% yields. The reduction of **18a–g** with stannous chloride dihydrate gave **19a–g** in 44–89% yields. Finally, the amine moiety of **19a–g** was converted to the corresponding *n*-butyl carbamates **20a–g** in 61–77% yields.



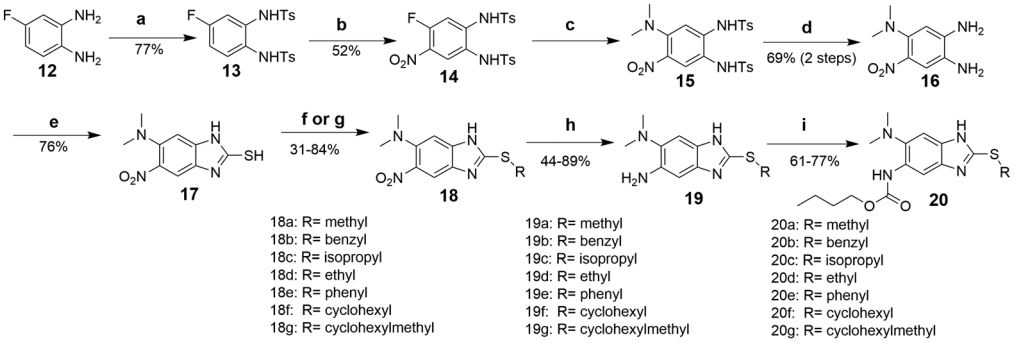
Scheme 1 Synthesis of 2,5,6-trisusbstituted benzimidazoles **5** and **6**. Reagents and conditions: a) dimethylamine, DIPEA, THF, 2 h, room temperature (rt); b) R^1COCl, pyridine, reflux, overnight; c) R^1COOH, PCl_3, acetonitrile, reflux, overnight; d) $SnCl_2{\cdot}2H_2O$, 4 M HCl, EtOH, reflux, 4 h; e) R^2COCl, Et_3N, 0 °C to rt, 4 h; f) R^2COOH, EDC.HCl, DMAP, CH_2Cl_2, reflux, overnight; g) (i) $R^2C(O)Cl$ or $R^2(O)Su$, DCM, RT, 24 h; (ii) aminomethylated polystyrene resin, DCM, RT, 6 h; h) (i) 1,1′-carbonyldiimidazole, CH_2Cl_2, reflux, 4 h; (ii) *n*-butanol, CH_2Cl_2, reflux, overnight.

Evaluation of antibacterial activities against *Mtb* H37Rv and SAR analysis

The library of 2,5,6-trisubstiutted benzimidazoles **5** and **6** (245 compounds) were screened against *Mtb* H37Rv at 5 μg mL^{-1} using microplate alamar blue assay (MABA).[25,26] Out of 245 compounds, 53 of them showed MIC ≤ 5 μg mL^{-1}. Furthermore, when 37 out of the 53 hits were resynthesized in analytically pure form and examined for their accurate



Scheme 2 Synthesis of 2,5,6-trisusbstituted benzimidazoles **10** and **11**. Reagents and conditions: a) dimethylamine, DIPEA, THF, 2 h, rt; b) chloroacetyl chloride, TEA, CH_2Cl_2, 0 °C to rt, overnight; c) dialkylamine, DIPEA, THF, reflux, overnight; d) $SnCl_2{\cdot}2H_2O$, 4 M HCl, EtOH, reflux, 4 h; e) R^2COCl, Et_3N, 0 °C to rt, 4 h; f) R^2COOH, EDC.HCl, DMAP, CH_2Cl_2, reflux, overnight; g) (i) 1,1′-carbonyldiimidazole, CH_2Cl_2, reflux, 4 h; (ii) *n*-Butanol, CH_2Cl_2, reflux, overnight.



Scheme 3 Synthesis of 2,5,6-trisubstituted benzimidazoles **20**. Reagents and conditions: a) *p*-toluenesulfonyl chloride, pyridine, 85 °C, overnight; b) fuming nitric acid, acetic acid, 65 °C, 40 minutes; c) dimethylamine, TEA, THF, 50 °C, 48 h; d) H_2SO_4, water, 85 °C, 1 h; e) carbon disulfide, TEA, MeOH, THF, 50 °C, 4 h; f) R–X, KOH, water, EtOH, rt, overnight; g) Ar–I, K_2CO_3, CuI, 1,10-phenanthroline, DMF, 140 °C, 22 h, pressure vessel; h) $SnCl_2{\cdot}2H_2O$, 4 M HCl, EtOH, reflux, 3 h; i) (i) 1,1′-carbonyldiimidazole, CH_2Cl_2, reflux, 4 h; (ii) *n*-butanol, CH_2Cl_2, reflux, overnight.

MIC values, there were a few compounds that exhibited higher MIC values than the initial cut off of 5 μg mL^{-1}. This could be attributed to the fact that the actual weight and purity of test compounds are not very accurate in a 96-well plate and could also be due to false positives in high throughput (HTP) screening. As Table 1 shows, the resynthesized compounds showed MIC values ranging from 0.078 to 50 μg mL^{-1} [note: MIC values were determined by two different laboratories using MABA with slightly different protocols, "A" and "B". Thus, Table 1 shows normalized MIC values with asterisks and also lists the original values in parentheses with superscript "b" marks].

Preliminary SAR of the hits from the library indicated that cyclopentyl, pent-3-yl, isopropyl and benzyl substituents at the 2 position were preferred. None of the compounds bearing a phenyl or 2-furyl substituents at the 2 position made the cut-off line in the initial screening. Although there was one hit with thien-2-yl substituent at the 2 position, this compound **6f** showed poor cell growth inhibitory activity (MIC >10 μg mL^{-1}) when assayed with the resynthesized compound. These results indicate that an sp^3 hybridized carbon is preferred over an sp^2 carbon at the 2 position.

Also, compounds bearing substituted benzamides at the 5 position fared better than their aliphatic amide counter parts.

Compounds bearing a cyclopentyl substituent at the 2 position (**5a-1**–**5a-8**, **6a** exhibited good inhibitory activity. The MIC values of trisubstituted benzimidazoles with pent-3-yl substituent at the 2 position (**5c-1**–**5c-7**, **c6b**) were comparable to the previously reported compounds bearing a cyclohexyl substituent at the 2 position.[13] These results suggest that pent-3-yl group is a close mimic of the cyclohexyl group at the 2 position. Compounds with isopropyl substitution at the 2 position (-15**d**, **6d**) were found to be less active than the ones with pent-3-yl substituent, suggesting the importance of the carbon chain length. In the case of 4-tetrahydropyranyl substituent at the 2 position (**5h-1**, **5h-2** and **6h**), there was a significant loss of inhibitory activity when compared to the cyclohexyl counterparts, indicating that the presence of an oxygen atom within the cyclohexyl moiety is unfavorable.

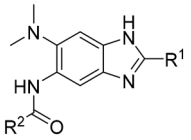
Next, we examined the effect of *N*,*N*-dialkylaminomethyl substitution at the 2-position. All the compounds were evaluated for their activity against *Mtb* H37Rv using MABA.[26,27] Compounds with an *N*,*N*-methyl(phenyl) aminomethyl group at the 2 position (**10e-1**–**10e-5**, **11e**) were far less active compared to ones with a pent-3-yl group with an exception of **10e-6**. Compound with *N*,*N*-dipropylaminomethyl (**11a**) showed a high activity (MIC 0.16 μg mL^{-1}). Morpholin-4-ylmethyl (**11b**) and pyrrolidin-1-ylmethyl (**11d**) groups at the 2 position resulted in diminished activities. 2,5,6-Trisubstituted benzimidazole with a *n*-butoxycarbonylamino group at the 5 position and a piperidin-1-ylmethyl group at the 2 position (**11c**) showed a high activity (MIC 0.09 μg mL^{-1}; normalized MIC 0.14 μg mL^{-1}).

In order to further diversify our library, a small series of 2,5,6-trisusbstituted benzimidazoles with alkyl/arylsulfanyl groups at the 2 position and *n*-butyl carbamate at the 5 position were designed and synthesized. All these sulfide-containing compounds were evaluated for their activities against *Mtb* H37Rv using MABA.[26,27] Results are summarized in Table 2.

Compounds **20b** and **20e**, bearing a benzylsulfanyl and a phenylsulfanyl groups, respectively, exhibited good activity (MIC 0.31 μg mL^{-1}). In the 2-alkylsulfanyl series of analogs, the size of alkyl groups is critical to their activities. Thus, cyclohexyl analog **20f** exhibited high activity (MIC <0.0081 μg mL^{-1}; normalized MIC <0.031 μg mL^{-1}) and isopropyl analog **20c** retained good activity (MIC 0.31 μg mL^{-1}), while ethyl analog **20d** displayed moderate activity (normalized MIC 1.2 μg mL^{-1}), a significant decrease in activity was observed for the methyl analog **20a** (normalized MIC 9.6 μg mL^{-1}). The introduction of a methylene group between the sulfur and cyclohexyl unexpectedly increased the activity. Thus, cyclohexylmethylsulfanyl analog **20g** exhibited remarkably enhanced activity (MIC 0.0039 μg mL^{-1}; normalized MIC 0.015 μg mL^{-1}), which marks the highest inhibitory activity so far to date.

Cytotoxicity in human cells (MTT assay). The cytotoxicity of top 10 compounds was evaluated against two human cell

Table 1 Anti-TB activity of 2,5,6-trisusbstituteid benzimidazoles **5**, **6**, **10** and **11** against *Mtb* H37Rv (MIC μg mL^{-1})



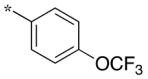
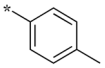
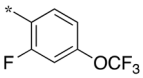
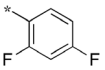
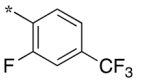
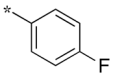
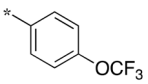
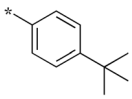
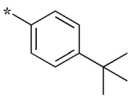
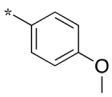
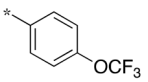
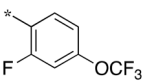
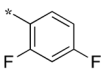
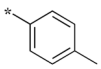
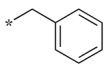
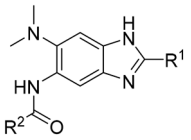
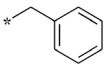
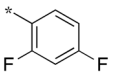
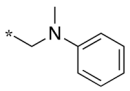
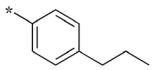
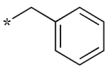
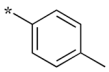
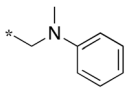
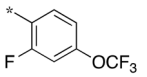
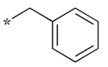
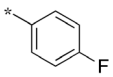
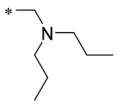
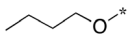
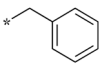
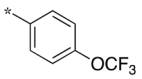
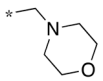
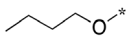
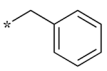
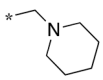
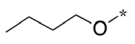
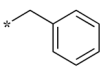
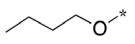
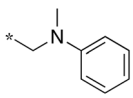
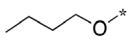
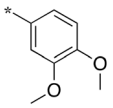
Compound	R^1	R^2	MIC[a]	Compound	R^1	R^2	MIC[a]
SB-P17G-C2			0.06 (0.008[b]) 0.064[c]	**5c-3**			1.56
SB-P17G-A20			0.16	**5c-4**			0.50
SB-P17G-A38			0.16 (0.019[b]) 0.15[c]	**5c-5**			0.71
SB-P17G-A42			0.16	**5c-6**			0.78
5a-1			1.56	**5c-7**			0.31
5a-2			0.78	**5d-1**			0.78
5a-3			1.25	**5h-1**			>10
5a-4			12.5	**5h-2**			10
5a-5			0.625	**6a**			0.31
5a-6			0.625	**6b**			<0.078
5a-7			0.78	**6c**			0.078
5a-8			1.56	**6d**			1.25
5a-9			50	**6f**			>10
5a-10			>10	**6h**			>2.5
5a-11			>50	**10e-1**			2.5
5b-1			1.25	**10e-2**			1.25
5b-2			0.33	**10e-3**			1.25
5b-3			1.25	**10e-4**			>10

