



Original article

Benzimidazole-based compounds kill *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis remains one of the deadliest infectious diseases, killing 1.4 million people annually and showing a rapid increase in cases resistant to multiple drugs. New antibiotics against tuberculosis are urgently needed. Here we describe the design, synthesis and structure–activity relationships of a series of benzimidazole-based compounds with activity against *Mycobacterium tuberculosis* (*Mtb*) in a replicating state, a physiologically-induced non-replicating state, or both. Compounds **49**, **67**, **68**, **69**, **70**, and **72**, which shared a 5-nitrofuranyl moiety, exhibited high potency and acceptable selectivity indices (SI). As illustrated by compound **70** (MIC₉₀ < 0.049 µg/mL, SI > 512), the 5-nitrofuranyl group was compatible with minimal cytotoxicity and good intra-macrophage killing, although it lacked non-replicating activity when assessed by CFU assays. Compound **70** had low mutagenic potential by SOS Chromotest assay, making this class of compounds good candidates for further evaluation and target identification.

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1. Introduction

Tuberculosis (TB), which is caused predominantly by *Mycobacterium tuberculosis* (*Mtb*), is the leading cause of death from a curable infectious disease, and has been identified by the World Health Organization (WHO) as one of the three priority diseases for drug research and development [1]. One-third of the world's population is estimated to be infected with *Mtb*, and in 2010 there were an estimated 8.8 million incident cases of TB globally, equivalent to 128 cases per 100,000 people.

The current TB treatment regimen requires patients to take a combination of four drugs, namely, isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB), for 2 months

and two of them (INH and RIF) for an additional 4 months. When *Mtb* is resistant to INH and RIF, optimal treatment with existing drugs averages 25–27 months with a total of 7 other drugs, a 21% rate of adverse effects serious enough to interrupt therapy, and an apparent short-term cure rate of only 54% [2]. The requirement for such prolonged treatment may be due in part to the presence of subpopulations of bacteria that are non-replicating (NR-*Mtb*) [3,4]. The lengthy course of treatment contributes to poor patient compliance, a high incidence of side effects, and the emergence of drug-resistant *Mtb* strains [5]. Most current drugs target replicating *Mtb* (R-*Mtb*), with only RIF, PZA and fluoroquinolones showing activity against NR-*Mtb* [6–8].

For these reasons, we have conducted an anti-*Mtb* whole-cell screening campaign using both R-*Mtb* and NR-*Mtb*, as recently described for a compound collection of known drugs [9]. *Mtb* was rendered non-replicating by a combination of four stresses designed to mimic an activated macrophage or a granuloma: mild acidity (pH 5.0), hypoxia (1% O₂), a fatty acid as the carbon source (butyrate), and mild nitrosative stress (a flux of nitric oxide). Here, we applied these same screening methods to a privileged-structure library centered on novel benzimidazoles. Compounds **1**, **2**, and **3**

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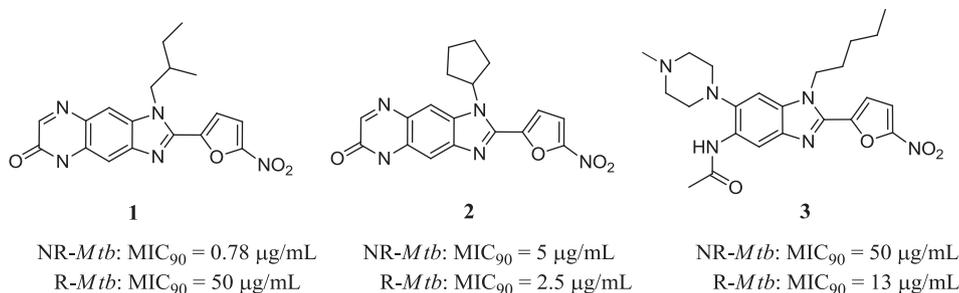


Fig. 1. Structures of active compounds in our benzimidazole library.

(Fig. 1) were identified to be potent actives against NR-*Mtb*, R-*Mtb*, or both. The common structural features of these active compounds were the benzimidazole core and a 5-nitrofuran substituent.

The benzimidazole moiety has long been recognized as a “privileged substructure” for drug design, and has been incorporated into many drugs with various therapeutic applications, including antiviral [10], antifungal [11], antitumor [12], and antihistaminic [13] agents. The benzimidazole scaffold has also shown antimycobacterial activity *in vitro* [14,15], making it a promising starting point for the discovery of new anti-TB drugs. Below we report the design, synthesis and biological actions of derivatives of three benzimidazole-based scaffolds (I, II, III, Fig. 2).

2. Chemistry

2.1. The synthesis of 1*H*-imidazo[4,5-*g*]quinoxalin-6(5*H*)-ones (Scaffold I)

1*H*-Imidazo[4,5-*g*]quinoxalin-6(5*H*)-ones (Scaffold I) were synthesized as outlined in Scheme 1 [16]. In the presence of diisopropylethylamine (DIPEA), one of the fluorine atoms of 1,5-difluoro-2,4-dinitrobenzene (DFDNB) was substituted by an equivalent α -amino acid methyl or ethyl ester hydrochlorate to afford compound 4, and then the other fluorine atom of DFDNB was substituted by a series of primary amines to give compound 5 in excellent yields. After reduction of the two nitro-groups using 10% palladium on activated carbon and subsequent acid-catalyzed cyclization, the expected 1*H*-imidazo[4,5-*g*]quinoxalin-6(5*H*)-ones I (1, 2, 6–27) were obtained with total yields of 15–35%.

2.2. The synthesis of 1,2,5,6-tetrasubstituted benzimidazoles (Scaffold II)

1,2,5,6-Tetrasubstituted benzimidazoles (Scaffold II) were obtained according to the synthetic route shown in Scheme 2. In the first step, secondary amines or alcohols were used as nucleophiles to replace quantitatively one fluorine atom of DFDNB in the presence of organic base. Then the remaining fluorine was substituted by primary amines to obtain compound 29. Simultaneous reduction of the aromatic *m*-dinitro group to generate 30 was achieved by use of HCOONH₄ and Pd/C or sodium hydrosulfite in a mixture of THF and EtOH. 2,4,5-Benzenetriamine 30 was found to be readily

oxidized. We herein adopted a one-pot strategy without purifying compound 30 [16], in which the reaction solution was directly filtered into another reaction vessel containing the aldehyde (R³CHO) to yield compound 31. The free aromatic amino group of compound 31 was modified *via* acylation with different acyl chlorides, isocyanates, or isothiocyanates to produce the expected 1,2,5,6-tetrasubstituted benzimidazoles II (3, 32–74).

2.3. The synthesis of 6*H*-imidazo[4',5':3,4]benzo[1,2-*d*]thiazole (Scaffold III)

6*H*-imidazo[4',5':3,4]benzo[1,2-*d*]thiazole (Scaffold III) was synthesized as outlined in Scheme 3. The intermediate 75 was obtained by reaction of compound 31 with potassium thiocyanate (KSCN) and bromine (Br₂) in acetic acid. Oxidation of the free aromatic amino group on compound 75 with sodium nitrite (NaNO₂) in acetic acid and water resulted in the desired compound 76. Acylation of 75 with acyl chloride produced the expected compounds 77 and 78.

3. Results and discussion

3.1. Chemistry

Twenty-four compounds of scaffold I were synthesized (Table 1). These compounds contained diversified substituent groups in which R¹ was derived from various methyl esters of natural amino acids, including alanine, glycine, leucine, isoleucine, phenylalanine, and valine. R² was introduced by various primary amines bearing alkyl, aryl, arylalkyl, and cycloalkyl groups. Four compounds (1, 6, 7 and 9) showed antibacterial activity, with MIC₉₀ values ranging from 0.78 to 6.3 μg/mL (Table 1). However, these active compounds were also toxic to human hepatoma HepG2 cells.

Eighteen compounds of scaffold II were then synthesized (Table 2). The diversity of R¹ was introduced by using primary amines; R² was from secondary amines or alcohols; R³ was kept constant as a 5-nitrofuran pharmacophore that appears in some antibiotics [17–19] although the 5-nitrofuran moiety may have off-target toxicity mediated by human aldehyde dehydrogenase (ALDH) 2 [20]; and R⁴ was derived from acyl chlorides, isocyanates, or isothiocyanates (Table 2).

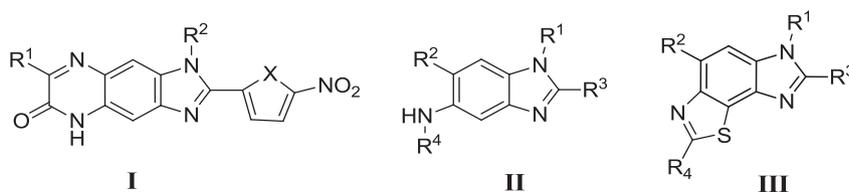
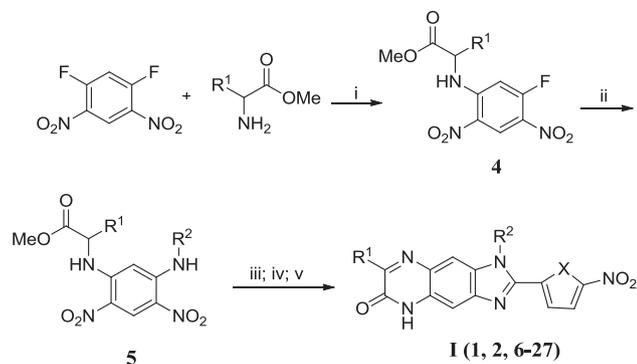
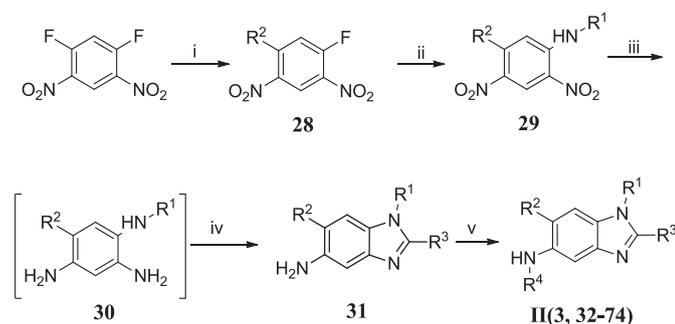


Fig. 2. Benzimidazole-based scaffolds.



Scheme 1. Synthesis of 1*H*-imidazo[4,5-*g*]quinoxalin-6(5*H*)-ones^a. ^aReagents and conditions: (i) DIPEA, THF, rt; (ii) R²NH₂, DIPEA, THF, rt; (iii) Pd/C–HCOONH₄, THF–EtOH, rt; (iv) 5-nitrofuraldehyde or 5-nitrothiophenolaldehyde, dioxane, THF–EtOH, 5% AcOH, rt; (v) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), reflux.



Scheme 2. Synthesis of 1,2,5,6-tetrasubstituted benzimidazoles^a. ^aReagents and conditions: (i) secondary amine or hydroxyalkyl, base, THF, rt; (ii) R¹NH₂, DIPEA, THF, rt; (iii) reducing agent, THF–EtOH; (iv) R³CHO, dioxane, THF–EtOH, 5% AcOH, rt; (v) RCOCl or RNCO, or RNCS, Et₃N, CH₂Cl₂, rt.

Table 2 indicates that compounds **33** (NR-*Mtb*: MIC₉₀ = 6.3 μg/mL; R-*Mtb*: MIC₉₀ = 1.6 μg/mL; SI(R) = 8.0) and **34** (NR-*Mtb*: MIC₉₀ = 13 μg/mL; R-*Mtb*: MIC₉₀ = 6.3 μg/mL; SI(R) > 8.0) showed activity against both R-*Mtb* and NR-*Mtb* with improved SI values. Compound **35** was only active against R-*Mtb*. While these data suggested that presence of the 5-nitrofuran pharmacophore at the 2-position of the benzimidazole core was effective, we also tested placement at the 6-position of the benzimidazole core (Table 3). These efforts resulted in compounds **49** (NR-*Mtb*:

MIC₉₀ = 3.1 μg/mL; R-*Mtb*: MIC₉₀ = 0.2 μg/mL; SI(R) = 64), **50** (NR-*Mtb*: MIC₉₀ = 0.78 μg/mL; R-*Mtb*: MIC₉₀ < 0.098 μg/mL; SI(R) > 16) and **59** (NR-*Mtb*: MIC₉₀ = 0.39 μg/mL; R-*Mtb*: MIC₉₀ < 0.098 μg/mL; SI(R) > 32) with significantly improved MIC and SI values from scaffold **II** (Table 3). Similar compounds with a thiazolobenzimidazole core were inactive and toxic to HepG2 cells (Table 4).

Subsequently, eight modified analogs of **49**, **50**, and **59** were designed and synthesized (Table 5). The introduced substituents included an aliphatic chain (R¹), such as *tert*-butyl, isopropyl, methyl, 1-ethoxybutanyl and 1,3-dimethylbutanyl, morpholine or morpholine mimics such as thiomorpholine for R², and methyl for R³. All of the new compounds were highly active against *Mtb* with significantly improved SI values, especially compound **70** (NR-*Mtb*: MIC₉₀ = 0.20 μg/mL; R-*Mtb*: MIC₉₀ < 0.049 μg/mL; SI(R) > 512) (Table 5). For these compounds, the 5-nitrofuran pharmacophore introduced at the 6-position of benzimidazole was particularly useful when R¹ was an aliphatic chain, R² was a morpholinyl or thiomorpholinyl substitution, and R³ was a methyl group. The activity of selected compounds (**49**, **70**) was confirmed with wild-type (wt) *Mtb*, and minimal inhibitory concentrations (MICs) were within 2–4 fold of those seen with the Δ *panCD1lysA* double auxotroph strain as described [9].

3.2. Biology

3.2.1. Cidal activity of benzimidazoles against wild-type *M. tuberculosis* (*Mtb*)

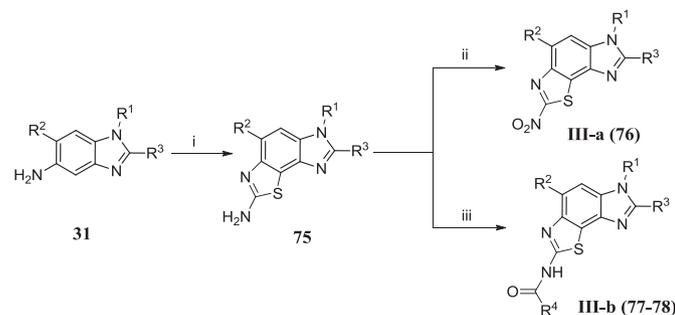
Our screening assay has the potential to assign provisional NR activity to a compound whose R activity is potent enough to be manifest after dilution from the NR screening phase into the R outgrowth phase. To definitively characterize activity as being manifest against NR-*Mtb*, R-*Mtb* or both, we tested selected benzimidazoles' (**49** and **70**) cidal activity in both conditions by a CFU-based assay (Fig. 3). **49** was cidal against replicating wt *Mtb*, reducing viable bacilli 1.4 log₁₀ at 0.74 μg/mL (Fig. 3B) but was inactive against NR-*Mtb* by a CFU assay (Fig. 3C). In one experiment, compound **70** likewise killed R-*Mtb* but was inactive against NR-*Mtb*.

3.2.2. Benzimidazoles kill wild-type *Mtb* infecting human macrophages

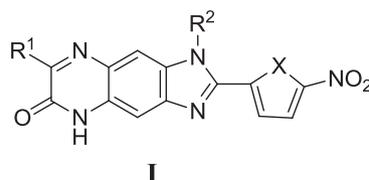
Given their cidal activity *in vitro* against wild-type *Mtb*, we next explored the activity of these compounds against *Mtb* in primary human macrophages, cells that provide an important niche for *Mtb* *in vivo*. Macrophages were differentiated *in vitro* from normal donors' blood monocytes and immunologically activated as reported [21]. At 2.5 μg/mL (8× MIC), compound **49** killed 1.9 log₁₀ of wt *Mtb* in macrophages when compared to the DMSO control (Fig. 4A). This killing activity was similar to that of rifampicin at 2.5 μg/mL (30× MIC), the most potent of the front-line drugs used to treat TB. Based on these results for compound **49**, we synthesized the compounds in Table 5. Given its improved potency and SI, compound **70** was also tested in human macrophages, and concentration-dependent bacterial killing was observed (Fig. 4B).

3.2.3. Selectivity of benzimidazoles

Compound **70** was tested against several different species of Gram-positive and Gram-negative bacteria to evaluate its selectivity. No activity was observed against *Escherichia coli* (Fig. 5A), *Salmonella* spp (Fig. 5B), *Staphylococcus aureus* (Fig. 5C) or *Pseudomonas aeruginosa* (Fig. 5D) at concentrations up to 10 μg/mL (≥250× MIC for *Mtb*). In contrast, both **49** and **70** were active against *Mycobacterium smegmatis* with MICs comparable to those



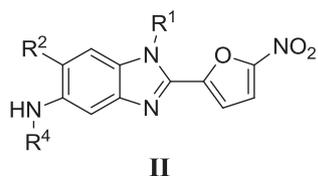
Scheme 3. Synthesis of 4,6,7-trisubstituted-2-nitro-6*H*-imidazo[4',5':3,4]benzo[1,2-*d*]thiazole or *N*-(4,6,7-trisubstituted-6*H*-imidazo[4',5':3,4]benzo[1,2-*d*]thiazol-2-yl)acetamide^a. ^aReagents and conditions: (i) KSCN, Br₂, HOAc, rt; (ii) NaNO₂, HOAc:H₂O = 1:1; (iii) RCOCl, Et₃N, CH₂Cl₂, rt.

Table 1Scaffold **I** compounds and their antibacterial activity.

Compound	R ¹	R ²	X	MIC ₉₀ (μg/mL)	MIC ₉₀ (μg/mL)	Toxicity ^a	SI(R) ^b
				NR-Mtb	R-Mtb		
1	H	*	O	0.78	50	1.6	0.031
2^c	H	*	O	5.0	2.5	ND ^d	ND ^d
6	H	*	O	1.6	6.3	1.6	0.26
7	* -	*	O	0.78	1.6	0.78	0.50
8	*	*	S	>50	>50	50	<1.0
9	H	*	O	6.3	6.3	6.3	1.0
10	H	*	O	>50	>50	1.6	<0.031
11	H	*	O	>10	>10	1.3	<0.13
12	H	*	O	>50	>50	>50	NC ^e
13	H	*	O	>50	>50	>50	NC ^e
14	* -	*	O	>10	>10	10	<1.0
15	*	*	O	>50	>50	>50	NC ^e
16	*	*	O	>50	>50	>50	NC ^e
17	*	*	O	>50	>50	>50	NC ^e
18	*	*	O	>50	>50	0.78	<0.016
19	*	*	O	>50	>50	3.1	<0.062
20	*	*	O	>25	>25	>25	NC ^e
21	*	*	O	>50	>50	25	<0.50
22	*	*	O	>50	>50	1.6	<0.031
23	*	*	O	>50	>50	25	<0.50
24	*	*	O	>25	>25	25	<1.0
25	*	*	O	>10	>10	>10	NC ^e
26	*	*	O	>50	>50	25	<0.50
27	*	*	O	>25	>25	3.1	<0.12

^a Toxicity = lethal dose (μg/mL) for 50% of HepG2 cells (LD₅₀).^b SI = selectivity index, lethal dose for 50% of HepG2 cells (LD₅₀)/MIC for R-Mtb. All values are rounded to two significant digits.^c This compound was tested in 96 well format vs. 384 well format for all the other compounds.^d ND: not determined.^e NC = cannot be calculated.

Table 2
Scaffold **II** compounds and their antibacterial activity.



Compound	R ¹	R ²	R ⁴	MIC ₉₀ (μg/mL)	MIC ₉₀ (μg/mL)	Toxicity ^a	SI(R) ^b
				NR- <i>Mtb</i>	R- <i>Mtb</i>		
3				50	13	6.3	0.50
32				>50	>50	25	<0.50
33				6.3	1.6	13	8.0
34				13	6.3	>50	>8.0
35				>50	6.3	>50	>8.0
36				>50	>50	>50	NC ^c
37^d				>10	>10	>10	NC ^c
38^d				>25	>25	25	<1.0
39				>50	>50	>50	NC ^c
40				13	13	13	1.0
41				>50	>50	50	<1.0
42				>50	>50	1.6	<0.031
43				>50	6.3	1.6	0.25

Table 2 (continued)

Compound	R ¹	R ²	R ⁴	MIC ₉₀ (μg/mL)	MIC ₉₀ (μg/mL)	Toxicity ^a	SI(R) ^b
				NR- <i>Mtb</i>	R- <i>Mtb</i>		
44				50	50	13	0.25
45				>50	>50	25	<0.50
46				>50	>50	6.3	<0.13
47				>50	25	6.3	0.25
48				50	6.3	3.1	0.50

^a Toxicity = lethal dose (μg/mL) for 50% of HepG2 cells (LD₅₀).

^b SI = selectivity index, lethal dose for 50% of HepG2 cells (LD₅₀)/MIC for R-*Mtb*. All values are rounded to two significant digits.

^c NC = cannot be calculated.

^d 5-nitrothiophenolaldehyde was used instead of 5-nitrofurfuraldehyde.

against *Mtb*: <0.039 μg/mL and 0.31–0.63 μg/mL, for **70** and **49** (Fig. 5E), respectively.

3.2.4. Evaluation of mutagenic potential of benzimidazoles

Mutagenicity is a concern in drug development efforts involving a nitrofuranyl moiety [22,23]. A convenient method for assessing the potential of a chemical compound to induce genetic damage is the SOS Chromotest [24–26]. This test was developed as a complement or alternative to the traditional Ames test for genotoxicity, and is a good screening tool given its quick turnaround time. We selected potent study compounds **67**, **70** for SOS Chromotest and clinical drugs metronidazole and isoniazid were evaluated as controls in the absence and presence of the metabolic activator (S9). The induction factors are shown in Fig. 6A (–S9) and Fig. 6B (+S9). Metronidazole and **67** (induction factor, IF > 2) were genotoxic either in the presence or in the absence of an exogenous metabolizing system from rat liver S9-mix. Isoniazid and **70** were classified as marginally genotoxic (IF = 1.5–2) in the absence of S9-mix [27]. The LOEC (lowest observed effect concentration) value of **70** was 333.3 μg/mL, which far exceeded its MIC value and was comparable to the LOEC value of isoniazid [28]. In the presence of S9-mix, **70** revealed no genotoxicity (IF < 1.5) while isoniazid was marginally genotoxic [27]. These results demonstrated a very low mutagenic potential of **70**.

4. Conclusions

In summary, a series of novel benzimidazole-based compounds were designed and synthesized in this study. A structure–activity relationship study evaluating activity against replicating and non-replicating *M. tuberculosis* (*Mtb*) was carried out and potent compounds with acceptable selectivity indices (SI) were discovered. Several nitrofuranyl benzimidazoles were selectively bactericidal for mycobacteria and killed *Mtb* in primary human macrophages, making them good leads for further study. Compound **70** potently killed replicating *Mtb*, although it lacked non-replicating activity when assessed by a CFU assay. Data further indicated that **70** had a very low mutagenic potential by SOS Chromotest assay, making this class of

compounds good candidates for further evaluation and target identification.

5. Experimental protocols

5.1. Biology

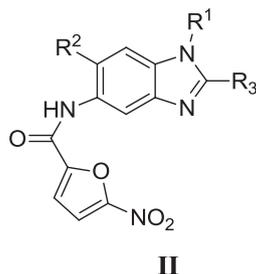
5.1.1. Bacterial strains and growth conditions

Mycobacterial strains used were *M. tuberculosis* H37Rv, which was grown in Middlebrook 7H9 supplemented with 0.2% glycerol, 0.02% tyloxapol, and 10% ADN (albumin, dextrose, NaCl) supplement. *M. tuberculosis* strain mc²6220 Δ panCD Δ lysA was grown in Middlebrook 7H9 supplemented with 0.5% glycerol, 0.02% tyloxapol, 10% OADC (oleic acid, albumin, dextrose, catalase), 0.05% casein hydrolyzate(CAS) amino acids, 240 μg/mL L-lysine and 24 μg/mL pantothenate. For R conditions, bacteria were plated in their respective growth media at 37 °C with 20% O₂ and 5% CO₂. NR media included 0.5 g of MgSO₄, 0.05 g of ferric ammonium citrate, 0.5 g of KH₂PO₄, 0.5% BSA, 0.085% NaCl, 0.02% tyloxapol, 50 μM butyrate and 0.5 mM NaNO₂ at pH 5.0. 240 μg/mL L-lysine and 24 μg/mL pantothenate was added for mc²6220 Δ panCD Δ lysA. For NR conditions, bacteria were incubated at 37 °C with 1% O₂ and 5% CO₂. *M. smegmatis* was grown in Middlebrook 7H9 supplemented with 0.2% glycerol, 0.02% tyloxapol at 37 °C in a shaking incubator. Other bacterial strains used included *Salmonella* spp (Luria Broth), uropathogenic *E. coli* (Luria Broth), *P. aeruginosa* PAO1 (Luria Broth) and *Staphylococcus aureus* ATCC 29213 (Mueller–Hinton broth).