Table 1 *(continued)*



Compound	R^1	R^2	MIC[a]	Compound	R^1	R^2	MIC[a]
5b-4			0.63	**10e-5**			5
5b-5			1.56	**10e-6**			0.29[c] (0.19[b])
5b-6			0.63	**11a**			0.16
5b-7			1.25	**11b**			2.4[c] (1.6[b])
5b-8			>10	**11c**			0.14[c] (0.09[b])
5b-9			>10	**11d**			2.4[c] (1.6[b])
5c-1			1.56	**11e**			0.16
5c-2			12.5				

MIC: minimum concentration of the compound required to inhibit growth of 99% of *Mtb* H37Rv cells. [a] MIC determined by protocol A. See Experimental section. [b] MIC determined by protocol B. See Experimental section. [c] Normalized MIC from the MIC determined by protocol B to the MIC by protocol A.

lines, WI38 (normal lung fibroblast) and HepG2 (liver cancer). The level of cytotoxicity was determined as% inhibition of cell growth, which gave sufficient information about potential systemic toxicity. Results are summarized in Table 3. The IC_{50} of **20g** appears to be very close to 20 μM since it shows 52% of cell growth inhibition against WI38 human normal cell line. Thus, the therapeutic window (IC_{50}/MIC) of **20g** is >100. In comparison, **20f** is considerably more cytotoxic (87% inhibition at 20 μM) than **20g**. Also, it is interesting that **20g** did not show any cytotoxicity against HepG2 human liver cancer cell line at 20 μM concentration. The least cytotoxic compound is **11a** (only 4% inhibition at 20 μM), while **11e** is most cytotoxic (96% inhibition) followed by **6b** (93% inhibition). It is interesting to note that **11a** and **11c** did not inhibit the cell growth of HepG2 cells, but even enhanced the cell growth at 20 μM.

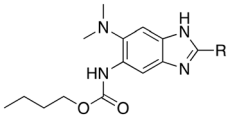
Computational analysis of SAR and toxicity prediction

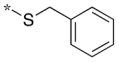
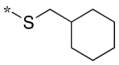
3D-QSAR analysis. The first 3D-QSAR study on 2,5,6-trisubstituted benzimidazole series was conducted and reported by Li *et al.* in 2016,[29] which used 67 compounds in our publication.[13] Since we have investigated a more diverse library of 2,5,6-trisubstituted benzimidazoles and obtained relevant data, we expanded the database to 148 compounds for building to a more robust 3D-QSAR model by building upon Li's model. First, Li's model was reproduced and confirmed with the 67 compounds he used (R^2 = 0.90; Q^2 = 0.97; RMSE = 0.19) using Schrödinger's AutoQSAR program. Then, we expanded it to 148 compounds, which also gave excellent results (R^2 = 0.90; Q^2 = 0.90; RMSE = 0.21) as illustrated in Fig. 3.

Next, we examined the validity of this 3D-QSAR model by applying it for the prediction of the MIC values of the top 12 and other two new benzimidazoles in the present work, as well as four of the lead compounds from our previously published work. The results are summarized in Table 4. As Table 4 shows, the predicted MIC values are all very close to those determined experimentally, except for **20f** (entry 6).

It should be noted that the most potent compound, **20g**, in this series, is actually predicted as the best compound (entry 5). The results clearly indicate that the prediction

Table 2 Anti-TB activity of 2-alkyl/arylsulfanylbenzimidazoles **20** against *Mtb* H37Rv (MIC μg mL^{-1})



Compound	R^1	MIC[a]
SB-P17G-C2		0.06 (0.008[b])
20a		9.6[c] (1.28[b])
20b		0.31
20c		0.31
20d		1.2[c] (0.16[b])
20e		0.31
20f		<0.031[c] (<0.0081[b])
20g		0.015[c] (0.0039[b])

[a] MIC determined by protocol A. See Experimental section. [b] MIC determined by protocol B. See Experimental section. [c] Normalized MIC from the MIC determined by protocol B to the MIC by protocol A.

based on the AutoQSAR model developed in the present work can be used for the selection of promising compounds from the designed compounds *in silico* for chemical synthesis and biological evaluations. Table 4 also lists calculated log*P* values using the ChemDraw Pro program. All compounds show the Clog*P* values of 3–5, which are in a good range in the Lipinski's rule of 5.[30]

We also carried out computational analysis of toxicity prediction using pKCSM program (Table S1†), as well as docking analysis of compound **20g** in the putative binding site of *Mtb* FtsZ (Fig. S1†). This information is available in the ESI.†

Conclusions

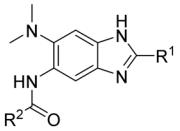
A library of 2,5,6-trisubstituted benzimidazoles with a variety of modifications at the 2 position were synthesized and tested for their antitubercular activities against *Mtb* H37Rv. Among the compounds with different functional groups at the 2 position, those having a butyl carbamate at the 5 position resulted in the most active compound within each series (**6a**, **6b**, **6c**, **6d**, **11e**), following a trend similar to our previous series of 2,5,6-trisubstituted benzimidazoles with a cyclohexyl substitution at the 2 position. Functional groups with an sp^3 carbon at the 2 position were preferred over ones with an sp^2 carbon. From this series, we identified 13 compounds (**5c-7**, **6a**, **6b**, **6c**, **10e-6**, **11a**, **11c**, **11e**, **20b**, **20c**, **20e**, **20f** and **20g**) that were highly potent. Overall SAR of 2,5,6-trisusbstituted benzimidazoles is summarized in Fig. 4. We built a robust 3DQSAR model by following up Li's model,[29] and used this updated model to predict the MIC of the top 13 compounds. Since almost all of the predicted MIC values are very close to those determined experimentally, this model can be used for the selection of promising compounds from the designed compounds *in silico* for chemical synthesis and biological evaluations in the future. Compound **20g** was found to be the most potent compound (MIC 0.0039 μg mL^{-1}; normalized MIC 0.015 μg mL^{-1}) from the current series, both experimentally and computationally from the 3DQSAR model. Not only was **20g** the most potent compound of the series, but also it was more potent than the previous lead SB-P17G-C2 (MIC 0.06 μg mL^{-1}). Also, the cytotoxicity (IC_{50}) of **20g** is found to be *ca.* 20 μM, which indicates the therapeutic index (IC_{50}/MIC) of **20g** is >100. Based on our current results, systematic SAR study on 2-alkylsulfanylbenzimidazoles with varying substituents at the 5 and 6 positions is clearly warranted.

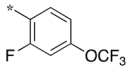
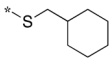
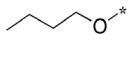
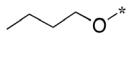
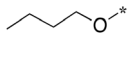
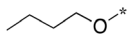
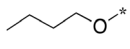
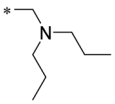
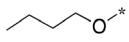
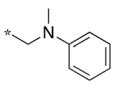
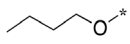
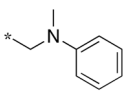
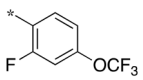
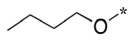
Experimental section

General methods

Melting points were measured on a Thomas Hoover Capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker Ascend 700 spectrometer operating at 700 MHz for ^{1}H and 175 MHz for ^{13}C, a Bruker 500 Advance spectrometer operating at 500 MHz and 125 MHz for ^{1}H and ^{13}C, respectively, or a Bruker 400 Nanobay spectrometer operating at 400 MHz, 100 MHz, and 376 MHz for ^{1}H, ^{13}C, and ^{19}F, respectively or a Bruker 300 Nanobay spectrometer operating at 300 MHz of ^{1}H. Chemical shifts were referenced to the residual proton and carbon-13 peaks of solvents used for ^{1}H and ^{13}C NMRs, respectively (^{1}H: $CDCl_3$, δ 7.26; ^{13}C: $CDCl_3$, δ 77.23; ^{1}H: DMSO-d_6, δ 2.50; ^{13}C: DMSO-d_6, δ 39.51). Signals are listed in ppm, and multiplicity identified as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet); *J* - coupling constants in Hz, and integration. Low resolution mass spectrometry (LRMS) analysis was carried out on an Agilent LC-MSD mass spectrometer and high resolution mass spectrometry (HRMS) analysis was carried out on an Agilent LC-UV-TOF mass spectrometer at the Institute of Chemical Biology and Drug Discovery, Stony Brook. Purity of the 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 μm, 250 mm Å ~ 4.6 mm column, isopropanol and hexanes, flow rate of 1 mL min^{-1}, *t* = 0–40 min, gradient of 5–50% isopropanol. (2): Kinetex PFP, 2.6 μm, 4.6 mm Å ~ 100 mm column, isopropanol and hexanes, flow rate of 0.3 mL min^{-1},, *t* = 0–25 min, gradient of 5–95% isopropanol. (3): Kinetex PFP, 2.6 μm, 4.6 mm Å ~ 100 mm column, isopropanol and hexanes,

Table 3 Cytotoxicity (% inhibition at 20 μM) of select 10 compounds with high potency



Entry	Compound	R^1	R^2	MIC[a] (μg mL^{-1})	WI38 % inhibition at 20 μM[d]	HepG2 % inhibition at 20 μM[d]
1	SB-P17G-A38			0.16	78	57
2	**20g**			0.015[c] (0.0039[b])	52	0
3	**20f**			<0.031[a,c] (<0.0081[b])	87	50
4	**6b**			<0.078	93	80
5	**6c**			0.078	77	32
6	**11c**			0.14[c] (0.09[b])	60	0[e]
7	**11a**			0.16	4.0	0[e]
8	**11e**			0.16	96	54
9	**10e-6**			0.29	59	30
10	**6a**			0.31	86	42

[a] MIC determined by protocol A. See Experimental section. [b] MIC determined by protocol B. See Experimental section. [c] Normalized MIC from the MIC determined by protocol B to the MIC by protocol A. [d] Average value of MTT assay in triplicate. [e] Enhancement of cell growth was observed.

flow rate of 0.3 mL min^{-1}, t = 0–20 min, gradient of 5–80% isopropanol. (4): Kinetex PFP, 2.6 μm, 4.6 mm Å ~ 100 mm column, isopropanol and hexanes, flow rate of 0.3 mL min^{-1}, t = 0–30 min, gradient of 5–80% isopropanol. Measurements were made at 254 and 303 nm.

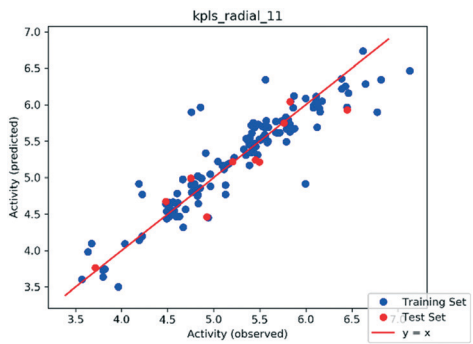
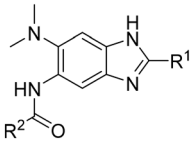


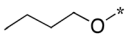
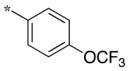
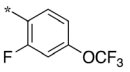
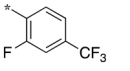
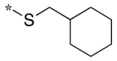
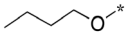
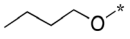
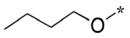
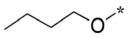
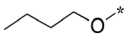
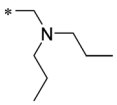
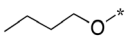
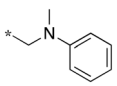
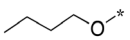
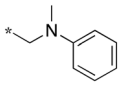
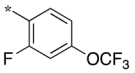
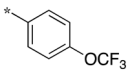
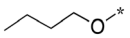
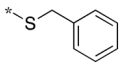
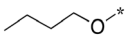
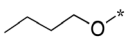
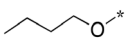
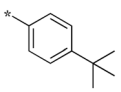
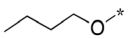
Fig. 3 Plot of experimental *vs.* predicted activities for the training set and test set compounds (AutoQSAR).