5.1.2. Testing activity of compounds

High throughput screening (HTS) was performed as described before [9]. Briefly, for R activity, log phase bacteria were diluted to an OD 0.01 and added (50 μL/well) to 384 well plates. Compound (500 nL in DMSO) was added (≤1% final DMSO concentration). Plates were incubated for 7 days, after which the OD₅₈₀ was read. To test NR activity, log phase bacteria were washed 2× with PBS/0.02% tyloxapol, resuspended in NR medium (as described above) and cells at a 0.1 OD₅₈₀ were dispensed into 384 well plates at 15 μL/well. Compound (150 nl) was added. Plates were incubated for 3 days at 37 °C with 1% O₂ and 5% CO₂. An outgrowth stage was performed by addition of 60 μL of R medium and incubation at

Table 3
Scaffold II compounds and their antibacterial activity.



Compound	R ¹	R ²	R ³	MIC ₉₀ (μg/mL)	MIC ₉₀ (μg/mL)	Toxicity ^a	SI(R) ^b
				NR- <i>Mtb</i>	R- <i>Mtb</i>		
49	*	*	* CH ₃	3.1	0.20	13	64
50	*	*	H	0.78	<0.098	1.6	>16
51	*	*	*	>50	>50	25	<0.50
52	*	*	* CH ₃	25	6.3	0.78	0.12
53	*	*	*	50	25	3.1	0.12
54 ^c	*	*	H	>50	>50	50	<1.0
55 ^c	*	*	H	>50	>50	>50	NC ^d
56	*	*	H	13	1.6	3.1	2.0
57	*	*	*	25	13	3.1	0.25
58	*	*	*	>50	25	3.1	0.12
59	*	*	* CH ₃	0.39	<0.098	3.1	>32
60	*	*	*	>50	>50	>50	NC ^d
61	* -	*	*	>50	>50	6.3	<0.12
62	*	*	*	>10	>10	>10	NC ^d
63 ^c	*	*	*	>50	>50	>50	NC ^d
64	*	*	*	>50	>50	3.1	<0.062
65	*	*	*	>25	>25	13	<0.50
66	*	*	*	25	13	1.6	0.12

^a Toxicity = lethal dose (μg/mL) for 50% of HepG2 cells (LD₅₀).

^b SI = selectivity index, lethal dose for 50% of HepG2 cells (LD₅₀)/MIC for R-*Mtb*. All values are rounded to two significant digits.

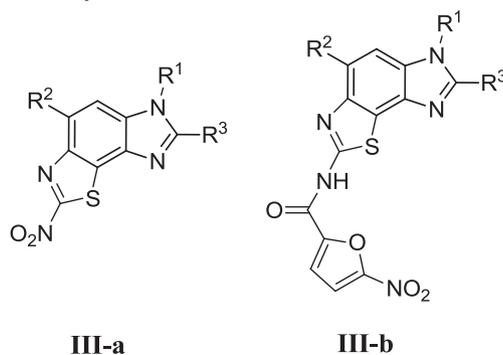
^c 5-nitrofuran was replaced by furan.

^d NC = cannot be calculated.

37 °C with 20% O₂ and 5% CO₂. OD₅₈₀ was read after 7 days. Initial screening was done using the mc²6220 Δ panCD Δ lysA strain. For select compounds, activity was confirmed against wt *Mtb*. MIC₉₀ (minimal inhibitory concentration) is the measure of activity of compound against *Mtb*, defined as 90% inhibition of growth when compared to vehicle control (DMSO).

To assay colony-forming units (CFU), wt *Mtb* (200 μL at OD 0.01 or 0.1 for R and NR conditions, respectively) was plated in 96 well plates and incubated with compound. After 7 days, bacteria were diluted and plated onto Middlebrook 7H11 agar plates containing 0.5% glycerol and 10% OADC. Colonies were counted after 3 weeks.

Table 4
Scaffold **III-a** and **III-b** compounds and their antibacterial activity.

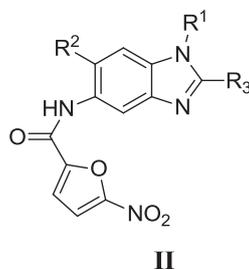


Compound	R ¹	R ²	R ³	MIC ₉₀ (μg/mL)		Toxicity ^a	SI(R) ^b
				NR- <i>Mtb</i>	R- <i>Mtb</i>		
76			* CH ₃	>50	50	3.1	0.062
77				>50	>50	1.6	<0.031
78			* CH ₃	>50	>50	0.78	<0.016

^a Toxicity = lethal dose (μg/mL) for 50% of HepG2 cells (LD₅₀).

^b SI = selectivity index, lethal dose for 50% of HepG2 cells (LD₅₀)/MIC for R-*Mtb*. All values are rounded to two significant digits.

Table 5
Compounds **49**, **50**, and **59** analogs.



Compound	R ¹	R ²	R ³	MIC ₉₀ (μg/mL)		Toxicity ^a	SI(R) ^b
				NR- <i>Mtb</i>	R- <i>Mtb</i>		
67			* CH ₃	0.78	<0.049	>25	>512
68			* CH ₃	0.39	<0.098	6.3	>64
69			* CH ₃	0.78	0.098	>25	>256
70			* CH ₃	0.20	<0.049	>25	>512
71			H	0.20	<0.10	1.6	>16
72	* CH ₃		H	0.63	0.039	>10	>256
73	* CH ₃		* CH ₃	0.20	<0.10	3.1	>32
74			* CH ₃	6.3	0.78	25	32

^a Toxicity = lethal dose (μg/mL) for 50% of HepG2 cells (LD₅₀).

^b SI = selectivity index, lethal dose for 50% of HepG2 cells (LD₅₀)/MIC for R-*Mtb*. All values are rounded to two significant digits.

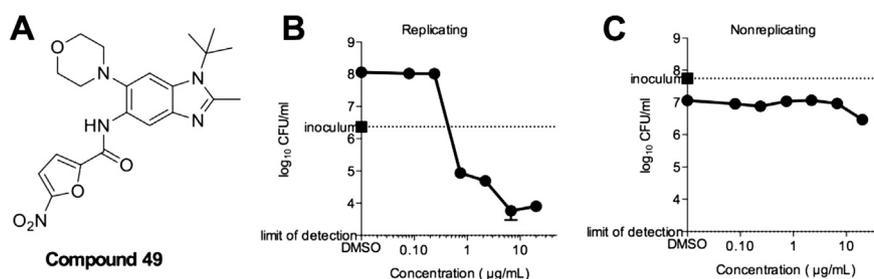


Fig. 3. Cidal activity of **49** against wt *Mtb*. Compound **49** (A) or vehicle (DMSO) was incubated for 7 days with wt *Mtb* was plated in R conditions (200 μ L of 5×10^6 /mL) (B) or NR conditions (200 μ L of 5×10^7 /mL) (C). After 7 days bacteria were resuspended and enumerated on 7H11 agar plates containing OADC and glycerol. Colonies were counted 2.5–3 weeks later. Results are means \pm SD of triplicates in one experiment representative of 2 (B) or 3 (C) independent experiments.

5.1.3. Toxicity assays

HepG2 cells, a human hepatocellular line, were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) supplemented with pyruvate, glutamine and non-essential amino acids. Three thousand cells per well in 384-well plates were incubated with compound ($\leq 1\%$ final DMSO concentration) for 2 days. Viability of HepG2 cells was measured by quantification of ATP content with the CellTiter-Glo kit from Promega. LD₅₀ was defined as the concentration of compound that caused a 50% decrease in the ATP signal when compared to the DMSO control. Selectivity index (SI) was calculated as LD₅₀ divided by MIC₉₀.

5.1.4. Macrophage assays

Human macrophages were generated as described [21]. Briefly, monocytes were isolated from healthy donors under an institutional review board-approved protocol and differentiated in 40% human plasma with 0.5 ng/mL GM-CSF and 0.5 ng/mL TNF- α under 10% O₂, 5% CO₂. After 2 weeks, cells were stimulated with 5 ng/mL IFN- γ and infected with wt *Mtb* at multiplicity of infection (MOI) of 0.1. 24 h later, compound was added. After 7 days of co-incubation, macrophages were visually inspected for viability and lysed with 0.55% triton-X. Lysates were plated for CFU.

5.1.5. Evaluation of mutagenic potential of benzimidazoles

Experimental compounds were screened for microbial mutagenicity activity using the SOS Chromotest in the presence and absence of an Aroclor 1254-induced rat-liver metabolic (S9) activation system. Dilutions of all test agents were prepared in DMSO. Reference compounds 4NQO (4-nitroquinoline oxide) and 2AA (2-

Aminoanthracene) were included as direct acting and metabolism dependent positive controls. All the test agents were dissolved in DMSO at 5 mg/mL and from this eight serial 1:3 dilutions were prepared.

In a 96-well plate 50 μ L of LB dosing stock was added to 50 μ L of bacteria with or without S9 (+/–S9). For each dose concentration duplicate cultures +/–S9 were tested for mutagenicity as well as a toxicity control. The plates were incubated at 37 °C with 100 rpm shaking for 2 h, protected from light.

Test agent mutagenicity and toxicity were determined by measuring reporter gene activity. Bacteria were lysed and enzyme activity determined after reaction with a colorimetric substrate. 100 μ L/well of ONPG Chromobuffer was added to all mutagenicity plates and 100 μ L/well of PNPP Chromobuffer was added to cytotoxicity plates. The plates were returned to the orbital shaker for 60 min (mutagenicity) or 30 min (toxicity) then absorbance was determined at 450 nm.

For each dose concentration the ratio of β -galactosidase units to alkaline phosphatase units was calculated. This ratio reflects the induction of the SOS repair gene. β -galactosidase activity is directly proportional to the extent of SOS gene induction and this value is divided by the constitutively expressed alkaline phosphatase activity to correct for inhibition of protein synthesis resulting from cytotoxicity.

$$R = \frac{OD_{450\beta} \times T_P}{OD_{450P} \times T_\beta}$$

where OD_{450 β} and T _{β} = absorbance and reaction times for the β -galactosidase assay and OD_{450P} and T_P = absorbance and reaction

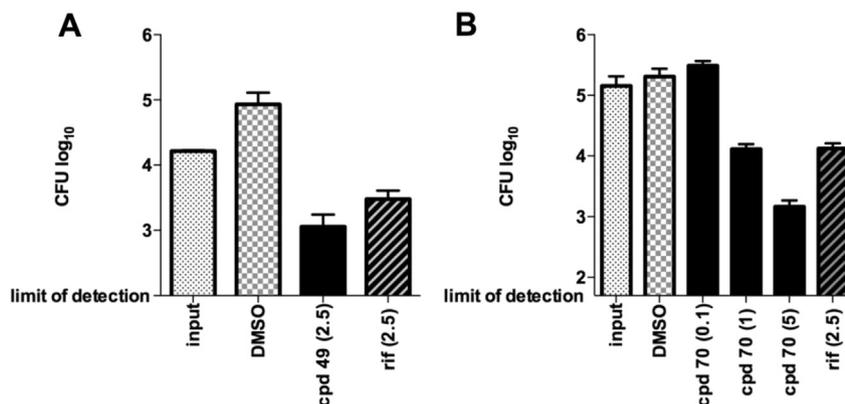


Fig. 4. Activity of **49** and **70** against wt *Mtb* infecting human macrophages. Differentiated and activated primary human macrophages were infected with *Mtb* at a multiplicity of infection (MOI) of 0.1 and treated with or without compounds for 7 days. Concentrations indicated in parenthesis are in μ g/mL. At the end of 7 days, macrophages showed no morphologic evidence of toxicity. Excess compound was removed by washing $2 \times$ with PBS, and macrophages were lysed to release intracellular bacteria. *Mtb* was enumerated by plating serial dilutions on 7H11 agar plates containing OADC and glycerol. Colonies were counted after 2.5–3 weeks incubation. Input was calculated by plating bacterial content of macrophages lysed at 24 h post infection, prior to adding any compound. Results are means \pm SD of triplicates of one of 3 similar experiments.

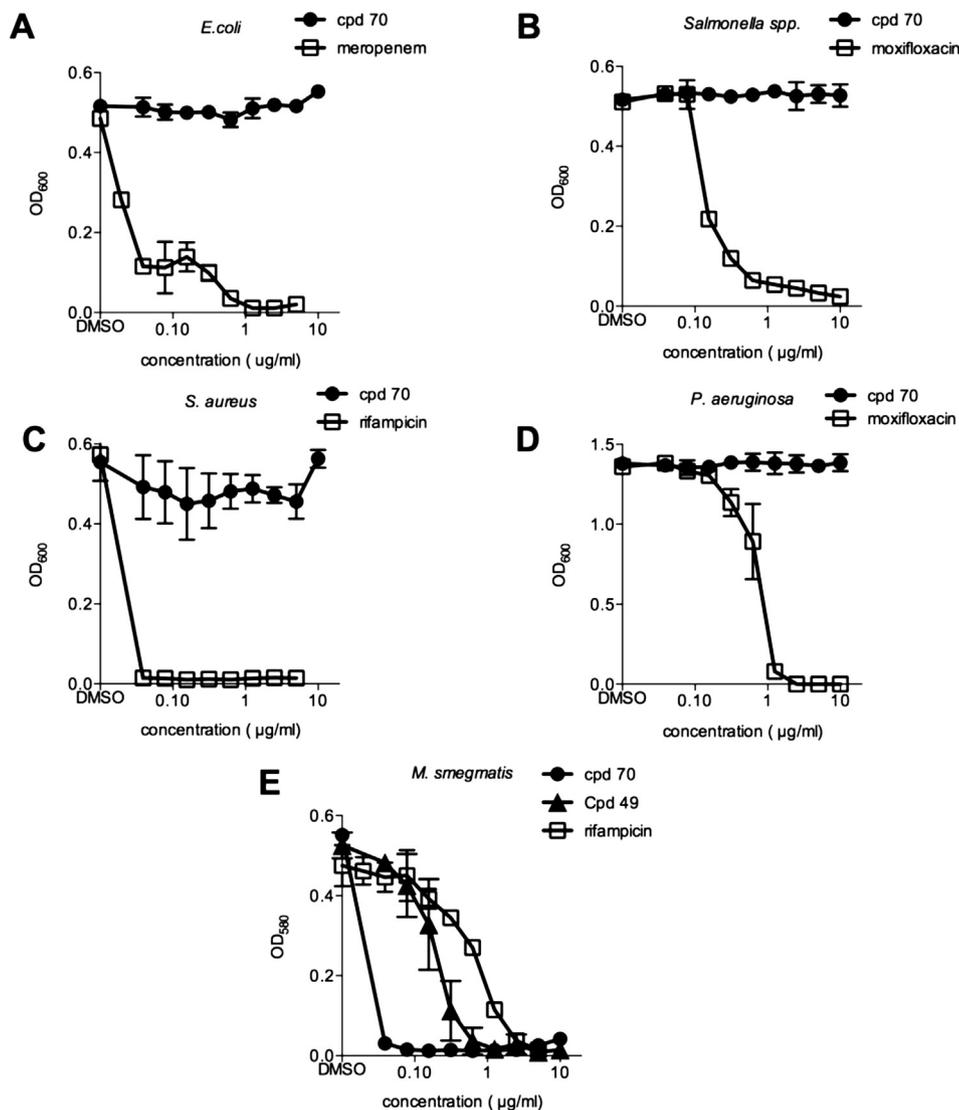


Fig. 5. Selectivity of benzimidazoles **49** and **70**. Log phase Gram-positive (*S. aureus*) and Gram-negative (*Salmonella spp.*, *E. coli*, *P. aeruginosa*) bacteria were diluted to an OD 0.01 and co-incubated with compounds for 6–10 h at doses up to 10 µg/mL. Compound **70** was inactive against these bacteria (A–D) when tested at >250× MIC for *Mtb*. In contrast, compounds **49** and **70** were potently active against *Mycobacterium smegmatis* after an overnight incubation, with an MIC <0.04 µg/mL and 0.31–0.625 µg/mL respectively (E). Results are means ± SD of triplicates and are representative of two similar experiments.

times for the alkaline phosphatase assay. To compare results between experiments or between +S9 and –S9 metabolic activation, R is normalized by dividing R at particular concentration by R(0), R at zero concentration. This is called the induction factor and is calculated for each dose concentration R(d).

$$I = \frac{R(d)}{R(0)}$$

A compound is classified as “not genotoxic” if the induction factor remains <1.5, as “marginal” if the induction factor is between

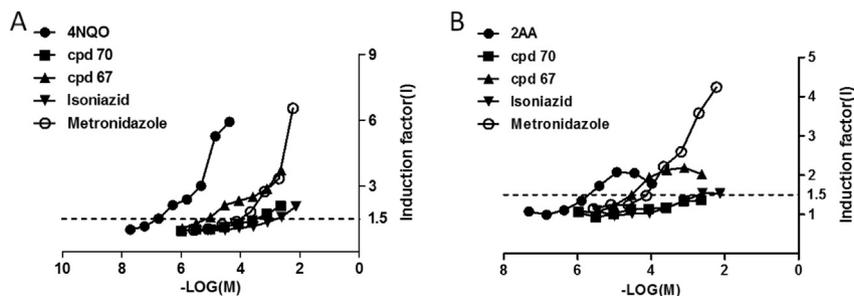


Fig. 6. Evaluation of the mutagenic potential of benzimidazoles **67** and **70**. The compounds **67** and **70** were evaluation in the absence or presence of metabolic activation (S9). In the absence of S9 (Fig. 6A), 4NQO was used as positive control. In the presence of S9 (Fig. 6B), 2AA was used as positive control.

1.5 and 2.0, and as “genotoxic” if the induction factor exceeds 2.0 and there's a dose-dependent increase in β -galactosidase activity is found [27]. The LOEC (lowest observed effect concentration) values and NOEC (no observed effect concentration) values were calculated as described [28].

5.2. Chemistry

Unless otherwise noted, all reagents and starting materials were purchased from commercial suppliers and used without further purification. NMR experiments were carried out on a Varian Mercury 300 or 400 or 600 MHz NMR spectrometer using DMSO- d_6 as the solvent. Chemical shifts were reported in ppm (δ) relative to the solvent and coupling constants (J) were reported in Hz. Melting points were determined without correction with a Yanaco micro-melting point apparatus. Automatic HPLC-MS analysis was performed on a Thermo Finnigan LCQ-Advantage mass spectrometer equipped with an Agilent pump, an Agilent detector, an Agilent liquid handler, and a fluent splitter. The column was a Kromasil C18 column (4.6 μ m, 4.6 mm \times 50 mm) from DIKMA for analysis. The eluent was a mixture of acetonitrile and water containing 0.05% HCOOH with a linear gradient from 5:95 (v/v) to 95:5 (v/v) of acetonitrile–water within 5 min at a 1.0 mL/min flow rate for analysis. The UV detection was carried out at a wavelength of 254 nm. The 5% of the eluent was split into the MS system. Mass spectra were recorded in positive ion mode using electrospray ionization (ESI). High resolution LC-MS was carried out by Agilent LC/MSD TOF using a column of Agilent ZORBAX SB-C18 (rapid resolution, 3.5 μ m, 2.1 mm \times 30 mm) at a flow of 0.40 mL/min. The solvent was methanol/water = 75:25 (v/v) containing 5 mmol/L ammonium formate. The ion source was electrospray ionization (ESI). Flash column chromatography was performed with silica gel 60 (200–300 mesh) from Qingdao Haiyang Chemical Factory. The purities of all tested compounds were \geq 95%, detected by HPLC under UV 254 nm wavelength, NMR, melting point, and HPLC-MS.

5.2.1. General procedure for the synthesis of compounds **1**, **6**–**27**

To a stirred solution of 1,5-difluoro-2,4-dinitrobenzene (DFDNB, 2.04 g, 10 mmol) in THF (50 mL), DIPEA (20 mmol) and $\text{NH}_2(\text{R}^1)\text{CH}_2\text{COOCH}_3\cdot\text{HCl}$ (10 mmol) was added. After vigorous stirring at room temperature until the total disappearance of DFDNB, compound **4** was obtained without purification. Then primary amine (R^2NH_2) (10 mmol) and DIPEA (10 mmol) were added and stirred under room temperature or refluxed conditions. The solvent was removed under reduced pressure to give crude compound **5** without purification. The two reactions above were traced by a fast LC-MS system until the all of the starting material disappeared. Compound **5** (1 g) was dissolved in a mixed solvent of THF (30 mL) and EtOH (30 mL), followed by addition of 10% Pd/C (1 g) and HCOONH_4 (2 g). The reaction mixture stirred at room temperature for 2 h. Then the reaction solution was directly filtered into another reaction vessel containing 5-nitro-furaldehyde (1.2 equiv) or 5-nitro-thiophenolaldehyde (1.2 equiv) in dioxane with 5% AcOH. The reaction mixture stirred at room temperature or refluxed conditions for 3–5 h. Then 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was added to the reaction mixture under reflux for 3–5 h and the expected compounds **1**, **6**–**27** were obtained. Following evaporation of the solvent *in vacuo*, the final products were characterized after purification by silica gel column chromatography.

5.2.1.1. 1-(2-Methylbutyl)-2-(5-nitrofuranyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (1). Yellow powder, total yield: 23%, m.p. 282–283 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.30 (s, 1H), 8.17 (s, 1H), 8.13 (d, J = 1.5 Hz, 1H), 7.91 (d, J = 3.6 Hz, 1H), 7.61 (d,

J = 3.9 Hz, 1H), 7.50 (s, 1H), 4.53–4.46 (m, 2H), 2.09–1.98 (m, 1H), 1.45–1.20 (m, 2H), 0.89–0.83 (m, 6H). HRMS (ESI): m/z ($\text{M} + \text{H}^+$) calcd for $\text{C}_{18}\text{H}_{18}\text{N}_5\text{O}_4$, 368.1353; found 368.1350.

5.2.1.2. 1-Cyclopentyl-2-(5-nitrofuranyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (2). Yellow powder, total yield 21%, m.p. 241–243 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.33 (s, 1H), 8.15 (d, J = 1.8 Hz, 1H), 8.04 (s, 1H), 7.93 (d, J = 3.9 Hz, 1H), 7.60 (d, J = 3.9 Hz, 1H), 7.57 (s, 1H), 5.47–5.36 (m, 1H), 2.25–2.20 (m, 4H), 2.05–2.03 (m, 2H), 1.79–1.76 (m, 2H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 154.5, 152.2, 150.7, 145.7, 145.0, 144.7, 130.5, 129.9, 128.8, 116.8, 114.1, 111.9, 104.1, 57.7, 30.0, 24.7. HRMS (ESI): m/z ($\text{M} + \text{H}^+$) calcd for $\text{C}_{18}\text{H}_{16}\text{N}_5\text{O}_4$, 366.1202; found 366.1194.

5.2.1.3. 1-(3-Methoxypropyl)-2-(5-nitrofuranyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (6). Brown powder, total yield: 23%, m.p. 268–270 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.33 (s, 1H), 8.16 (d, J = 2.4 Hz, 1H), 8.14 (s, 1H), 7.95 (d, J = 3.9 Hz, 1H), 7.63 (d, J = 3.9 Hz, 1H), 7.55 (s, 1H), 4.70 (t, J = 6.6 Hz, 2H), 3.34–3.33 (m, 2H), 3.14 (s, 3H), 2.11 (t, J = 6.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 154.5, 152.0, 150.4, 146.1, 144.3, 143.9, 133.1, 130.4, 129.0, 116.0, 114.3, 110.3, 103.4, 68.6, 57.8, 42.3, 29.5. HRMS (ESI): m/z ($\text{M} + \text{H}^+$) calcd for $\text{C}_{17}\text{H}_{16}\text{N}_5\text{O}_5$, 370.1146; found 370.1148.