Materials

All air- and moisture-insensitive reactions were carried out under an ambient atmosphere, magnetically stirred, and monitored by thin layer chromatography (TLC) using Agilent Technologies TLC plates pre-coated with 250 μm thickness silica gel 60 F254 plates and visualized by fluorescence quenching under UV light. Flash chromatography was performed on SiliaFlash® Silica Gel 40–63 μm 60 Å particle size using a forced flow of eluent at 0.3–0.5 bar pressure. All air- and moisture-sensitive manipulations were performed using oven-dried glassware using the standard Schlenk techniques under nitrogen. Diethyl ether and tetrahydrofuran (THF) were distilled from deep purple sodium benzopheone ketyl. Dichloromethane (DCM), chloroform and acetonitrile were dried over calcium hydride and distilled. DCM was degassed *via* three freeze-pump-thaw cycles. All other chemicals were used as received. All deuterated solvents were purchased from Cambridge Isotope Laboratories. 5-*N*,*N*-Dimethylamino-2,4-dinitroaniline (**2**) was synthesized using

Table 4 Prediction of MIC values of top 13 compounds and other selected compounds using AutoQSAR



Entry	Compound	R^1	R^2	MIC[a]	Predicted MIC	Clog *P*
1	SB-P17G-C2			0.06	0.16	4.456
2	SB-P17G-A20			0.16	0.24	4.936
3	SB-P17G-A38			0.16	0.28	4.669
4	SB-P17G-A42			0.16	0.21	4.524
5	**20g**			0.015[c] (0.0039[b])	0.03	5.675
6	**20f**			0.031 (<0.0081[b])	0.62	5.056
7	**6b**			<0.078	0.17	3.903
8	**6c**			0.078	0.08	4.321
9	**11c**			0.14[c] (0.09[b])	0.22	3.093
10	**11a**			0.16	0.26	4.016
11	**11e**			0.16	0.13	3.999
12	**10e-6**			0.29[c] (0.19[b])	0.10	4.212
13	**5c-7**			0.31	0.43	4.801
14	**6a**			0.31	0.60	3.897
15	**20b**			0.31	0.31	4.593
16	**20c**			0.31	0.68	3.863
17	**20e**			0.31	0.67	4.804
18	**5b-2**			0.33	0.33	5.181
19	**20d**			1.2[c] (0.16[b])	0.5	3.554
20	**20a**			9.6[c] (1.28[b])	3.96	3.025

[a] MIC determined by Protocol A. See Experimental Section. [b] MIC determined by Protocol B. See Experimental Section. [c] Normalized MIC from the MIC determined by Protocol B to the MIC by Protocol A.

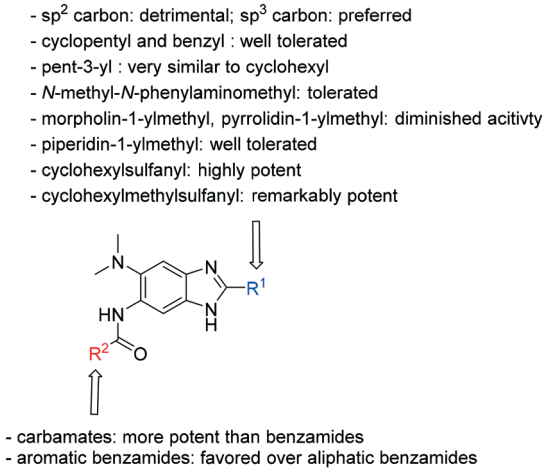


Fig. 4 SAR of 2,5,6-trisubstituted benzimidazoles.

the procedure previously published by us.[13] 4-Fluoro-1,2-di(4-methylbenzenesulfonamido)benzene (**13**) and 4-fluoro-5-nitro-1,2-di(4-methylbenzene-sulfonamido)benzene (**14**) were synthesized by literature methods.[31]

5-Benzamido-2-cyclopentyl-6-dimethylamino-1*H*-benzo[*d*] imidazole (5a-1). Compound **4a** (0.100 g, 0.4 mmol), benzoic acid (0.060 g, 1.2 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) (0.100 g, 0.53 mmol) and *N*,*N*-4-dimethylaminopyridine (DMAP) (0.065 g, 1.3 mmol) were dissolved in DCM (2 mL) and the reaction mixture was refluxed overnight. Then, the reaction mixture was cooled to room temperature and washed with aqueous saturated $NaHCO_3$ (20 mL × 2) followed by water (20 mL × 2). The organic layer was separated, dried with $MgSO_4$, filtered and concentrated on a rotary evaporator to give a crude product. The crude product was purified by column chromatography on silica get, using 1 : 1 AcOEt : hexanes as eluent to afford **5a-1** as an off-white solid (134 mg, 94% yield); mp 176–180 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.46–1.48 (m, 2 H), 1.62–1.68 (m, 2 H), 1.83–1.96 (m, 4 H), 2.72 (s, 6 H), 3.09 (quintet, J = 8.5 Hz, 1 H), 7.52–7.58 (m, 3 H), 7.61 (s, 1 H), 7.97 (d, J = 7.1 Hz, 2 H), 8.94 (s, 1 H), 9.98 (s, 1 H), 11.66 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.5, 32.5, 39.9, 46.1, 101.8, 110.6, 127.1, 128.9, 129.0, 131.8, 131.9, 135.8, 139.3, 139.9, 159.74, 165.7; HRMS (ESI-TOF) m/z calcd for $C_{21}H_{24}N_4OH^+$: 349.2023, found: 349.2013 (Δ = 2.78 ppm); HPLC (1): t = 10.0 min, purity >96%.

The same procedure was used for the synthesis and characterization of **5a-3**, **5a-7**, **5a-8**, **5b-1**, **5b-3**, **5b-6**, **5b-7**, **5c-1**, **5c-2**, **5h-1**, **5h-2** and **10e-6**.

5-(4-*tert*-Butylbenzamido)-2-cyclopentyl-6-dimethyl-amino-1*H*-benzo[*d*]imidazole (5a-2). A solution of **4a** (0.100 g, 0.4 mmol) and 4-*tert*-butylbenzoyl chloride (0.072 g, 0.36 mmol) in DCM (4 mL) was stirred at 0 °C for 2 h. After the completion of the reaction, the reaction mixture was washed with saturated aqueous $NaHCO_3$ (20 mL × 2) followed by water (20 mL × 2). The organic layer was separated, dried over $MgSO_4$, filtered and concentrated on a rotary evaporator to give a crude product. The crude product was purified by column chromatography on alumina, using 1 : 1 AcOEt : hexanes as eluent to afford **5a-2** as beige solid (132 mg, 80% yield); mp 207–210 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 9 H), 1.65–1.70 (m, 2 H), 1.82–1.85 (m, 2 H), 1.87–1.93 (m, 4 H), 2.73 (s, 6 H), 3.03–3.12 (quintet, J = 8.4 Hz, 1 H), 7.56 (d, J = 8.4 Hz, 2 H), 7.61 (s, 1 H), 7.91 (d, J = 8.4 Hz, 2 H), 8.96 (s, 1 H), 9.96 (s, 1 H), 11.39 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.6, 31.4, 32.5, 35.2, 39.9, 46.2, 101.7, 110.4, 126.0, 127.0, 129.1, 131.9, 133.1, 139.2, 139.9, 155.5, 159.6, 165.9; HRMS (ESI-TOF) m/z calcd for $C_{25}H_{32}N_4OH^+$: 405.2649, found: 405.2641 (Δ = 1.87 ppm). HPLC (1): t = 9.98 min, purity >99%.

The same procedure was used for the synthesis and characterization of compounds **5a-4**, **5a-5**, **5a-6**, **5a-9**, **5a-10**, **5a-11**, **5b-2**, **5b-4**, **5b-5**, **5b-8**, **5b-9**, **5c-3**, **5c-4**, **5c-5**, **5c-6**, **5c-7**, **5d-1**, **10e-1**, **10e-2**, **10e-3**, **10e-4** and **10e-5**.

2-Cyclopentyl-6-dimethylamino-5-(4-methoxy-benzamido)-1*H*-benzo[*d*]-imidazole (5a-3). White solid (53% yield); mp 205–208 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60–1.65 (m, 2 H), 1.71–1.81 (m, 2 H), 1.87–1.95 (m, 2 H), 2.04 2.06 (m, 2 H), 2.73 (s, 6 H), 3.18 (quintet, J = 6.7 Hz, 1 H), 3.89 (s, 3 H), 7.03 (d, J = 7.0 Hz, 2 H), 7.59 (s, 1 H), 7.93 (d, J = 7 Hz, 2 H), 8.81 (s, 1 H), 9.80 (s, 1 H), 10.07 (bs, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.7, 32.5, 39.9, 46.2, 55.7, 101.4, 110.9, 114.3, 128.0, 129.0, 129.5, 139.5, 159.1, 162.6, 165.1; HRMS (ESI-TOF) m/z calcd for $C_{22}H_{26}N_4O_2H^+$: 379.2129, found: 379.2129 (Δ = −0.19 ppm). HPLC (1): t = 15.1 min, purity >99%.

2-Cyclopentyl-6-dimethylamino-5-(2-methoxybenzamido)-1*H*-benzo[*d*]imidazole (5a-4). White solid (76% yield); mp 214–217 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39–1.48 (m, 2 H), 1.57–1.65 (m, 2 H), 1.80–1.89 (m, 2 H), 1.91 1.99 (m, 2 H), 2.73 (s, 6 H), 3.09 (quintet, J = 8.5 Hz 1 H), 4.07 (s, 3 H), 7.06 (d, J = 7.5 Hz, 1 H), 7.14 (t, J = 7.5 Hz, 1 H), 7.51 (t, J = 7.8 Hz, 1 H), 7.57 (s, 1 H), 8.37 (dd, J = 7.8, 1.8 Hz, 1 H), 9.04 (s, 1 H), 11.19 (s, 1 H), 11.39 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.5, 32.5, 39.9, 46.1, 55.9, 102.6, 110.4, 111.6, 121.4, 122.6, 129.9, 131.7, 132.4, 133.3, 139.7, 139.9, 157.6, 159.5, 163.6; HRMS (ESI-TOF) m/z calcd for $C_{22}H_{26}N_4O_2H^+$: 379.2129, found: 379.2119 (Δ = 2.62 ppm). HPLC (1): t = 12.7 min, purity >99%.

2-Cyclopentyl-5-(2,4-difluorobenzamido)-6-dimethylamino-1*H*-benzo[*d*]imidazole (5a-5). White solid (92% yield); mp 213–214 °C ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.58–1.61 (m, 2 H), 1.73–1.77 (m, 2 H), 1.87–1.94 (m, 2 H), 2.03–2.07 (m, 2 H), 2.71 (s, 6 H), 3.19 (quintet, J = 8.0 Hz, 1 H), 6.94–6.98 (m, 2 H), 7.03–7.07 (m, 1 H), 7.60 (s, 1 H), 8.21–8.26 (m, 1 H), 8.82 (s, 1 H), 10.29 (s, 1 H), 10.33 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.6, 32.5, 39.9, 46.0, 102.1, 104.5, 104.8, 105.0, 110.9, 112.5, 112.5, 112.69, 112.71, 118.91, 118.94, 119.01, 119.03, 129.3, 131.5, 133.7, 133.8, 139.7, 140.0, 159.6, 159.9, 160.0, 160.5, 161.9, 162.0, 164.0, 164.1, 166.0, 166.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ −103.58 (d, 1 F), −108.92 (d, 1 F); HRMS (ESI-TOF) m/z calcd for $C_{21}H_{22}F_2N_4OH^+$: 385.1834, found: 385.1829 (Δ = 1.46 ppm). HPLC (1): t = 8.5 min, purity >99%.