5.2.1.4. 1-(3-Methoxypropyl)-7-methyl-2-(5-nitrofuranyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (7). Brown powder, total yield: 27%, m.p. >280 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.20 (s, 1H), 8.00 (s, 1H), 7.92 (d, J = 3.6 Hz, 1H), 7.58 (d, J = 3.6 Hz, 1H), 7.48 (s, 1H), 4.65–4.62 (m, 2H), 3.34–3.31 (m, 2H), 3.15 (s, 3H), 2.41 (s, 3H), 2.09–2.07 (m, 2H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 157.9, 154.5, 151.9, 146.2, 143.7, 142.9, 132.9, 130.1, 129.3, 115.6, 114.4, 109.1, 103.0, 68.7, 57.8, 42.2, 29.4, 20.6. HRMS (ESI): m/z ($\text{M} + \text{H}^+$) calcd for $\text{C}_{18}\text{H}_{18}\text{N}_5\text{O}_5$, 384.1302; found 384.1304.

5.2.1.5. 7-Isobutyl-1-(3-methoxypropyl)-2-(5-nitrothiophen-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (8). Yellow powder, total yield: 32%, m.p. >280 °C. ^1H NMR (400 MHz, DMSO- d_6) δ 12.24 (s, 1H), 8.26 (d, J = 4.4 Hz, 1H), 8.11 (s, 1H), 7.88 (d, J = 4.4 Hz, 1H), 7.49 (s, 1H), 4.66 (t, J = 6.7 Hz, 2H), 3.36–3.33 (m, 2H), 3.19 (s, 3H), 2.70 (d, J = 7.0 Hz, 2H), 2.35–2.22 (m, 1H), 2.07 (t, J = 6.1 Hz, 2H), 0.97 (d, J = 6.6 Hz, 6H). HRMS (ESI): m/z ($\text{M} + \text{H}^+$) calcd for $\text{C}_{21}\text{H}_{24}\text{N}_5\text{O}_4\text{S}$, 442.1544; found 442.1543.

5.2.1.6. 1-(tert-Butyl)-2-(5-nitrofuranyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (9). Brown powder, total yield: 27%, m.p. >270 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.32 (s, 1H), 8.37 (s, 1H), 8.18 (s, 1H), 7.91 (d, J = 3.6 Hz, 1H), 7.55 (s, 1H), 7.39 (d, J = 3.6 Hz, 1H), 1.71 (s, 9H). HRMS (ESI): m/z ($\text{M} + \text{H}^+$) calcd for $\text{C}_{17}\text{H}_{16}\text{N}_5\text{O}_4$, 354.1197; found 354.1203.

5.2.1.7. 2-(5-Nitrofuranyl)-1-(2-phenylpropyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (10). Yellow powder, total yield: 31%, m.p. 277–279 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.28 (s, 1H), 8.11 (d, J = 1.5 Hz, 1H), 8.04 (s, 1H), 7.86 (d, J = 3.6 Hz, 1H), 7.46–7.45 (m, 2H), 7.08–7.04 (m, 5H), 4.88–4.69 (m, 2H), 3.39–3.35 (m, 1H), 1.31 (d, J = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 154.4, 151.7, 150.3, 146.1, 144.2, 143.7, 142.5, 133.0, 130.2, 128.8, 128.0, 127.2, 126.6, 116.0, 114.2, 111.0, 103.1, 51.5, 18.0. HRMS (ESI): m/z ($\text{M} + \text{H}^+$) calcd for $\text{C}_{22}\text{H}_{18}\text{N}_5\text{O}_4$, 416.1353; found 416.1354.

5.2.1.8. 1-(3,4-Dimethoxyphenethyl)-2-(5-nitrofuranyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (11). Yellow powder, total yield: 34%, m.p. 279–281 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.33 (s, 1H), 8.21 (s, 1H), 8.16 (s, 1H), 7.86 (d, J = 3.9 Hz, 1H), 7.53 (s, 1H), 7.45 (d, J = 3.9 Hz, 1H), 6.70–6.67 (m, 2H), 6.56 (d, J = 7.8 Hz, 1H), 4.87–4.85

(m, 2H), 3.62 (s, 3H), 3.59 (s, 3H), 3.04–3.02 (m, 2H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 154.5, 151.9, 150.4, 148.5, 147.6, 146.3, 144.4, 144.0, 132.7, 130.3, 129.8, 129.1, 121.0, 115.9, 114.2, 112.7, 111.7, 110.8, 103.3, 55.5, 55.2, 46.6, 35.1. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{23}\text{H}_{20}\text{N}_5\text{O}_6$, 462.1408; found 462.1406.

5.2.1.9. 2-(5-Nitrofuranyl-2-yl)-1-nonyl-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**12**). Yellow powder, total yield: 25%, m.p. 233–235 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.32 (s, 1H), 8.19 (s, 1H), 8.14 (d, $J = 2.1$ Hz, 1H), 7.93 (d, $J = 3.9$ Hz, 1H), 7.61 (d, $J = 3.9$ Hz, 1H), 7.52 (s, 1H), 4.62 (t, $J = 7.2$ Hz, 2H), 1.83–1.81 (m, 2H), 1.33–1.18 (m, 12H), 0.81 (d, $J = 6.5$ Hz, 3H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 154.5, 152.0, 150.3, 146.1, 144.1, 143.9, 133.0, 130.3, 129.0, 116.0, 114.3, 110.4, 103.3, 44.9, 31.1, 29.6, 28.8, 28.5, 28.4, 25.9, 21.9, 13.8. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{22}\text{H}_{26}\text{N}_5\text{O}_4$, 424.1979; found 424.1985.

5.2.1.10. 1-(3,3-Diphenylpropyl)-2-(5-nitrofuranyl-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**13**). Yellow powder, total yield: 26%, m.p. >260 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.31 (s, 1H), 8.14 (d, $J = 2.1$ Hz, 1H), 7.90 (s, 1H), 7.84 (d, $J = 4.2$ Hz, 1H), 7.50 (s, 1H), 7.42 (d, $J = 4.2$ Hz, 1H), 7.30–7.10 (m, 10H), 4.54 (t, $J = 7.0$ Hz, 2H), 4.06 (t, $J = 7.8$ Hz, 1H), 2.66 (q, $J = 7.5$ Hz, 2H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 154.5, 151.9, 150.4, 145.9, 144.2, 144.0, 143.9, 132.6, 130.2, 129.0, 128.5, 127.4, 126.2, 116.1, 114.2, 110.4, 103.5, 48.1, 43.9, 34.3. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{28}\text{H}_{22}\text{N}_5\text{O}_4$, 492.1666; found 492.1668.

5.2.1.11. 7-Methyl-2-(5-nitrofuranyl-2-yl)-1-phenethyl-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**14**). Yellow powder, total yield: 18%, m.p. 255–256 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.23 (s, 1H), 8.11 (s, 1H), 7.86 (d, $J = 4.2$ Hz, 1H), 7.49 (s, 1H), 7.44 (d, $J = 4.2$ Hz, 1H), 7.17–7.14 (m, 5H), 4.85 (t, $J = 6.6$ Hz, 2H), 3.13–3.11 (m, 2H), 2.40 (s, 3H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{22}\text{H}_{18}\text{N}_5\text{O}_4$, 416.1353; found 416.1359.

5.2.1.12. 1-(3,4-Dimethoxyphenethyl)-7-isopropyl-2-(5-nitrofuranyl-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**15**). Yellow powder, total yield: 23%, m.p. 265–267 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.23 (s, 1H), 8.16 (s, 1H), 7.84 (d, $J = 4.2$ Hz, 1H), 7.50 (s, 1H), 7.40 (d, $J = 4.2$ Hz, 1H), 6.69–6.53 (m, 3H), 4.86 (t, $J = 6.9$ Hz, 2H), 3.62 (s, 3H), 3.59 (s, 3H), 3.52–3.45 (m, 1H), 3.03 (t, $J = 6.9$ Hz, 2H), 1.05 (d, $J = 6.9$ Hz, 6H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 164.8, 154.3, 152.3, 149.0, 148.1, 147.0, 144.4, 143.6, 133.1, 130.4, 130.3, 129.5, 121.5, 116.1, 114.8, 113.3, 112.2, 110.5, 103.4, 56.0, 55.7, 46.9, 35.6, 30.4, 20.7. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{26}\text{H}_{26}\text{N}_5\text{O}_6$, 504.1878; found 504.1882.

5.2.1.13. 7-Isopropyl-2-(5-nitrofuranyl-2-yl)-1-(3-phenylpropyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**16**). Yellow powder, total yield: 22%, m.p. 269–270 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.22 (s, 1H), 8.13 (s, 1H), 7.89 (d, $J = 4.2$ Hz, 1H), 7.52 (d, $J = 4.2$ Hz, 1H), 7.50 (s, 1H), 7.23–7.19 (m, 5H), 4.66 (t, $J = 6.0$ Hz, 2H), 3.51–3.47 (m, 1H), 2.74–2.71 (m, 2H), 2.17–2.15 (m, 2H), 1.24 (d, $J = 6.6$ Hz, 6H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 164.3, 153.7, 152.0, 146.1, 143.6, 143.0, 141.0, 132.8, 129.9, 129.0, 128.2, 128.1, 125.8, 115.6, 114.3, 109.6, 102.9, 44.7, 32.1, 31.4, 29.8, 20.1. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{25}\text{H}_{24}\text{N}_5\text{O}_4$, 458.1823; found 458.1831.

5.2.1.14. 1-Heptyl-7-isopropyl-2-(5-nitrofuranyl-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**17**). Yellow powder, total yield: 16%, m.p. 210–211 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.23 (s, 1H), 8.14 (s, 1H), 7.93 (d, $J = 4.2$ Hz, 1H), 7.59 (d, $J = 4.2$ Hz, 1H), 7.50 (s, 1H), 4.62 (t, $J = 7.2$ Hz, 2H), 3.51–3.45 (m, 1H), 1.83–1.80 (m, 2H), 1.36–1.01 (m, 11H), 0.83 (d, $J = 7.2$ Hz, 6H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 164.3, 153.7, 151.9, 146.2, 143.5, 142.9, 132.9, 129.9, 129.0, 115.7,

114.3, 109.6, 102.8, 44.9, 31.1, 29.8, 29.7, 28.1, 25.9, 21.9, 20.1, 13.8. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{23}\text{H}_{28}\text{N}_5\text{O}_4$, 438.2136; found 438.2144.

5.2.1.15. 1-Cyclohexyl-7-isopropyl-2-(5-nitrofuranyl-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**18**). Yellow powder, total yield: 12%, m.p. 251–253 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.22 (s, 1H), 8.18 (s, 1H), 7.91 (d, $J = 3.9$ Hz, 1H), 7.53 (d, $J = 3.9$ Hz, 1H), 7.51 (s, 1H), 5.00–4.87 (m, 1H), 3.48–3.45 (m, 1H), 2.49–2.48 (m, 2H), 2.07–1.90 (m, 4H), 1.78–1.75 (m, 1H), 1.53–1.51 (m, 3H), 1.24 (d, $J = 6.6$ Hz, 6H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 164.5, 153.7, 151.9, 146.0, 143.8, 143.7, 131.1, 129.5, 128.6, 116.4, 114.1, 111.7, 103.2, 57.3, 30.7, 29.8, 25.4, 24.2, 20.1. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{22}\text{H}_{24}\text{N}_5\text{O}_4$, 422.1823; found 422.1825.

5.2.1.16. 7-Isobutyl-1-(3-methoxypropyl)-2-(5-nitrofuranyl-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**19**). Yellow powder, total yield: 23%, m.p. 249–250 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.23 (s, 1H), 8.08 (s, 1H), 7.95 (d, $J = 3.9$ Hz, 1H), 7.61 (d, $J = 3.9$ Hz, 1H), 7.52 (s, 1H), 4.68 (t, $J = 6.9$ Hz, 2H), 3.38–3.32 (m, 2H), 3.16 (s, 3H), 2.70 (d, $J = 6.6$ Hz, 2H), 2.31–2.23 (m, 1H), 2.13–2.09 (m, 2H), 0.97 (d, $J = 6.9$ Hz, 6H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{21}\text{H}_{24}\text{N}_5\text{O}_5$, 426.1772; found 426.1776.