2-Cyclopentyl-6-dimethylamino-5-(4-methylbenzamido)-1*H*-benzo[*d*]imidazole (5a-6). White solid (76% yield); mp 187–

190 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.47–1.48 (m, 2 H), 1.66–1.76 (m, 2 H), 1.87–1.93 (m, 4 H), 2.44 (s, 3 H), 2.72 (s, 6 H), 3.04–3.11 (quintet, J = 8.3 Hz, 1 H), 7.34 (d, J = 7.7 Hz, 2 H), 7.60 (s, 1 H), 7.87 (d, J = 7.7 Hz, 2 H), 8.92 (s, 1 H), 9.92 (s, 1 H), 11.52 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.7, 25.5, 32.5, 39.9, 46.1, 101.7, 110.6, 127.1, 128.9, 129.7, 131.8, 133.0, 139.2, 139.8, 142.4, 159.6, 165.8; HRMS (ESI-TOF) m/z calcd for $C_{22}H_{26}N_4OH^+$: 363.2179, found: 363.2171 (Δ = 2.3 ppm). HPLC (1): t = 12.98 min, purity >99%.

2-Cyclopentyl-6-dimethylamino-5-(4-fluoro-benzamido)-1*H*-benzo[*d*]imidazole (5a-7). White solid (75% yield); mp 198–199 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.47–1.54 (m, 2 H), 1.68–1.70 (m, 2 H), 1.84–1.88 (m, 2 H), 1.90–1.96 (m, 2 H), 2.71 (s, 6 H), 3.09–3.15 (quintet, J = 8.6 Hz, 1 H), 7.21 (t, J = 8.3 Hz, 2 H), 7.59 (s, 1 H), 7.95–7.98 (m, 2 H), 8.82 (s, 1 H), 9.90 (s, 1 H), 11.36 (bs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.6, 32.5, 39.9, 46.1, 101.7, 110.7, 116.0, 116.2, 128.8, 129.4, 129.5, 131.7, 131.88, 131.90, 139.3, 139.9, 159.7, 164.0, 164.5, 166.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ −107.42 (s, 1 F); HRMS (ESI-TOF) m/z calcd for $C_{21}H_{23}FN_4OH^+$: 367.1929, found: 367.1919 (Δ = 2.51 ppm). HPLC (1): t = 12.0 min, purity >99%.

2-Cyclopentyl-6-dimethylamino-5-(4-trifluoromethoxy-benzamido)-1*H*-benzo[*d*] imidazole (5a-8). White solid (50% yield); mp 182–186 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.53–1.57 (m, 2 H), 1.68–1.75 (m, 2 H), 1.86–1.93 (m, 2 H), 1.97–2.03 (m, 2 H), 2.73 (s, 6 H), 3.11–3.18 (m, 1 H), 7.38 (d, J = 8.3 Hz, 2 H), 7.62 (s, 1 H), 8.0 (d, J = 8.3 Hz, 2 H), 8.81 (s, 1 H), 9.94 (s, 1 H), 10.77 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.6, 32.5, 39.9, 46.2, 101.6, 111.0, 117.5, 119.5, 121.1, 121.6, 123.6, 131.6, 134.2, 139.4, 140.0, 151.8, 159.6, 164.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ −57.69 (s, 3 F). HRMS (ESI-TOF) m/z calcd for $C_{22}H_{23}F_3N_4O_2H^+$: 433.1846, found: 433.1843 (Δ = 0.77 ppm). HPLC (1): t = 11.1 min, purity >98%.

2-Cyclopentyl-6-dimethylamino-5-(pivalamido)-1*H*-benzo[*d*] imidazole (5a-9). White solid (94% yield); mp >220 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 (s, 9H), 1.55–1.57 (m, 2 H), 1.68–1.76 (m, 2 H), 1.85–1.90 (m, 2 H), 1.99–2.05 (m, 2 H), 2.64 (s, 6 H), 3.19 (quintet, J = 8.4 Hz, 1 H), 7.46 (s, 1 H), 8.53 (s, 1 H), 9.25 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.6, 27.8, 32.4, 39.7, 40.1, 45.7, 102.0, 110.0, 128.8, 131.7, 139.3, 159.4, 176.7; HRMS (ESI-TOF) m/z calcd for $C_{19}H_{28}N_4OH^+$: 329.2336, found: 329.2327 (Δ = 2.83 ppm). HPLC (1): t = 10.6 min, purity >99%.

5-Cyclobutanecarboxamido-2-cyclopentyl-6-dimethylamino-1*H*-benzo[*d*]imidazole (5a-10). Brown solid (75% yield); mp 206–209 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64–1.69 (m, 2 H), 1.76–1.84 (m, 2 H), 1.88–1.99 (m, 3 H), 2.01–2.13 (m, 3 H), 2.26–2.34 (m, 2 H), 2.37–2.47 (m, 2 H), 2.64 (s, 6 H), 3.21–3.34 (m, 2 H), 7.51 (s, 1 H), 8.59 (s, 1 H), 8.87 (s, 1 H), 10.63 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.3, 25.76, 25.80, 32.5, 39.8, 41.6, 45.8, 101.7, 110.5, 128.9, 131.4, 139.1, 159.3, 176.3; HRMS (ESI-TOF) m/z calcd for $C_{19}H_{26}N_4OH^+$: 327.2179, found: 327.2171 (Δ = 2.65 ppm). HPLC (3): t = 10.3 min, purity >98%.

2-Cyclopentyl-6-dimethylamino-5-(2-ethylbutamido)-1*H*-benzo[*d*]imidazole (5a-11). Beige solid (66% yield); mp 210–212 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (t, J = 7.4 Hz, 6 H), 1.59–1.79 (m, 6 H), 1.81–1.86 (m, 2 H), 1.92–2.01 (m, 2 H), 2.09–2.21 (m, 3 H), 2.67 (s, 6 H), 3.22–3.30 (m, 1 H), 7.57 (s, 1 H), 8.59 (s, 1 H), 8.91 (s, 1 H), 9.84 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.4, 25.8, 26.2, 32.5, 39.8, 46.0, 53.1, 101.5, 110.7, 129.2, 131.2, 139.2, 139.5, 159.1, 174.3; HRMS (ESI-TOF) m/z calcd for $C_{20}H_{30}N_4OH^+$: 343.2411, found: 343.2483 (Δ = 2.44 ppm). HPLC (1): t = 10.3 min, purity >99%.

5-Benzamido-2-benzyl-6-dimethylamino-1*H*-benzo[*d*] imidazole (5b-1). Light yellow solid (57% yield); mp 212–213 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.72 (s, 6 H), 4.12 (s, 2 H), 7.13–7.23 (m, 5 H,), 7.48–7.51 (m, 2 H), 7.54–7.57 (m, 2 H), 7.92–7.93 (m, 2 H), 8.84 (s, 1 H), 9.89 (s, 1 H), 10.99 (bs, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 35.9, 46.1, 101.8, 111.0, 127.0, 127.1, 128.88, 128.90, 129.1, 129.4, 131.9, 135.8, 137.0, 139.7, 140.1, 154.0, 165.5; HRMS (ESI-TOF) m/z calcd for $C_{23}H_{22}N_4$-OH^+: 371.1866, found: 371.1864 (Δ = 0.7 ppm). HPLC (1): t = 12.67 min, purity >98%.

2-Benzyl-5-(4-*tert*-butylbenzamido)-6-dimethylamino-1*H*-benzo[*d*]imidazole (5b-2). Beige solid (79% yield); mp 164–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (s, 9 H), 2.73 (s, 6 H), 4.16 (s, 2 H), 7.15–7.25 (m, 5 H,), 7.51 (d, J = 8.5 Hz, 2 H), 7.59 (s, 1 H), 7.86 (d, J = 8.5 Hz, 2 H), 8.82 (s, 1 H), 9.85 (s, 1 H), 10.45 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.4, 35.2, 36.0, 46.1, 101.6, 111.0, 126.1, 127.0, 127.1, 128.9, 129.0, 129.7, 131.8, 132.9, 136.9, 139.7, 139.9, 153.7, 155.5, 165.5; HRMS (ESI-TOF) m/z calcd for $C_{27}H_{30}N_4OH^+$: 427.2492, found: 427.2496 (Δ = 1.51 ppm). HPLC (1): t = 10.5 min, purity >98%.

2-Benzyl-6-dimethylamino-5-(4-methoxybenzamido)-1*H*-benzo[*d*]imidazole (5b-3). Light yellow solid (55% yield); mp 204–206 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.71 (s, 6 H), 3.85 (s, 3 H), 4.11 (s, 2 H), 6.97 (d, J = 8.8 Hz, 2 H), 7.12–7.20 (m, 5 H,), 7.55 (s, 1 H), 7.88 (d, J = 8.8 Hz, 2 H), 8.82 (s, 1 H), 9.80 (s, 1 H), 11.26 (bs, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.8, 46.0, 55.6, 101.7, 110.8, 114.3, 126.9, 127.9, 128.8, 129.0, 129.5, 131.8, 137.1, 139.6, 139.9, 153.9, 162.6, 165.1; HRMS (ESI-TOF) m/z calcd for $C_{24}H_{24}N_4O_2H^+$: 401.1972, found: 401.1967 (Δ = 1.26 ppm). HPLC (1): t = 14.6 min, purity >98%.

2-Benzyl-6-dimethylamino-5-(2,4-difluorobenzamido)-1*H*-benzo[*d*]imidazole (5b-4). Light yellow solid (92% yield); mp 180–182 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.68 (s, 6 H), 4.11 (s, 2 H), 6.90–6.94 (m, 1 H), 6.95–6.99 (m, 1 H), 7.11–7.16 (m, 5 H), 7.49 (s, 1 H), 8.13–8.18 (m, 1 H), 8.80 (s, 1 H), 10.27 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.0, 45.9, 102.1, 104.4, 104.7, 104.9, 111.2, 112.6, 112.6, 112.72, 112.74, 118.8, 118.9, 127.3, 129.0, 129.11, 130.0, 134.0, 136.7, 140.0, 153.8, 159.90, 159.97, 160.3, 161.9, 162.0, 164.0, 164.1, 166.0, 166.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ −103.53 (d, 1 F), −108.97 (d, 1 F); HRMS (ESI-TOF) m/z calcd for $C_{23}H_{20}F_2N_4OH^+$: 407.1678, found: 407.1672 (Δ = 1.49 ppm). HPLC (1): t = 10.9 min, purity >99%.

2-Benzyl-6-dimethylamino-5-(4-methylbenzamido)-1*H*-benzo[*d*]imidazole (5b-5). Off white solid (68% yield); mp 218–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3 H), 2.73

(s, 6 H), 4.12 (s, 2 H), 7.12–7.22 (m, 5 H,), 7.29 (d, J = 8.1 Hz, 2 H), 7.58 (s, 1 H), 7.82 (d, J = 8.1 Hz, 2 H), 8.82 (s, 1 H), 9.85 (s, 1 H), 11.02 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.7, 35.9, 46.1, 101.8, 110.9, 127.0, 127.1, 128.9, 129.5, 129.8, 131.8, 132.9, 137.06, 139.6, 140.0, 142.5, 153.9, 165.6; HRMS (ESI-TOF) m/z calcd for $C_{24}H_{24}N_4OH^+$: 385.2023, found: 385.2023 (Δ = 0.08 ppm). HPLC (1): t = 11.4 min, purity >96%.