5.2.1.17. 1-(2-(Cyclohex-1-en-1-yl)ethyl)-7-isobutyl-2-(5-nitrofuranyl-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**20**). Yellow powder, total yield: 13%, m.p. 255–256 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.22 (s, 1H), 8.06 (s, 1H), 7.95 (d, $J = 4.2$ Hz, 1H), 7.60 (d, $J = 4.2$ Hz, 1H), 7.49 (s, 1H), 5.04 (s, 1H), 4.71 (t, $J = 6.0$ Hz, 2H), 2.68 (d, $J = 6.9$ Hz, 2H), 2.38–2.36 (m, 2H), 2.30–2.23 (m, 1H), 1.95–1.92 (m, 2H), 1.68–1.65 (m, 2H), 1.33–1.31 (m, 4H), 0.95 (d, $J = 6.3$ Hz, 6H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 159.8, 154.2, 151.6, 146.4, 143.6, 142.8, 133.3, 132.5, 129.8, 128.9, 123.8, 115.5, 114.3, 109.6, 102.7, 43.7, 41.3, 37.2, 27.4, 26.0, 24.4, 22.3, 21.9, 21.2. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{25}\text{H}_{28}\text{N}_5\text{O}_4$, 462.2136; found 462.2139.

5.2.1.18. 1-(1-Benzylpiperidin-4-yl)-7-isobutyl-2-(5-nitrofuranyl-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**21**). Yellow powder, total yield: 21%, m.p. >260 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.24 (s, 1H), 8.07 (s, 1H), 7.93 (d, $J = 3.9$ Hz, 1H), 7.55 (d, $J = 3.9$ Hz, 1H), 7.53 (s, 1H), 7.41–7.26 (m, 5H), 5.03–5.01 (m, 1H), 3.59 (s, 2H), 3.05–3.04 (m, 2H), 2.72 (d, $J = 6.9$ Hz, 2H), 2.58–2.56 (m, 2H), 2.31–2.22 (m, 3H), 2.02–2.00 (m, 2H), 0.97 (d, $J = 6.6$ Hz, 6H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{29}\text{H}_{31}\text{N}_6\text{O}_4$, 527.2401; found 527.2390.

5.2.1.19. 1-(Heptan-2-yl)-7-isobutyl-2-(5-nitrofuranyl-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**22**). Yellow powder, total yield: 14%, m.p. 219–220 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.23 (s, 1H), 8.17 (s, 1H), 7.93 (d, $J = 4.2$ Hz, 1H), 7.57 (d, $J = 4.2$ Hz, 1H), 7.53 (s, 1H), 5.15–5.10 (m, 1H), 2.68 (d, $J = 6.9$ Hz, 2H), 2.28–2.18 (m, 2H), 1.99–1.95 (m, 1H), 1.70 (d, $J = 6.9$ Hz, 3H), 1.15–1.14 (m, 4H), 0.96–0.94 (m, 8H), 0.71 (t, $J = 6.6$ Hz, 3H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 160.3, 154.4, 152.0, 145.9, 144.1, 143.8, 130.7, 129.7, 128.8, 116.6, 114.1, 111.4, 103.4, 53.7, 41.5, 34.0, 30.5, 26.4, 25.4, 22.6, 21.7, 19.5, 13.6. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{24}\text{H}_{30}\text{N}_5\text{O}_4$, 452.2292; found 452.2300.

5.2.1.20. 1-(Benzo[d][1,3]dioxol-5-ylmethyl)-7-isobutyl-2-(5-nitrofuranyl-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (**23**). Yellow powder, total yield: 27%, m.p. >260 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 12.21 (s, 1H), 8.08 (s, 1H), 7.85 (d, $J = 4.2$ Hz, 1H), 7.52 (d, $J = 4.2$ Hz, 1H), 7.50 (s, 1H), 6.80–6.60 (m, 3H), 5.89 (s, 2H), 5.78 (s, 2H), 2.62 (d, $J = 6.6$ Hz, 2H), 2.27–2.18 (m, 1H), 0.90 (d, $J = 6.6$ Hz, 6H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 160.2, 154.3, 151.9, 147.5, 146.7,

145.8, 143.7, 143.0, 132.9, 130.1, 130.0, 129.3, 120.1, 116.0, 114.3, 109.8, 108.4, 107.3, 103.1, 101.1, 47.6, 41.5, 26.2, 22.5. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{25}H_{22}N_5O_6$, 488.1565; found 488.1566.

5.2.1.21. 1-Benzyl-7-isobutyl-2-(5-nitrofur-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (24). Yellow powder, total yield: 30%, m.p. >260 °C. 1H NMR (300 MHz, DMSO- d_6) δ 12.25 (s, 1H), 8.11 (s, 1H), 7.88 (d, $J = 3.9$ Hz, 1H), 7.54 (d, $J = 3.9$ Hz, 1H), 7.53 (s, 1H), 7.34–7.18 (m, 5H), 5.97 (s, 2H), 2.66 (d, $J = 6.9$ Hz, 2H), 2.28–2.30 (m, 1H), 0.90 (d, $J = 6.6$ Hz, 6H). HRMS (ESI): m/z ($M + H^+$) calcd for $C_{24}H_{22}N_5O_4$, 444.1666; found 444.1668.

5.2.1.22. 7-Benzyl-1-cyclohexyl-2-(5-nitrofur-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (25). Yellow powder, total yield: 24%, m.p. 237–239 °C. 1H NMR (300 MHz, DMSO- d_6) δ 12.32 (s, 1H), 8.25 (s, 1H), 7.92 (d, $J = 3.9$ Hz, 1H), 7.54 (d, $J = 3.9$ Hz, 1H), 7.53 (s, 1H), 7.38–7.13 (m, 5H), 4.83–4.88 (m, 1H), 4.15 (s, 2H), 2.38–2.34 (m, 2H), 1.99–1.90 (m, 4H), 1.69–1.67 (m, 1H), 1.51–1.48 (m, 3H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 159.0, 154.0, 151.8, 145.8, 143.8, 143.7, 137.3, 131.0, 129.4, 128.8, 128.7, 128.1, 126.1, 116.4, 113.9, 111.7, 103.1, 57.1, 30.5, 25.3, 24.0. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{26}H_{24}N_5O_4$, 470.1823; found 470.1825.

5.2.1.23. 7-Benzyl-2-(5-nitrofur-2-yl)-1-nonyl-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (26). Yellow powder, total yield: 26%, m.p. 230–231 °C. 1H NMR (300 MHz, DMSO- d_6) δ 12.31 (s, 1H), 8.14 (s, 1H), 7.93 (d, $J = 4.2$ Hz, 1H), 7.61 (d, $J = 4.2$ Hz, 1H), 7.50 (s, 1H), 7.36–7.18 (m, 5H), 4.60 (t, $J = 6.9$ Hz, 2H), 4.14 (s, 2H), 1.81–1.79 (m, 2H), 1.36–1.18 (m, 12H), 0.80 (d, $J = 6.3$ Hz, 3H). HRMS (ESI): m/z ($M + H^+$) calcd for $C_{29}H_{32}N_5O_4$, 514.2449; found 514.2445.

5.2.1.24. 7-Benzyl-1-(2-(cyclohex-1-en-1-yl)ethyl)-2-(5-nitrofur-2-yl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (27). Yellow powder, total yield: 18%, m.p. 135–137 °C. 1H NMR (300 MHz, DMSO- d_6) δ 12.30 (s, 1H), 8.07 (s, 1H), 7.94 (d, $J = 4.2$ Hz, 1H), 7.61 (d, $J = 4.2$ Hz, 1H), 7.50 (s, 1H), 7.35–7.17 (m, 5H), 5.02 (s, 1H), 4.70 (t, $J = 6.6$ Hz, 2H), 4.14 (s, 2H), 2.36–2.35 (m, 2H), 1.91–1.90 (m, 2H), 1.65–1.63 (m, 2H), 1.31–1.27 (m, 4H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 159.1, 154.2, 151.9, 146.6, 144.0, 143.3, 137.5, 133.5, 132.8, 130.0, 129.3, 129.2, 128.3, 126.3, 124.1, 115.8, 114.5, 110.0, 103.0, 44.0, 37.4, 32.4, 27.7, 24.6, 22.2, 21.4. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{28}H_{26}N_5O_4$, 496.1979; found 496.1971.

5.2.2. General procedure for 1H-benzo[d]imidazole compounds

5.2.2.1. General procedure A for the synthesis of compounds 3, 32–38, 41, 45, 46, 49–51, 59–64, 67, 70–74. To a stirred solution of DFDNB (2.04 g, 10 mmol) in THF (50 mL), DIPEA (10 mmol) and secondary amine (10 mmol) were added. After vigorous stirring at room temperature until the total disappearance of DFDNB, compound **28** was obtained without purification. Then primary amine R^1NH_2 (10 mmol) and DIPEA (10 mmol) were added and stirred at room temperature overnight. The solvent was removed under reduced pressure to yield compound **29**. The above two reactions were traced by a fast LC-MS system until all of the starting material was changed to the anticipated compound. Compound **29** (1 g) was dissolved in a mixed solvent of THF (30 mL) and EtOH (30 mL), followed by the addition of 10% Pd/C (1 g) and $HCOONH_4$ (2 g). The formation of compound **30** was traced by a fast LC-MS system. Then the reaction solution was directly filtered into another reaction vessel containing the aldehyde (R^3CHO , 1.2 equiv) in dioxane with 5% HOAc to yield compound **31**. After the reaction was completed, the solvent was removed *in vacuo*. To a stirred solution of compound **31** (1 mmol) (without purification) in dry CH_2Cl_2 , various acyl chlorides (1.2 mmol) and Et_3N (1.2 mmol) were added. The reaction mixture was stirred at room temperature for 3–5 h and the

expected product **3, 32–38, 41, 45, 46, 49–51, 59–64, 67, 70–74** were obtained. After the reaction was completed, the solvent was evaporated *in vacuo*. The final products were characterized after purification by silica gel column chromatography.

5.2.2.2. General procedure B for the synthesis of compounds 42–44. According to the general procedure **A**, the intermediate **31** was obtained. To a stirred solution of compound **31** (1 mmol) (without purification) in dry CH_2Cl_2 , various isocyanates (2 mmol) or isothiocyanates (2 mmol) were added. The reaction mixture was refluxed for 3–5 h, the expected product **42–44** was obtained. After the reaction was completed, the solvent was evaporated *in vacuo*. The final products were characterized after purification by silica gel column chromatography.

5.2.2.3. General procedure C for the synthesis of compounds 65, 68 and 69. To a stirred solution of DFDNB (2.04 g, 10 mmol) in THF (50 mL), DIPEA (10 mmol) and thiomorpholine (10 mmol) were added. After vigorous stirring at room temperature until DFDNB completely disappeared, compound **28** ($R^2 =$ thiomorpholine) was obtained without purification. Then primary amine R^1NH_2 (10 mmol) and DIPEA (10 mmol) were added and stirred at room temperature overnight. The solvent was removed under reduced pressure to give compound **29**. Compound **29** (1 mmol) was dissolved in a mixed solvent of THF (30 mL) and EtOH (30 mL), followed by the addition of sodium hydrosulfite ($Na_2S_2O_4$, 20 mmol), K_2CO_3 (20 mmol) and H_2O (30 mL) at 50 °C, the reaction mixture was stirred at 50 °C for 2 h. The formation of compound **30** was traced by a fast LC-MS system. The solvent was evaporated *in vacuo* and extracted with EtOAc (3 \times 30 mL). The organic layer was washed with saturated NaCl, dried with anhydrous Na_2SO_4 and evaporated. The residual solid **30** (1 mmol) was dissolved in a mixed solvent of THF (25 mL), EtOH (25 mL) after which dioxane (10 mL), aldehyde (R^3CHO , 1.2 mmol) and 5% HOAc were added. The mixture was neutralized with $NaHCO_3$ and extracted with CH_2Cl_2 . The organic layer was washed with H_2O , dried with anhydrous Na_2SO_4 and evaporated, resulting in **31**. To a stirred solution of compound **31** (1 mmol) (without purification) in dry CH_2Cl_2 , various acyl chlorides (1.2 mmol) and Et_3N (1.2 mmol) were added. The reaction mixture was stirred at room temperature for 3–5 h and the expected product **65, 68 and 69** was obtained. After the reaction was completed, the solvent was evaporated *in vacuo*. The final products were characterized after purification by silica gel column chromatography.