2-Benzyl-6-dimethylamino-5-(4-fluorobenzamido)-1*H*-benzo[*d*]imidazole (5b-6). Yellow solid (89% yield); mp 169–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.71 (s, 6 H), 4.16 (s, 2 H), 7.14–7.25 (m, 7 H), 7.56 (s, 1 H), 7.89–7.93 (m, 2 H), 8.73 (s, 1 H), 9.79 (s, 1 H), 10.62 (bs, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.9, 46.1, 101.8, 111.1, 116.0, 116.2, 127.2, 128.9, 129.0, 129.4, 129.5, 131.79, 131.81, 136.9, 139.7, 153.9, 163.8, 164.3, 166.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ −107.42 (s, 1 F); HRMS (ESI-TOF) m/z calcd for $C_{23}H_{21}FN_4OH^+$: 389.1772, found: 389.1764 (Δ = 2.18 ppm). HPLC (1): t = 13.2 min, purity >99%.

2-Benzyl-6-dimethylamino-5-(4-trifluoromethoxy-benzamido)-1*H*-benzo[*d*]imidazole (5b-7). Light yellow solid (66% yield); mp 196–197 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.71 (s, 6 H), 4.12 (s, 2 H), 7.11–7.19 (m, 5 H,), 7.32 (d, J = 8.5 Hz, 2 H), 7.53 (s, 1 H), 7.94 (d, J = 8.5 Hz, 2 H), 8.76 (s, 1 H), 9.85 (s, 1 H), 11.39 (bs, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 35.6, 45.9, 101.7, 110.8, 117.3, 119.3, 120.9, 121.4, 123.5, 126.9, 128.7, 129.0, 131.7, 133.8, 136.8, 139.6, 139.8, 151.6, 154.0, 163.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ −57.68 (s, 3 F); HRMS (ESI-TOF) m/z calcd for $C_{24}H_{21}F_3N_4O_2H^+$: 455.1619, found: 455.1690 (Δ = −0.42 ppm). HPLC (1): t = 9.7 min, purity >98%.

2-Benzyl-6-dimethylamino-5-(pivalamido)-1*H*-benzo[*d*]imidazole (5b-8). White solid (92% yield); mp >220 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 9 H), 2.65 (s, 6 H), 4.12 (s, 2 H), 7.16–7.18 (m, 5 H), 7.47 (s, 1 H), 8.54 (s, 1 H), 9.29 (s, 1 H), 11.24 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.9, 35.8, 40.1, 45.7, 101.7, 110.5, 126.9, 128.8, 128.9, 129.4, 131.6, 137.1, 139.5, 153.8, 176.7; HRMS (ESI-TOF) m/z calcd for $C_{21}H_{26}N_4OH^+$: 351.2179, found: 351.2172 (Δ = 2.18 ppm). HPLC (1): t = 11.1 min, purity >98%.

2-Benzyl-6-dimethylamino-5-(2-ethylbutanamido)-1*H*-benzo[*d*]imidazole (5b-9). White solid (67% yield); mp 175–177 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, J = 7.4 Hz, 6 H), 1.53–1.62 (m, 2 H), 1.66–1.75 (m, 2 H), 2.09–2.16 (m, 1 H), 2.67 (s, 6 H), 4.24 (s, 2 H), 7.21–7.30 (m, 5 H), 7.54 (s, 1 H), 8.58 (s, 1 H), 8.91 (s, 1 H), 10.09 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.4, 26.3, 36.1, 45.9, 53.1, 101.7, 110.8, 127.2, 129.0, 129.1, 129.5, 131.5, 136.9, 139.4, 139.7, 153.5, 174.4; HRMS (ESI-TOF) m/z calcd for $C_{22}H_{28}N_4OH^+$: 365.2336, found: 365.2331 (Δ = 1.42 ppm). HPLC (1): t = 11.3 min, purity >97%.

5-Benzamido-6-dimethylamino-2-(pent-3-yl)-1*H*-benzo[*d*]imidazole (5c-1). White solid (85% yield); mp 208–210 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.76 (t, J = 7.40 Hz, 6 H), 1.58–1.78 (m, 4 H), 2.54–2.59 (m, 1 H), 2.74 (s, 6 H), 7.52–7.60 (m, 3 H), 7.63 (s, 1 H), 7.97 (dd, J = 7.8, 1.4 Hz, 2 H), 8.91 (s, 1 H), 9.94 (s, 1 H), 10.99 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 27.5, 44.1, 46.1, 101.8, 110.9, 127.2, 129.0, 129.1, 131.3, 131.9, 135.9, 139.3, 140.1, 159.3, 165.8; HRMS (ESI-TOF) m/z calcd for $C_{21}H_{26}N_4OH^+$: 351.2179, found: 351.2173 (Δ = 1.94 ppm). HPLC (1): t = 8.8 min, purity >99%.

5-(3,4-Dimethoxybenzamido)-2-(pent-3-yl)-6-dimethylamino-1*H*-benzo[*d*]imidazole (5c-2). White solid (58% yield); mp 184–186 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77 (t, J = 7.4 Hz, 6 H,), 1.64–1.79 (m, 4 H), 2.56–2.62 (m, 1 H), 2.73 (s, 6 H), 3.97 (s, 6 H), 6.99 (d, J = 8.4 Hz, 1 H), 7.49 (d, J = 8.4 Hz, 1 H,), 7.60 (s, 2 H), 8.80 (s, 1 H), 9.85 (s, 1 H), 10.40 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.3, 27.6, 44.2, 46.1, 56.2, 56.3, 101.5, 110.80, 110.84, 110.9, 119.7, 128.3, 129.2, 131.1, 139.3, 139.8, 149.4, 152.2, 158.9, 165.2; HRMS (ESI-TOF) m/z calcd for $C_{23}H_{30}N_4O_3H^+$: 411.2391, found: 411.2387 (Δ = 0.79 ppm). HPLC (1): t = 14.1 min, purity >79%.

2-(Pent-3-yl)-6-dimethylamino-5-(2-methoxybenzamido)-1*H*-benzo[*d*]imidazole (5c-3). Light brown solid (60% yield); mp 214–215 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.75 (t, J = 7.3 Hz, 6 H), 1.59–1.77 (m, 4 H), 2.53–2.59 (m, 1 H), 2.74 (s, 6 H), 4.08 (s, 3 H), 7.07 (d, J = 7.9 Hz, 1 H), 7.15 (t, J = 7.9 Hz, 1 H), 7.52 (t, J = 7.8 Hz, 1 H), 7.58 (s, 1 H), 8.37 (dd, J = 7.8, 1.8 Hz, 1 H), 9.01 (s, 1 H), 10.60 (s, 1 H), 11.36 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.2, 27.5, 44.1, 46.0, 56.0, 102.6, 110.5, 111.7, 121.5, 122.7, 130.0, 131.1, 132.6, 133.2, 139.7, 140.0, 157.7, 158.95, 163.6; HRMS (ESI-TOF) m/z calcd for $C_{22}H_{28}N_4O_2H^+$: 381.2285, found: 381.228 (Δ = 1.24 ppm).

2-(Pent-3-yl)-6-dimethylamino-5-(4-methylbenzamido)-1*H*-benzo[*d*]imidazole (5c-4). White solid (61% yield); mp >230 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.76 (t, J = 7 Hz, 6 H), 1.61–1.75 (m, 4 H), 2.45 (s, 3 H), 2.54–2.60 (m, 1 H), 2.73 (s, 6 H), 7.36 (d, J = 7.8 Hz, 2 H), 7.61 (s, 1 H), 7.86 (d, J = 7.8 Hz, 2 H), 8.89 (s, 1 H), 9.89 (s, 1 H), 10.91 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.2, 21.7, 27.5, 44.0, 46.1, 101.7, 110.8, 127.2, 129.1, 129.7, 131.3, 133.0, 139.3, 140.0, 142.5, 159.2, 165.8; HRMS (ESI-TOF) m/z calcd for $C_{22}H_{28}N_4OH^+$: 365.2336, found: 365.2332 (Δ = 1.02 ppm). HPLC (1): t = 10.2 min, purity >97%.

5-(2,4-Difluorobenzamido)-6-dimethylamino-2-(pent-3-yl)-1*H*-benzo[*d*]imidazole (5c-5). Beige solid (62% yield); mp 207–210 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (t, J = 7.2 Hz, 6 H), 1.69–1.78 (m, 4 H), 2.59–2.66 (m, 1 H), 2.70 (s, 6 H), 6.96 (t, J = 9.5 Hz, 1 H), 7.05 (t, J = 9.5 Hz, 1 H), 7.60 (s, 1 H), 8.21–8.26 (m, 1 H), 8.83 (s, 1 H), 10.32–10.37 (m, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.3, 27.6, 44.2, 45.9, 102.1, 104.5, 104.7, 105.00, 111.0, 112.5, 112.6, 112.7, 118.97, 119.03, 129.4, 131.0, 133.9, 139.7, 140.1, 159.0, 159.88, 159.98, 160.5, 161.9, 162.0, 164.0, 164.1, 166.05, 166.14; ^{19}F NMR (376 MHz, $CDCl_3$) δ −103.63 (d, 1 F), −108.95 (d, 1 F); HRMS (ESI-TOF) m/z calcd for $C_{21}H_{24}F_2N_4OH^+$: 387.1991, found: 387.1988 (Δ = 0.76 ppm). HPLC (1): t = 8.98 min, purity >98%.

6-Dimethylamino-5-(4-fluorobenzamido)-2-(pent-3-yl)-1*H*-benzo[*d*]imidazole (5c-6). White solid (44% yield); mp 192–195 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77 (t, J = 7.4 Hz, 6 H), 1.63–1.78 (m, 4 H), 2.58–2.62 (m, 1 H), 2.71 (s, 6 H), 7.19–

7.25 (m, 2 H), 7.59 (s, 1 H), 7.95–7.98 (m, 2 H), 8.83 (s, 1 H), 9.86 (s, 1 H), 11.10 (bs, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.2, 27.5, 44.1, 46.1, 101.8, 110.7, 116.0, 116.2, 128.8, 129.4, 129.5, 131.3, 131.89, 131.91, 139.3, 140.0, 159.3, 164.0, 164.5, 166.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ −107.54 (s, 1 F); HRMS (ESI-TOF) *m*/*z* calcd for $C_{21}H_{25}FN_4OH^+$: 369.2085, found: 369.2082 (Δ = 0.84 ppm). HPLC (1): *t* = 10.7 min, purity >99%.

6-Dimethylamino-2-(pent-3-yl)-5-(4-trifluoromethoxybenzamido)-1*H*-benzo[*d*]imidazole (5c-7). White solid (76% yield); mp 194–198 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80 (t, *J* = 7.4 Hz, 6 H,), 1.67–1.80 (m, 4 H), 2.58–2.64 (m, 1 H), 2.74 (s, 6 H), 7.38 (d, *J* = 8.5 Hz, 2 H), 7.64 (s, 1 H), 8.00 (d, *J* = 8.5 Hz, 2 H), 8.79 (s, 1 H), 9.9 (s, 1 H), 10.26 (bs, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.3, 27.6, 44.2, 46.2, 101.6, 111.1, 119.5, 121.2, 121.6, 129.1, 131.1, 134.2, 139.4, 140.1, 151.8159.1, 164.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ −57.70 (s, 3 F); HRMS (ESI-TOF) *m*/*z* calcd for $C_{22}H_{25}F_3N_4O_2H^+$: 435.2002, found: 435.2 (Δ = 0.62 ppm). HPLC (1): *t* = 10.8 min, purity >97%.