5.2.2.4. General procedure D for the synthesis of compounds 47, 48, 52–58 and 66. To a stirred solution of alcohols (1 mmol) in THF NaH (1 mmol) was added at 0 °C, then the reaction mixture was refluxed for 2 h to give the sodium alcoholate. To a solution of DFDNB (1 mmol) in THF (20 mL), the above sodium alcoholate solvent was added dropwise. The reaction mixture was refluxed for 3–5 h with stirring and then washed with saturated NH_4Cl aqueous, followed by extraction with DCM. The organic layer was condensed *in vacuo* and dried with anhydrous Na_2SO_4 . Compound **28** ($R^2 =$ alcohol) was obtained after purification. To a solution of **28** (1 mmol) in THF (20 mL), various primary amines (10 mmol) and DIPEA (10 mmol) were added. The mixture was stirred at room temperature overnight and compound **29** was obtained after evaporating the solvent *in vacuo*. Compound **29** (1 g) was dissolved in a mixed solvent of THF (30 mL) and EtOH (30 mL), followed by the addition of 10% Pd/C (1 g) and $HCOONH_4$ (2 g). The formation of compound **30** was monitored by a fast LC-MS system. Then the reaction solution was directly filtered into another reaction vessel containing the aldehydes (R^3CHO , 1.2 equiv) in dioxane with 5% HOAc to yield intermediate **31**. After the reaction was completed,

the solvent was condensed *in vacuo*. To a stirred solution of intermediate **31** (1 mmol) (without purification) in dry CH_2Cl_2 , various acyl chlorides (1.2 mmol) and Et_3N (1.2 mmol) were added. The reaction mixture was stirred at room temperature for 3–5 h, and the expected products **47**, **48**, **52–58** and **66** were obtained. After the reaction was completed, the solvent was evaporated *in vacuo*. The final products were characterized after purification by silica gel column chromatography.

5.2.2.5. General procedure E for the synthesis of compounds 39 and 40. According to the general procedure **D**, intermediate **31** ($R^2 = \text{alcohol}$) was obtained. To a stirred solution of compound **31** (1 mmol) (without purification) in dry CH_2Cl_2 , various isocyanates (2 mmol) or isothiocyanates (2 mmol) were added. The reaction mixture was refluxed for 3–5 h, and the expected product **39** and **40** were obtained. After the reaction was completed, the solvent was condensed *in vacuo*. The final products were characterized after purification by silica gel column chromatography.

5.2.2.5.1. N-(6-(4-Methylpiperazin-1-yl)-2-(5-nitrofurano-2-yl)-1-pentyl-1H-benzod[jimidazol-5-yl]acetamide (3). Procedure **A** was used. Yellow powder, total yield: 33%, m.p. 186–187 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 8.87 (s, 1H), 8.23 (s, 1H), 7.91 (d, $J = 3.9$ Hz, 1H), 7.47 (d, $J = 3.9$ Hz, 2H), 4.54 (t, $J = 7.4$ Hz, 2H), 2.91 (br, 4H), 2.58–2.56 (m, 4H), 2.28 (s, 3H), 2.14 (s, 3H), 1.81–1.80 (m, 2H), 1.34–1.32 (m, 4H), 0.85 (t, $J = 6.9$ Hz, 3H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{23}\text{H}_{31}\text{N}_5\text{O}_4$, 454.2329; found 454.2335.

5.2.2.5.2. N-(1-(3-Methoxypropyl)-6-morpholino-2-(5-nitrofurano-2-yl)-1H-benzod[jimidazol-5-yl]butyramide (32). Procedure **A** was used. Yellow powder, total yield: 23%, m.p. 182–183 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 8.95 (s, 1H), 8.27 (s, 1H), 7.91 (d, $J = 3.9$ Hz, 1H), 7.49 (d, $J = 3.9$ Hz, 1H), 7.47 (s, 1H), 4.59 (t, $J = 6.8$ Hz, 2H), 3.84–3.82 (m, 4H), 3.31–3.28 (m, 2H), 3.16 (s, 3H), 2.90–2.80 (m, 4H), 2.41 (t, $J = 7.4$ Hz, 2H), 2.09–2.05 (m, 2H), 1.68–1.61 (m, 2H), 0.93 (t, $J = 7.4$ Hz, 3H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{23}\text{H}_{30}\text{N}_5\text{O}_6$, 472.2191; found 472.2195.

5.2.2.5.3. 4-Fluoro-N-(1-(3-methoxypropyl)-6-morpholino-2-(5-nitrofurano-2-yl)-1H-benzod[jimidazol-5-yl]benzamide (33). Procedure **A** was used. Yellow powder, total yield: 18%, m.p. 221–222 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.79 (s, 1H), 8.44 (s, 1H), 8.06 (dd, $J = 8.7$ Hz, 5.4 Hz, 2H), 7.92 (d, $J = 4.2$ Hz, 1H), 7.63 (s, 1H), 7.52 (d, $J = 4.2$ Hz, 1H), 7.43 (t, $J = 8.9$ Hz, 2H), 4.62 (t, $J = 6.8$ Hz, 2H), 3.81–3.80 (m, 4H), 3.34–3.31 (m, 2H), 3.17 (s, 3H), 2.96–2.94 (m, 4H), 2.11–2.07 (m, 2H). ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 163.3, 151.7, 146.9, 142.2, 141.1, 139.2, 133.1, 131.1, 129.8 (d, $^3J_{\text{C-F}} = 9.0$ Hz), 129.6, 115.9, 115.8, 114.7, 114.4, 111.7, 103.4, 68.6, 66.8, 58.0, 52.7, 41.9, 29.7. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{26}\text{H}_{27}\text{FN}_5\text{O}_6$, 524.1940; found 524.1944.

5.2.2.5.4. N-(1-(3-Methoxypropyl)-6-morpholino-2-(5-nitrofurano-2-yl)-1H-benzod[jimidazol-5-yl]furan-2-carboxamide (34). Procedure **A** was used. Yellow powder, total yield: 33%, m.p. 226–227 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.73 (s, 1H), 8.58 (s, 1H), 8.02 (s, 1H), 7.92 (d, $J = 3.9$ Hz, 1H), 7.65 (s, 1H), 7.51 (d, $J = 3.9$ Hz, 1H), 7.29 (d, $J = 3.0$ Hz, 1H), 6.74–6.73 (m, 1H), 4.61–4.60 (m, 2H), 3.87–3.86 (m, 4H), 3.32–3.31 (m, 2H), 3.17 (s, 3H), 2.96–2.95 (m, 4H), 2.09–2.07 (m, 2H). ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 155.2, 151.7, 147.6, 146.9, 145.9, 141.2, 140.6, 139.3, 132.6, 129.3, 114.8, 114.7, 114.4, 112.7, 109.3, 103.7, 68.6, 66.8, 58.0, 52.7, 41.9, 29.7. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{24}\text{H}_{26}\text{N}_5\text{O}_7$, 496.1827; found 496.1830.

5.2.2.5.5. 3-Cyano-N-(1-(3-methoxypropyl)-6-morpholino-2-(5-nitrofurano-2-yl)-1H-benzod[jimidazol-5-yl]benzamide (35). Procedure **A** was used. Yellow powder, total yield: 19%, m.p. 226–227 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.95 (s, 1H), 8.38 (s, 1H), 8.35 (s, 1H), 8.28 (d, $J = 8.1$ Hz, 1H), 8.09 (d, $J = 8.1$ Hz, 1H), 7.92 (d, $J = 4.2$ Hz, 1H), 7.81 (t, $J = 8.1$ Hz, 1H), 7.61 (s, 1H), 7.52 (d,

$J = 4.2$ Hz, 1H), 4.63 (t, $J = 6.3$ Hz, 2H), 3.79–3.78 (m, 4H), 3.34–3.31 (m, 2H), 3.17 (s, 3H), 2.96–2.95 (m, 4H), 2.12–2.07 (m, 2H). ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 162.8, 151.7, 146.9, 142.8, 141.2, 139.0, 135.7, 135.1, 133.5, 131.8, 131.0, 130.2, 129.2, 118.2, 114.7, 114.4, 112.8, 111.9, 103.2, 68.6, 66.7, 58.0, 52.6, 41.9, 29.6. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{27}\text{H}_{27}\text{N}_6\text{O}_6$, 531.1987; found 531.1997.

5.2.2.5.6. 3-(2-Chlorophenyl)-N-(1-(3-methoxypropyl)-6-morpholino-2-(5-nitrofurano-2-yl)-1H-benzod[jimidazol-5-yl]propanamide (36). Procedure **A** was used. Yellow powder, total yield: 21%, m.p. 253–254 °C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.33 (s, 1H), 8.53 (s, 1H), 7.95–7.91 (m, 3H), 7.58–7.52 (m, 3H), 7.47–7.45 (m, 2H), 7.26 (d, $J = 15.6$, 1H), 4.62 (t, $J = 6.7$ Hz, 2H), 3.91–3.89 (m, 4H), 3.35–3.33 (m, 2H), 3.19 (s, 3H), 2.95–2.93 (m, 4H), 2.13–2.07 (m, 2H). ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 162.9, 151.6, 147.0, 141.7, 140.9, 138.9, 135.2, 133.4, 132.8, 132.5, 131.1, 129.9, 129.3, 127.8, 127.6, 125.6, 114.6, 114.2, 111.5, 102.5, 68.5, 66.1, 57.9, 52.6, 41.7, 29.6. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{28}\text{H}_{29}\text{ClN}_5\text{O}_6$, 566.1801; found 566.1807.

5.2.2.5.7. 3-(2-Chlorophenyl)-N-(1-(3-methoxypropyl)-6-morpholino-2-(5-nitrothiophen-2-yl)-1H-benzod[jimidazol-5-yl]propanamide (37). Procedure **A** was used. Brown powder, total yield: 26%, m.p. 280 °C decom. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.32 (s, 1H), 8.48 (s, 1H), 8.22–8.21 (m, 1H), 7.94–7.89 (m, 2H), 7.76–7.75 (m, 1H), 7.54–7.52 (m, 2H), 7.45–7.43 (m, 2H), 7.27–7.22 (m, 1H), 4.58–4.57 (m, 2H), 3.89–3.88 (m, 4H), 3.32–3.31 (m, 2H), 3.21 (s, 3H), 2.92–2.91 (m, 4H), 2.04–2.02 (m, 2H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{28}\text{H}_{29}\text{ClN}_5\text{O}_5\text{S}$, 582.1572; found 582.1574.

5.2.2.5.8. 3-Cyano-N-(1-(3-methoxypropyl)-6-morpholino-2-(5-nitrothiophen-2-yl)-1H-benzod[jimidazol-5-yl]benzamide (38). Procedure **A** was used. Brown powder, total yield: 23%, m.p. 255–256 °C decom. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.96 (s, 1H), 8.40 (s, 1H), 8.34 (s, 1H), 8.29 (d, $J = 7.8$ Hz, 1H), 8.24 (d, $J = 4.5$ Hz, 1H), 8.10 (d, $J = 7.8$ Hz, 1H), 7.84–7.79 (m, 2H), 7.62 (s, 1H), 4.61 (t, $J = 6.9$ Hz, 2H), 3.80 (t, $J = 4.2$ Hz, 4H), 3.34–3.32 (m, 2H), 3.21 (s, 3H), 2.96 (t, $J = 4.2$ Hz, 4H), 2.05 (t, $J = 6.3$ Hz, 2H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{27}\text{H}_{27}\text{N}_6\text{O}_5\text{S}$, 547.1758; found 547.1759.

5.2.2.5.9. 1-(6-Methoxy-1-(3-methoxypropyl)-2-(5-nitrofurano-2-yl)-1H-benzod[jimidazol-5-yl]-3-(4-methoxyphenyl)urea (39). Procedure **E** was used. Brown powder, total yield: 17%, m.p. 255–256 °C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.15 (s, 1H), 8.42 (s, 1H), 8.20 (s, 1H), 7.90 (d, $J = 4.0$ Hz, 1H), 7.44 (d, $J = 4.0$, 1H), 7.37 (d, $J = 9.0$ Hz, 2H), 7.28 (s, 1H), 6.87 (d, $J = 9.0$ Hz, 2H), 4.59 (t, $J = 6.8$ Hz, 2H), 4.01 (s, 3H), 3.71 (s, 3H), 3.34–3.31 (m, 2H), 3.17 (s, 3H), 2.11–2.04 (m, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 154.4, 152.7, 151.4, 147.5, 147.4, 139.3, 136.5, 132.9, 130.9, 126.6, 119.7, 114.8, 114.0, 113.4, 108.0, 92.3, 68.6, 57.9, 56.4, 55.2, 41.7, 29.7. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{24}\text{H}_{26}\text{N}_5\text{O}_7$, 496.1827; found 496.1821.