5-(4-*tert*-Butylbenzamido)-2-isopropyl-6-dimethylamino-1*H*-benzo[*d*]imidazole (5d-1). White solid (87% yield); mp 198–200 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30 (d, *J* = 6.1 Hz, 6 H), 1.38 (s, 9 H), 2.72 (s, 6 H), 3.01–3.03 (m, 1 H), 7.55–7.62 (m, 3 H), 7.90 (d, *J* = 8.1 Hz, 2 H), 8.90 (s, 1 H), 9.96 (s, 1 H), 10.87 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.7, 29.1, 31.4, 35.2, 46.2, 101.8, 110.9, 126.0, 127.1, 129.3, 131.6, 133.0, 139.4, 139.7, 155.5, 160.7, 165.9; HRMS (ESI-TOF) *m*/*z* calcd for $C_{23}H_{30}N_4OH^+$: 379.2492, found: 379.2495 (Δ = 1.89 ppm).

6-Dimethylamino-2-tetrahydropyranyl-5-(4-trifluoromethoxybenzamido)-1*H*-benzo[*d*]imidazole (5h-1). White solid (75% yield); mp 210–213 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.96–2.07 (m, 4 H), 2.73 (s, 6 H), 3.09–3.14 (m, 1 H), 3.42–3.47 (m, 2 H), 4.00–4.03 (m, 2 H), 7.40 (d, *J* = 8.1 Hz, 2 H), 7.59 (bs, 1 H), 8.00 (d, *J* = 8.1 Hz, 2 H), 8.80 (s, 1 H), 9.88 (bs, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.5, 36.1, 46.1, 67.7, 102.5, 110.5, 119.5, 121.2, 121.6, 123.6, 129.0, 129.3, 134.1, 139.8, 151.90, 151.92, 158.0, 164.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ −57.66 (s, 3 F); HRMS (ESI-TOF) *m*/*z* calcd for $C_{22}H_{23}F_3N_4O_3H^+$: 449.1795, found: 449.1792 (Δ = 0.57 ppm). HPLC (3): *t* = 12.3 min, purity >98%.

6-Dimethylamino-5-(2-fluoro-4-trifluoromethoxybenzamido)-2-tetrahydropyranyl-1*H*-benzo[*d*]imidazole (5h-2). White solid (73% yield); mp 213–214 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.59–2.15 (m, 4 H), 2.71 (s, 6 H), 3.12–3.20 (m, 1 H), 3.47–3.54 (m, 2 H), 4.03–4.07 (m, 2 H), 7.12 (dd, *J* = 14.5, 1.4 Hz, 1 H), 7.22 (d, *J* = 11.1 Hz, 1 H), 7.58 (s, 1 H), 8.26 (t, *J* = 11.1 Hz, 1 H), 8.82 (s, 1 H), 10.33 (d, *J* = 14.5 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.5, 36.1, 46.0, 67.7, 109.1, 109.4, 117.1, 119.1, 120.9, 121.0, 121.7, 129.8, 133.6, 134.0, 140.2, 152.3, 152.4, 157.8, 159.3, 160.20, 160.24, 161.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ −57.66 (s, 3 F), −109.38 (s, 1 F); HRMS (TOF) *m*/*z* calcd for $C_{22}H_{22}F_4N_4O_3H^+$: 467.1701, found: 467.1699 (Δ = 0.47 ppm). HPLC (4): *t* = 14.7 min, purity >97%.

5-Butoxycarbonylamino-2-cyclopentyl-6-dimethylamino-1*H*-benzo[*d*]imidazole (6a. To a solution of **4** (100 mg, 0.4 mmol) in DCM (2 mL), carbonyldiimidazole (73.2 mg, 0.45 mmol) was added. The reaction mixture was refluxed for 4 h, followed by the addition of *n*-BuOH (66 μL), and the reaction mixture was refluxed overnight. After the completion of the reaction, the reaction mixture was washed with $NaHCO_3$ (50 mL × 2) and water (50 mL × 2). The organic layer was separated, dried over $MgSO_4$, filtered and concentrated on a rotary evaporator to give a crude product. The crude product was purified by column chromatography on alumina, using 1 : 1 AcOEt : hexanes as eluent to afford **6a** as a white solid (98 mg, 70% yield); mp 174–175 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, *J* = 7.4 Hz,3 H), 1.39–1.47 (m, 2 H), 1.63–1.71 (m, 4 H), 1.75–1.82 (m, 2 H), 1.87–1.95 (m, 2 H), 2.07–2.13 (m, 2 H), 2.62 (s, 6 H), 3.23 (quintet, *J* = 8.4 Hz,1 H,), 4.18 (t, *J* = 6.7 Hz, 2 H), 7.5 (s, 1 H), 8.19 (s, 2 H), 9.99 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 19.3, 25.7, 31.3, 32.5, 39.8, 45.8, 65.1, 99.7, 110.7, 129.5, 131.3, 138.8, 154.4, 158.7; HRMS (ESI-TOF) *m*/*z* calcd for $C_{19}H_{28}N_4O_2H^+$: 345.2285, found: 345.2279 (Δ = 1.87 ppm). HPLC (2): *t* = 22.1 min, purity >96%.

Compounds **6b–6d**, **6f**, **6h**, **11a–11e** and **20a–20h** were synthesized in a similar manner and characterized.

2-Benzyl-5-butoxycarbonylamino-6-dimethylamino-1*H*-benzo[*d*]imidazole (6b). Light brown solid (44% yield); mp 193–196 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (t, *J* = 9.2 Hz, 3 H), 1.37–1.51 (m, 2 H), 1.63–1.70 (m, 2 H), 2.63 (s, 6 H), 4.14–4.19 (m, 4 H), 7.21–7.30 (m, 5 H,), 7.44 (s, 1 H), 8.15 (s, 1 H), 9.71 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 19.3, 31.3, 36.1, 45.8, 65.1, 99.6, 111.1, 127.4, 129.1, 129.2, 130.1, 136.7, 139.1, 153.1, 154.3; HRMS (ESI-TOF) *m*/*z* calcd for $C_{21}H_{26}N_4O_2H^+$: 367.2129, found: 367.2122 (Δ = 1.82 ppm). HPLC (1): *t* = 10.3 min, purity >98%.

5-Butoxycarbonylamino-6-dimethylamino-2-(3-pentyl)-1*H*-benzo[*d*]imidazole (6c). Light brown solid (57% yield); mp 208–210 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83 (t, *J* = 7.4 Hz, 6 H,), 0.95 (t, *J* = 7.3 Hz, 3 H), 1.40–1.46 (m, 2 H), 1.65–1.70 (m, 2 H), 1.72–1.81 (m, 4 H), 2.62 (s, 6 H), 2.67–2.69 (m, 1 H), 4.19 (t, *J* = 6.7 Hz, 2 H), 7.25 (s, 1 H), 7.51 (s, 1 H), 8.19 (s, 1 H), 10.18 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.3, 14.0, 19.3, 27.7, 31.2, 44.2, 45.8, 65.2, 99.8, 110.8, 129.4, 131.0, 138.7, 138.8, 154.5, 158.3; HRMS (ESI-TOF) *m*/*z* calcd for $C_{19}H_{30}N_4O_2H^+$: 347.2442, found: 347.2434 (Δ = 2.12 ppm).

5-Butoxycarbonylamino-6-dimethylamino-2-isopropyl-1*H*-benzo[*d*]imidazole (6d). Brown solid (79% yield); mp 112–115 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.4 Hz, 3 H), 1.34–1.41 (m, 2 H), 1.58–1.64 (m, 8 H), 2.61 (s, 6 H), 3.76 (quintet, *J* = 7.0 Hz, 1 H), 4.09 (t, *J* = 6.7 Hz, 2 H), 7.64 (s, 1 H), 8.01 (s, 1 H), 8.48 (s, 1 H), 14.7 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.9, 19.3, 21.2, 27.8, 31.1, 45.3, 65.5, 102.6, 106.3, 126.4, 128.4, 132.8, 142.0, 153.7, 157.8; HRMS (ESI-TOF) *m*/*z* calcd for $C_{17}H_{26}N_4O_2H^+$: 319.2129, found: 319.2121 (Δ = 2.27 ppm). HPLC (3): *t* = 7.3 min, purity >99%.

5-Butoxycarbonylamino-6-dimethylamino-2-thienyl-1*H*-benzo[*d*]imidazole (6f). White solid (57% yield); mp 175–177 °C ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, *J* = 7.4 Hz, 3 H), 1.38–1.45 (m, 2 H,), 1.64–1.70 (m, 2 H), 2.64 (s, 6 H), 4.18 (t, *J* = 6.7

Hz, 2 H), 7.03–7.05 (m, 1 H), 7.37 (dd, J = 5.0, 1.1 Hz, 1 H), 7.53–7.56 (m, 2 H), 8.23 (s, 1 H), 8.26 (s, 1 H), 10.32 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 19.3, 31.2, 45.8, 65.3, 99.7, 111.2, 125.9, 127.8, 128.1, 130.6, 131.7, 133.7, 139.5, 139.7, 147.2, 154.5. HRMS (ESI-TOF) m/z calcd for $C_{18}H_{22}N_4O_2SH^+$: 359.1536, found: 359.1537 (Δ = −0.13 ppm). HPLC (1): t = 8.8 min, purity >98%.

5-Butoxycarbonylamino-6-dimethylamino-2-tetrahydropyranyl-1*H*-benzo[*d*] imidazole (6h). White solid (59% yield); mp 176–178 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.96 (t, J = 7.4 Hz, 3 H,), 1.41–1.48 (m, 2 H), 1.67–1.72 (m, 2 H), 2.00–2.03 (m, 4 H), 2.64 (s, 6 H), 3.14–3.20 (m, 1 H), 3.52–3.57 (m, 2 H), 4.06–4.09 (m, 2 H), 4.20 (t, J = 6.7 Hz, 2 H,), 7.50 (s, 1 H), 8.17 (s, 1 H), 8.25 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 19.4, 31.3, 31.5, 35.9, 45.8, 65.25, 67.7, 130.2, 139.4, 154.4, 156.8; HRMS (ESI-TOF) m/z calcd for $C_{19}H_{28}N_4O_3H^+$: 361.2234, found: 361.2229 (Δ = 1.56 ppm). HPLC (4): t = 14.8 min, purity > 98%.

5-(4-*tert*-Butylbenzamido)-6-dimethylamino-2-(*N*-methyl-*N*-phenylamino)-methyl-1*H*-benzo[*d*]imidazole (10e-1). Yellow solid (75% yield); mp 111–112 °C ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.37 (s, 9 H), 2.75 (s, 6 H), 3.02 (s, 3 H), 4.68 (s, 2 H), 6.76–6.84 (m, 3 H), 7.23 (dd, J = 8.9, 7.3 Hz, 2 H), 7.52 (d, J = 8.5 Hz, 2 H), 7.60 (bs, 1 H), 7.87 (d, J = 8.5 Hz, 2 H), 8.70 (bs, 1 H), 9.76 (bs, 1 H), 9.89 (bs, 1 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 31.2, 35.0, 39.6, 45.8, 52.6, 101.5, 110.8, 113.5, 118.4, 125.8, 126.9, 129.5, 130.0, 132.6, 139.8, 149.5, 152.9, 155.1, 165.0; HRMS (ESI-TOF) m/z calculated for $C_{28}H_{33}N_5OH^+$: 456.2758, found 456.2757 (Δ 0.13 ppm). HPLC (3): t = 6.5 min, purity >99%.

6-Dimethylamino-5-(4-methylbenzamido)-2-(*N*-methyl-*N*-phenylamino)methyl-1*H*-benzo[*d*]imidazole (10e-2). Yellowish brown solid (50% yield); mp 178–181 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.43 (s, 3 H), 2.72 (s, 6 H), 2.99 (s, 3 H), 4.64 (s, 2 H), 6.66–6.86 (m, 3 H), 7.12–7.24 (m, 2 H), 7.29 (d, J = 8.1 Hz, 2 H), 7.51 (bs, 1 H) 7.82 (d, J = 8.1 Hz, 2 H), 8.72 (s, 1 H), 9.71 (bs, 1 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 21.5, 39.5, 45.8, 52.4, 102.6, 109.9, 113.4, 118.2, 127.0, 129.4, 129.5, 129.7, 132.6, 139.8, 142.1, 149.5, 153.0, 165.1; HRMS (ESI-TOF) m/z calculated for $C_{25}H_{27}N_5OH^+$: 414.2288, found 414.229 (Δ −0.29 ppm). HPLC (3): t = 7.7 min, purity >99%.

6-Dimethylamino-5-(2,4-difluorobenzamido)-2-(*N*-methyl-*N*-phenylamino)methyl-1*H*-benzo[*d*]imidazole (10e-3). Yellow solid (49% yield); mp 130–134 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.71 (s, 6 H), 2.98 (s, 3 H), 4.63 (s, 2 H), 6.63–6.82 (m, 3 H), 6.85–7.05 (m, 2 H), 7.18–7.21 (m, 2 H), 7.51 (bs, 1 H), 8.07–8.26 (m, 1 H), 8.76 (bs, 1 H) 10.18–10.22 (m, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 39.3, 45.5, 52.2, 104.0, 104.2, 104.3, 104.5, 112.2, 112.3, 112.4, 113.1, 117.9, 118.4, 118.5, 129.1, 129.6, 133.5, 133.6, 133.7, 139.9, 149.3, 153.3, 159.5, 159.6, 159.90, 159.93, 161.5, 161.6, 163.6, 163.7, 165.6, 165.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ −103.61 (d, 1 F), −109.01 (d, 1 F); HRMS (ESI-TOF) m/z calculated for $C_{24}H_{23}F_2N_5OH^+$: 436.1943, found 436.1946 (Δ −0.55 ppm). HPLC (3): t = 6.8 min, purity >99%.

6-Dimethylamino-2-(*N*-methyl-*N*-phenylamino)methyl-5-(4-methoxyphenyl)-ethanamido-1*H*-benzo[*d*]imidazole (10e-4). Yellow solid (29% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.36 (s, 6 H), 3.00 (s, 3 H), 3.66 (s, 2 H), 3.83 (s, 3 H), 4.65 (s, 2 H), 6.73–6.84 (m, 3 H), 6.93 (d, J = 8.5 Hz, 2 H), 7.14–7.26 (m, 4 H), 7.38 (bs, 1 H), 8.58 (bs, 1 H), 8.85 (bs, 1 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 39.4, 44.4, 45.2, 52.5, 55.3, 113.4, 114.3, 118.3, 126.7, 129.4, 129.5, 130.8, 139.4, 149.5, 152.7, 159.0, 169.6; HRMS (ESI-TOF) m/z calculated for $C_{26}H_{29}N_5O_2H^+$: 444.2394, found 444.2374 (Δ 4.6 ppm). HPLC (3): t = 8.9 min, purity >99%.

6-Dimethylamino-2-(*N*-methyl-*N*-phenylamino)methyl-5-(4-propylbenzamido)-1*H*-benzo[*d*]imidazole (10e-5). Yellow solid (66% yield); mp 164–166 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3 H), 1.67 (sextet, J = 7.5 Hz, 2 H), 2.64 (t, J = 7.5 Hz, 2 H), 2.71 (s, 6 H), 2.94 (s, 3 H), 4.59 (s, 2 H), 6.65–6.78 (m, 3 H), 7.13–7.16 (m, 2 H), 7.24–7.31 (m, 2 H), 7.55 (bs, 1 H), 7.82 (d, J = 8.2 Hz, 2 H), 8.72 (bs, 1 H), 9.72 (bs, 1 H), 10.80 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 13.7, 24.2, 37.8, 39.3, 45.7, 52.2, 101.8, 110.4, 113.3, 117.9, 126.9, 129.2, 129.5, 132.7, 139.6, 146.7, 149.4, 153.0, 165.0; HRMS (ESI-TOF) m/z calculated for $C_{27}H_{31}N_5OH^+$: 442.2601, found 442.2602 (Δ −0.12 ppm). HPLC (3): t = 67.0 min, purity >99%.

6-Dimethylamino-5-(2-fluoro-4-trifluoro-methoxybenzamido)-2-(*N*-methyl-*N*-phenylamino)methyl-1*H*-benzo[*d*]imidazole (10e-6). Beige solid (73% yield); mp 73–75 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.72 (s, 6 H), 2.99 (s, 3 H), 4.66 (s, 2 H), 6.73–6.80 (m, 3 H), 7.07 (d, J = 12.2 Hz, 1 H), 7.14 (d, J = 8.9 Hz, 1 H), 7.17–7.23 (m, 2 H), 7.52 (s, 1 H), 8.20 (t, J = 8.9 Hz, 1 H), 8.75 (s, 1 H), 10.23 (d, J = 12.2 Hz, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 39.4, 45.6, 52.3, 103.7, 108.7, 108.9, 109.2, 113.3, 116.7, 117.1, 118.3, 119.1, 120.5, 120.6, 121.2, 123.2, 129.4, 129.8, 132.5, 133.5, 133.5, 137.7, 140.1, 149.3, 151.8, 151.9, 153.2, 159.2, 159.6, 159.7, 161.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ −57.85 (s, 3 F), −109.42 (s, 1 F); HRMS (ESI-TOF) m/z calculated for $C_{25}H_{23}F_4N_5O_2H^+$: 502.1861, found 502.1859 (Δ 0.23 ppm). HPLC (3): t = 6.4 min, purity >95%.

5-Butoxycarbonylamino-6-dimethylamino-2-*N*,*N*-dipropylaminomethyl-1*H*-benzo[*d*]imidazole (11a). Light brown solid (54% yield); mp 176–178 °C; ^{1}H NMR (500 MHz, CDCl3) δ 0.80 (t, J = 7.3 Hz, 6 H), 0.89 (t, J = 7.5 Hz, 3 H), 1.31–1.48 (m, 6 H), 1.59–1.65 (m, 2 H), 2.30–2.46 (m, 4 H), 2.58 (s, 6 H), 3.75 (s, 2 H), 4.12 (t, J = 6.7 Hz, 2 H), 7.43 (bs, 1 H), 8.12 (s, 1 H), 8.15 (s, 1 H), 9.52 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 11.8, 13.8, 19.1, 20.3, 31.1, 45.6, 53.1, 56.8, 64.9, 99.4, 110.8, 129.9, 130.5, 138.6, 138.9, 154.0, 154.2; HRMS (ESI-TOF) m/z calculated for $C_{21}H_{35}N_5O_2H^+$: 390.2864, found 390.2864 (Δ −0.09 ppm). HPLC (3): t = 6.0 min, purity >95%.

5-Butoxycarbonylamino-6-dimethylamino-2-morpholin-1-ylmethyl-1*H*-benzo[*d*]imidazole (11b). Light brown solid (54% yield); mp 146–147 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (t, J = 7.3 Hz, 3 H), 1.41–1.46 (m, 2 H), 1.64–1.76 (m, 2 H), 2.45–2.60 (m, 4 H), 2.66 (s, 6 H), 3.54–3.76 (m, 4 H), 3.78 (s, 2 H), 4.20 (t, J = 6.7 Hz, 2 H), 7.52 (bs, 1 H), 8.21 (bs, 2 H), 9.72 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.0, 19.3, 31.3, 45.8, 54.0, 57.1, 65.1, 67.1, 99.6, 111.2, 130.4, 131.1, 138.7, 139.1, 151.5,

154.3; HRMS (ESI-TOF) *m*/*z* calculated for $C_{19}H_{29}N_5O_3H^+$: 376.2343, found 376.2347 (*Δ* −0.89 ppm). HPLC (3): *t* = 8.1 min, purity >99%.

5-Butoxycarbonylamino-6-dimethylamino-2-piperidin-1-ylmethyl-1*H*-benzo[*d*]imidazole (11c). Beige solid (63% yield); mp 160–161 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 0.97 (t, *J* = 7.3 Hz, 3 H), 1.39–1.52 (m, 4 H), 1.58–1.60 (m, 4 H), 1.66–1.74 (m, 2 H), 2.47 (bs., 4 H), 2.66 (s, 6 H), 3.73 (s, 2 H), 4.20 (t, *J* = 6.7 Hz, 2 H), 7.51 (bs, 1 H), 8.20 (bs, 1 H), 9.73 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 13.8, 19.1, 23.9, 26.0, 31.1, 45.6, 54.9, 57.2, 64.9, 99.4, 110.9, 129.0, 130.0, 130.8, 138.7, 152.5, 154.0; HRMS (ESI-TOF) *m*/*z* calculated for $C_{20}H_{31}N_5O_2H^+$: 374.2551, found 374.2551 (*Δ* −0.23 ppm). HPLC (3): *t* = 6.1 min, purity >99%.

5-Butoxycarbonylamino-6-dimethylamino-2-pyrrolidin-1-ylmethyl-1*H*-benzo[*d*]imidazole (11d). Light brown solid (41% yield); mp 135–137 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 0.96 (t, *J* = 7.5 Hz, 3 H), 1.38–1.50 (m, 2 H), 1.63–1.73 (m, 2 H), 1.81–1.83 (m, 4 H), 2.55–2.72 (m, 10 H), 3.91 (s, 2 H), 4.19 (t, *J* = 6.7 Hz, 2 H), 7.50 (bs, 1 H), 8.18 (bs, 2 H), 10.19 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 14.0, 19.3, 23.9, 31.3, 45.8, 54.1, 54.6, 65.1, 99.7, 111.1, 130.1, 131.2, 139.0, 152.8, 154.2; HRMS (ESI-TOF) *m*/*z* calculated for $C_{19}H_{29}N_5O_2H^+$: 360.2394, found 360.2396 (*Δ* −0.58 ppm). HPLC (3): *t* = 6.2 min, purity >95%.

5-Butoxycarbonylamino-6-dimethylamino-2-(*N*-methyl-*N*-phenylamino)methyl-1*H*-benzo[*d*]imidazole (11e). Light brown solid (59% yield); ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 0.96 (t, *J* = 7.5 Hz, 3 H), 1.36–1.54 (m, 2 H), 1.57–1.77 (m, 2 H), 2.65 (s, 6 H), 3.00 (s, 3 H), 4.16 (t, *J* = 6.7 Hz, 2 H), 4.63 (s, 2 H), 6.70–6.87 (m, 3 H), 7.18–7.26 (m, 2 H), 7.45 (bs, 1 H), 8.13 (bs, 1 H), 8.19 (bs, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 13.9, 19.3, 31.2, 39.7, 45.7, 52.6, 65.1, 99.9, 110.7, 113.4, 118.4, 129.6, 130.1, 131.1, 139.1, 139.3, 149.6, 152.7, 154.2; HRMS (ESI-TOF) *m*/*z* calculated for $C_{22}H_{29}N_5O_2H^+$: 396.2394, found 396.2395 (*Δ* −0.16 ppm). HPLC (3): *t* = 6.6 min, purity >99%.

5-Butoxycarbonylamino-6-dimethylamino-2-(methylthio)-1*H*-benzo[*d*]imidazole (20a). White solid (66% yield); mp 159–160 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 0.97 (t, *J* = 7.3 Hz, 3 H), 1.34–1.55 (m, 2 H), 1.58–1.82 (m, 2 H), 2.64 (bs, 6 H), 2.72 (s, 3 H), 4.28–4.30 (m, 2 H), 7.52 (bs, 1 H), 8.30 (s, 1 H), 8.28 (s, 1 H), 11.30 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 13.7, 14.8, 19.1, 30.9, 45.6, 65.3, 99.2, 109.7, 129.1, 132.7, 138.4, 139.3, 150.8, 154.4; HRMS (ESI-TOF) *m*/*z* calculated for $C_{15}H_{22}N_4O_2SH^+$: 323.1536, found 323.1533 (*Δ* 0.86 ppm). HPLC (2): *t* = 4.4 min, purity >95%.

2-Benzylsulfanyl-5-butyloxycarbonylamino-6-dimethylamino-1*H*-benzo[*d*]imidazole (20b). White solid (77% yield); mp 126–127 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 0.95 (t, *J* = 7.3 Hz, 3 H), 1.41–1.44 (m, 2 H), 1.57–1.78 (m, 2 H), 2.66 (s, 6 H), 4.26 (t, *J* = 6.7 Hz, 2 H), 4.52 (s, 2 H), 7.20–7.31 (m, 3 H), 7.32–7.40 (m, 2 H), 7.56 (bs, 1 H), 8.30 (bs, 2 H), 10.84 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 13.7, 19.1, 30.9, 37.4, 45.6, 65.3, 99.2, 110.0, 127.5, 128.6, 128.9, 129.4, 132.4, 136.8, 138.7, 139.4, 149.0, 154.3; HRMS (ESI-TOF) *m*/*z* calculated for $C_{21}H_{26}N_4O_2SH^+$: 399.1849, found 399.1848 (*Δ* 0.25 ppm). HPLC (3): *t* = 3.97 min, purity >95%.

5-Butoxycarbonylamino-6-dimethylamino-2-(isopropylsulfanyl)-1*H*-benzo[*d*]-imidazole (20c). White solid (62% yield); mp 165–166 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 0.98 (t, *J* = 7.3 Hz, 3 H), 1.39 (d, *J* = 7.02 Hz, 6 H), 1.43–1.54 (m, 2 H), 1.71–1.75 (m, 2 H), 2.65 (s, 6 H), 3.91–3.97 (m, 1 H), 4.30 (t, *J* = 6.4 Hz, 2 H), 7.53 (bs, 1 H), 8.32 (bs, 2 H), 11.05 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 13.8, 19.2, 23.6, 31.0, 38.9, 45.7, 65.4, 99.3, 110.1, 129.5, 132.2, 138.8, 139.9, 148.7, 154.4; HRMS (ESI-TOF) *m*/*z* calculated for $C_{17}H_{26}N_4O_2SH^+$: 351.1849, found 351.1846 (*Δ* 0.79 ppm). HPLC (3): *t* = 3.9 min, purity >99%.

5-Butoxycarbonylamino-6-dimethylamino-2-(ethylsulfanyl)-1*H*-benzo[*d*]imidazole (20d). Beige solid (76% yield); mp 136–138 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 0.98 (t, *J* = 7.2 Hz, 3 H), 1.40 (t, *J* = 7.5 Hz, 3 H), 1.47–1.51 (m, 2 H), 1.62–1.81 (m, 2 H), 2.65 (s, 6 H), 3.28 (q, *J* = 7.5 Hz, 2 H), 4.29 (bs, 2 H), 7.53 (bs, 1 H), 8.30 (s, 2 H), 10.96 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 13.8, 15.0, 19.1, 27.1, 31.0, 45.6, 65.4, 99.2, 109.9, 129.2, 132.4, 138.5, 139.5, 149.5, 154.4; HRMS (ESI-TOF) *m*/*z* calculated for $C_{16}H_{24}N_4O_2SH^+$: 337.1693, found 337.1692 (*Δ* 0.35 ppm). HPLC (3): *t* = 6.5 min, purity >95%.

5-Butoxycarbonylamino-6-dimethylamino-2-(phenylsulfanyl)-1*H*-benzo[*d*]imidazole (20e). White solid (77% yield); mp 172–173 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 0.95 (t, *J* = 7.3 Hz, 3 H), 1.33–1.51 (m, 2 H), 1.57–1.77 (m, 2 H), 2.63 (s, 6 H), 4.21 (t, *J* = 6.7 Hz, 2 H), 7.14–7.25 (m, 3 H), 7.39–7.45 (m, 3 H), 8.20 (bs, 1 H), 8.28 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 13.7, 19.1, 30.9, 45.4, 65.1, 77.2, 127.9, 129.3, 130.0, 131.2, 131.8, 139.2, 146.4, 154.2; HRMS (ESI-TOF) *m*/*z* calculated for $C_{20}H_{24}N_4O_2SH^+$: 385.1693, found 385.1691 (*Δ* 0.52 ppm). HPLC (3): *t* = 4.5 min, purity >95%.

5-Butoxycarbonylamino-2-(cyclohexylsulfanyl)-6-dimethylamino-1*H*-benzo[*d*]imidazole (20f). White solid (73% yield); mp 129–132 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 0.98 (t, *J* = 7.3 Hz, 3 H), 1.19–1.31 (m, 1 H), 1.31–1.42 (m, 2 H), 1.42–1.53 (m, 4 H), 1.56–1.59 (m, 1 H), 1.66–1.80 (m, 4 H), 2.01–2.15 (m, 2 H), 2.66 (s, 6 H), 3.65–3.81 (m, 1 H), 4.30 (s, 2 H), 7.54 (bs, 1 H), 8.29 (bs, 2 H), 10.55 (bs, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 13.8, 19.1, 25.5, 25.8, 31.0, 33.6, 45.6, 46.7, 65.3, 99.1, 110.2, 129.5, 132.1, 138.6, 139.6, 148.3, 154.4; HRMS (TOF) *m*/*z* calculated for $C_{20}H_{30}N_4O_2SH^+$: 391.2162, found 391.2161 (*Δ* 0.23 ppm). HPLC (3): *t* = 12.6 min, purity >99%.

5-Butoxycarbonylamino-2-(cyclohexylmethylsulfanyl)-6-dimethylamino-1*H*-benzo[*d*]imidazole (20g). White solid (61% yield); mp 109–110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.91–1.06 (m, 5 H), 1.07–1.29 (m, 3 H),1.37–1.55 (m, 2 H), 1.56–1.79 (m, 6 H), 1.84–1.86 (m, 2 H), 2.59 (bs, 1 H), 2.65 (s, 5H), 3.22 (d, *J* = 7.0 Hz, 2 H), 4.31 (t, *J* = 6.6 Hz, 2 H), 7.52 (s, 1 H), 8.27 (bs, 1 H), 8.31 (s, 1 H), 10.81 (s, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 13.8, 19.1, 25.9, 26.2, 31.0, 32.5, 37.7,39.7, 45.6, 65.4, 99.1, 109.8, 129.1, 132.4, 138.4, 139.4, 150.3, 154.4; HRMS (ESI-TOF) *m*/*z* calculated for $C_{21}H_{32}N_4O_2SH^+$: 405.2319, found 405.2317 (*Δ* 0.43 ppm). HPLC (3): *t* = 18.6 min, purity >96%.

***In silico* predictions of pharmacological properties with pkCSM.** All of the tested compounds were drawn and

translated into the SMILES format using the ChemDraw Prime 16.0 program. Then, the compounds in the SMILES format were processed and analyzed by the pkCSM online tool.[28] Results are summarized in Table S1 in the ESI.†

3D-QSAR. All 2,4,6-trisubstituted benzimidazoles we have studied are fully characterized and MIC values were determined by the standard assay method. All these benzimidazoles are assumed to bind the same putative allosteric site in a similar manner. For the 3D-QSAR analysis, the MIC value of each compound was converted to the negative logarithmic scale (−log pM). Then, all benzimidazole molecules were drawn using the ChemDraw Prime 16.0 program and saved individually as MOL files. Each compound's structural and biological information was transferred to the Schödinger's AutoQSAR program.[32] The original dataset was divided into training and test sets by given ratio. The sampling process of these training and test sets occurs randomly selected, while the ratio between training and test sets remains the same. With given information along with a default set of descriptors, a large number of models were built using the AutoQSAR. Then, their predictive powers were estimated using test set to choose 10 best models. All the predictions were proceeded using all top ten models and the predicted values were simply arithmetically averaged from these 10 different models has been used.

Molecular docking. The 2,4,6-trisubstituted benzimidazole molecules were drawn as 2D structures using ChemDraw Prime 16.0. Then, the Avogadro program was used to build 3D structures and to optimize the internal energy of each compounds. The force field chosen to perform during the energy optimization was Merck Molecule Force Field (MMFF94). Once this was completed, individual compounds were saved as MOL2 files. Molecular docking was performed with the AutoDock 4.2 program (with default settings) and each compound was docked on the *Mtb*-FtsZ homology model, reproduced from the published Li's model.[29] Analysis of predicted binding poses and their associated scores was performed with the UCSF Chimera program. The results on **20g** are shown in Fig. S1 in the ESI.†

Bacterial strains and growth.[14,33] For evaluation of drug sensitivity, Mtb H37Rv was grown in 7H9 media containing 10% oleic acid/albumin/catalase (OADC) enrichment and 0.05% Tween-80 and assessed at mid log phase growth.

Antibacterial activity. Protocol A:[25] The minimum inhibitory concentration (MIC) was determined by the microplate Alamar Blue assay (MABA) as described previously.[25] Stock solutions of the compounds were prepared in DMSO and were serially diluted 2-fold in 96-well microtiter plates. *Mtb* H37Rv was added to each well to an OD_{600} of 0.005. The plates were incubated for 6 days at 37 °C. Alamar Blue (Invitrogen) 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^{-1}) of compound that inhibited bacterial growth and prevented a color change.

Protocol B:[27] Each compound was dissolved in DMSO at a final concentration of 12 mg mL^{-1} and serial dilutions were performed to generate test concentrations ranging from 32 mg mL^{-1} to 0.488 ng mL^{-1}. M. tuberculosis strain H37Rv at the midlogarithmic stage of growth (OD_{580} = 0.4) was diluted 1 : 100, and 0.1 mL was added to each well of a 96-well plate along with 0.1 mL of test compound solution. After 6 days of incubation at 37 °C, Alamar blue (Invitrogen) reagent was added along with 12.5 mL of 20% Tween 80 (Sigma) to evaluate bacterial cell viability. Plates were scanned 24 h later at 570 nm with a reference wavelength of 600 nm utilizing a Biotek Instruments ELX 808. Inoculum control wells of untreated H37Rv were used to create a survival-inhibition curve with each assay. Rifampicin was used as a positive control (MIC = 0.0125–0.05 μg mL^{-1}).

Cell growth inhibition assay. Human liver cancer cell line HepG2 was cultured and maintained in Roswell Park Memorial Institute growth medium RPMI1640 (with L-glutamine) supplemented by 10% fetal bovine serum (FBS) and 1% penicillin (PenStrip). Normal human lung fibroblast cell line WI-38 was cultured and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented by 10% fetal bovine serum (FBS) and 1% penicillin (PenStrip). Cells were plated at a density of 2500 cells per well in 96-well plates and allowed to attach overnight and reach 70–90% confluency. Benzimidazoles were dissolved in sterilized DMSO and further diluted with either RPMI-1640 medium or DMEM medium to reach the concentration of 20 uM. The concentration of DMSO was kept at 0.5% (*i.e.*, 99.5% culture medium and 0.5% DMSO). After 72 h of incubation with test compounds, supernatant was aspirated to remove the medium. Then MTT solution was added into each well. Following 4 h incubation avoiding light, the remaining MTT solution was aspirated. The dye was dissolved in DMSO with 100 μL per well. Then, the 96-well plate was shaken for 10 min. Optical density (OD) was measured at 570 nm. The percentage inhibition was calculated as follows: % inhibition = 1 − (OD of compound/OD of DMSO control) × 100.

Author contributions

K. H.: experimental design and synthesis, data collections, data analysis, manuscript preparation; S. T. design, synthesis, data collections, partial manuscript preparation; S. K.: 3D-QSAR model building and analysis, pkCSM analysis, data collections, partial manuscript preparation; M. A.: molecular docking analysis, partial 3D-QSAR model building; L. C.: cytotoxicity assay; S. E. K., E. S. and R. R. MABA assay, MIC determination; R. A. S. and N. C. supervision of MABA assays. I. O.: research design, supervision of whole project, manuscript preparation.

Conflicts of interest

The authors declare no competing financial interest.

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