5.2.2.5.10. 1-(4-Fluorophenyl)-3-(6-methoxy-1-(3-methoxypropyl)-2-(5-nitrofurano-2-yl)-1H-benzod[jimidazol-5-yl]urea (40). Procedure **E** was used. Brown powder, total yield: 25%, m.p. 204 °C decom. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.39 (s, 1H), 8.43 (s, 1H), 8.28 (s, 1H), 7.92 (d, $J = 3.0$ Hz, 1H), 7.48–7.46 (m, 3H), 7.30 (s, 1H), 7.14 (t, $J = 8.6$ Hz, 2H), 4.60 (t, $J = 6.0$ Hz, 2H), 4.02 (s, 3H), 3.32–3.31 (m, 2H), 3.18 (s, 3H), 2.09 (t, $J = 6.0$ Hz, 2H). ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 157.2 (d, $^1J_{\text{C-F}} = 236.4$ Hz), 152.5, 151.3, 147.42, 147.4, 139.4, 136.4, 136.1 (d, $^4J_{\text{C-F}} = 1.7$ Hz), 131.0, 126.3, 119.5 (d, $^3J_{\text{C-F}} = 7.5$ Hz), 115.2 (d, $^2J_{\text{C-F}} = 21.9$ Hz), 114.8, 113.4, 108.1, 92.4, 68.5, 57.9, 56.4, 41.7, 29.6. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{23}\text{H}_{23}\text{FN}_5\text{O}_6$, 484.1627; found 484.1629.

5.2.2.5.11. N-(6-(Cyclohexyl(methyl)amino)-1-(3-methoxypropyl)-2-(5-nitrofurano-2-yl)-1H-benzod[jimidazol-5-yl]-4-fluorobenzamide (41). Procedure **A** was used. Yellow powder, total yield: 21%, m.p. 183–184 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.83 (s, 1H), 8.43 (s, 1H), 8.02 (dd, $J = 8.7$ Hz, 5.4 Hz, 2H), 7.92 (d, $J = 3.9$ Hz,

1H), 7.59 (s, 1H), 7.52 (d, $J = 3.9$ Hz, 1H), 7.41 (t, $J = 8.9$ Hz, 2H), 4.61 (t, $J = 6.6$ Hz, 2H), 3.34–3.31 (m, 2H), 3.17 (s, 3H), 2.81–2.77 (m, 1H), 2.73 (s, 3H), 2.10–2.06 (m, 2H), 1.76–1.72 (m, 2H), 1.64–1.62 (m, 2H), 1.50–1.49 (m, 2H), 1.33–1.30 (m, 2H), 1.07–1.04 (m, 2H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 164.0 (d, $^1J_{\text{C-F}} = 247.9$ Hz), 163.1, 151.6, 147.0, 142.0, 140.9, 138.9, 132.9, 131.1 (d, $^4J_{\text{C-F}} = 2.4$ Hz), 130.6, 129.5 (d, $^3J_{\text{C-F}} = 9.3$ Hz), 115.7 (d, $^2J_{\text{C-F}} = 21.9$ Hz), 114.6, 114.2, 111.1, 105.0, 68.6, 62.0 (s, 5H), 41.7, 37.1, 29.7, 29.5, 25.3, 24.8. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{29}\text{H}_{33}\text{FN}_5\text{O}_5$, 550.2460; found 550.2463.

5.2.2.5.12. 1-(4-Methoxyphenyl)-3-(1-(3-methoxypropyl)-6-(4-methylpiperazin-1-yl)-2-(5-nitrofuranyl)-1H-benzodjimidazol-5-yl)urea (**42**). Procedure **B** was used. Red powder, total yield: 24%, m.p. 221–222 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 9.27 (s, 1H), 8.32 (s, 1H), 8.00 (s, 1H), 7.90 (d, $J = 3.6$ Hz, 1H), 7.47 (d, $J = 3.6$ Hz, 1H), 7.44 (s, 1H), 7.39 (d, $J = 8.4$ Hz, 2H), 6.90 (d, $J = 8.4$ Hz, 2H), 4.57 (m, 2H), 3.72 (s, 3H), 3.33–3.31 (m, 2H), 3.17 (s, 3H), 2.88 (s, 4H), 2.58 (s, 4H), 2.28 (s, 3H), 2.07–2.05 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 154.7, 152.9, 151.5, 147.3, 140.9, 140.3, 139.3, 132.7, 131.4, 130.9, 120.9, 114.7, 114.1, 113.9, 108.9, 102.1, 68.6, 58.0, 55.1, 54.6, 52.1, 45.7, 41.6, 29.7. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{28}\text{H}_{34}\text{N}_7\text{O}_6$, 564.2565; found 564.2566.

5.2.2.5.13. 1-(4-Fluorophenyl)-3-(1-(3-methoxypropyl)-6-(4-methylpiperazin-1-yl)-2-(5-nitrofuranyl)-1H-benzodjimidazol-5-yl)urea (**43**). Procedure **B** was used. Red powder, total yield: 27%, m.p. 207–208 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 9.52 (s, 1H), 8.29 (s, 1H), 8.04 (s, 1H), 7.89 (d, $J = 3.3$ Hz, 1H), 7.50–7.46 (m, 4H), 7.13 (t, $J = 8.7$ Hz, 2H), 4.58–4.57 (m, 2H), 3.30–3.28 (m, 2H), 3.18 (s, 3H), 2.90 (br.s, 4H), 2.63 (br.s, 4H), 2.30 (s, 3H), 2.07–2.05 (m, 2H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 157.3 (d, $^1J_{\text{C-F}} = 236.6$ Hz), 152.6, 151.5, 147.2, 141.0, 140.4, 139.2, 136.1, 131.5, 130.5, 120.1 (d, $^3J_{\text{C-F}} = 7.4$ Hz), 115.2 (d, $^2J_{\text{C-F}} = 22.1$ Hz), 114.7, 114.0, 109.2, 102.0, 68.5, 57.9, 54.5, 52.0, 45.7, 41.6, 29.6. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{27}\text{H}_{31}\text{FN}_7\text{O}_5$, 552.2365; found 552.2361.

5.2.2.5.14. 1-(4-Methoxyphenyl)-3-(1-(3-methoxypropyl)-6-(4-methylpiperazin-1-yl)-2-(5-nitrofuranyl)-1H-benzodjimidazol-5-yl)thiourea (**44**). Procedure **B** was used. Yellow powder, total yield: 21%, m.p. 186–187 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 9.94 (s, 1H), 9.19 (s, 1H), 8.82 (s, 1H), 7.92 (s, 1H), 7.51–7.32 (m, 4H), 7.00–6.98 (m, 2H), 4.58 (br.s, 2H), 3.77 (s, 3H), 3.30 (s, 2H), 3.16 (s, 3H), 2.79 (s, 4H), 2.49 (s, 4H), 2.18–2.07 (m, 5H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{28}\text{H}_{34}\text{N}_7\text{O}_5\text{S}$, 580.2337; found 580.2329.

5.2.2.5.15. *N*-(1-(3-Methoxypropyl)-6-(4-methylpiperazin-1-yl)-2-(5-nitrofuranyl)-1H-benzodjimidazol-5-yl)acetamide (**45**). Procedure **A** was used. Yellow powder, total yield: 19%, m.p. 186–187 °C. ^1H NMR (400 MHz, DMSO- d_6) δ 8.86 (s, 1H), 8.21 (s, 1H), 7.91 (d, $J = 4.0$ Hz, 1H), 7.48 (d, $J = 4.0$ Hz, 1H), 7.42 (s, 1H), 4.58 (t, $J = 6.7$ Hz, 2H), 3.33–3.31 (m, 2H), 3.16 (s, 3H), 2.91–2.89 (m, 4H), 2.60–2.58 (m, 4H), 2.28 (s, 3H), 2.13 (s, 3H), 2.08–2.05 (m, 2H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{22}\text{H}_{29}\text{N}_6\text{O}_5$, 457.2194; found 457.2198.

5.2.2.5.16. *N*-(2-(5-Nitrofuranyl)-1-pentyl-6-(piperidin-1-yl)-1H-benzodjimidazol-5-yl)acetamide (**46**). Procedure **A** was used. Yellow powder, total yield: 6%, m.p. 149–150 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 8.88 (s, 1H), 8.25 (s, 1H), 7.90 (d, $J = 3.9$ Hz, 1H), 7.48 (s, 1H), 7.45 (d, $J = 3.9$ Hz, 1H), 4.53 (t, $J = 6.9$ Hz, 2H), 2.84 (s, 4H), 2.15 (s, 3H), 1.76–1.74 (m, 6H), 1.58–1.56 (m, 2H), 1.32–1.29 (m, 4H), 0.84 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 168.0, 151.6, 147.3, 143.2, 140.3, 138.7, 132.5, 129.6, 114.8, 114.1, 111.3, 102.1, 53.5, 44.5, 29.6, 28.3, 25.9, 24.2, 23.8, 21.7, 13.9. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{23}\text{H}_{30}\text{N}_5\text{O}_4$, 440.2292; found 440.2291.

5.2.2.5.17. *N*-(1-(3-Ethoxypropyl)-2-(5-nitrofuranyl)-6-(2-(piperidin-1-yl)ethoxy)-1H-benzodjimidazol-5-yl)butyramide (**47**). Procedure **D** was used. Yellow powder, total yield: 18%, m.p. 123–125 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 8.94 (s, 1H), 8.22 (s, 1H), 7.91 (d, $J = 3.9$ Hz, 1H), 7.47 (d, $J = 3.9$ Hz, 1H), 7.36 (s, 1H), 4.61–4.57 (m,

2H), 4.25–4.23 (m, 2H), 3.33–3.31 (m, 4H), 2.79–2.76 (m, 2H), 2.37 (t, $J = 7.2$ Hz, 2H), 2.08–1.98 (m, 4H), 1.66–1.41 (m, 8H), 1.03 (t, $J = 6.9$ Hz, 3H), 0.93 (t, $J = 7.5$ Hz, 3H), 0.84–0.82 (m, 2H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{27}\text{H}_{38}\text{N}_5\text{O}_6$, 528.2817; found 528.2816.

5.2.2.5.18. *N*-(1-(4-Methylpentan-2-yl)-2-(5-nitrofuranyl)-6-(2-(piperidin-1-yl)ethoxy)-1H-benzodjimidazol-5-yl)cyclopropanecarboxamide (**48**). Procedure **D** was used. Brown powder, total yield: 23%, m.p. 109–111 °C. ^1H NMR (400 MHz, DMSO- d_6) δ 9.31 (s, 1H), 8.25 (s, 1H), 7.91 (d, $J = 3.9$ Hz, 1H), 7.44 (d, $J = 3.9$ Hz, 1H), 7.41 (s, 1H), 5.15–5.09 (m, 1H), 4.31 (t, $J = 5.8$ Hz, 2H), 2.79 (br.s, 2H), 2.51–2.50 (m, 2H), 2.09–1.99 (m, 1H), 1.83–1.76 (m, 1H), 1.67 (d, $J = 6.8$ Hz, 3H), 1.55–1.52 (m, 4H), 1.41–1.40 (m, 2H), 1.23–1.21 (m, 2H), 0.84–0.75 (m, 12H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{28}\text{H}_{38}\text{N}_5\text{O}_5$, 524.2867; found 524.2859.

5.2.2.5.19. *N*-(1-(tert-Butyl)-2-methyl-6-morpholino-1H-benzodjimidazol-5-yl)-5-nitrofuranyl-2-carboxamide (**49**). Procedure **A** was used. Yellow powder, total yield: 21%, m.p. 240 °C decom. ^1H NMR (300 MHz, DMSO- d_6) δ 10.15 (s, 1H), 8.36 (s, 1H), 7.85 (d, $J = 3.9$ Hz, 1H), 7.62 (s, 1H), 7.55 (d, $J = 3.9$ Hz, 1H), 3.89–3.87 (m, 4H), 2.95–2.93 (m, 4H), 2.70 (s, 3H), 1.78 (s, 9H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 153.3, 152.4, 151.1, 147.9, 139.8, 136.9, 131.9, 126.8, 116.4, 113.9, 108.6, 107.7, 66.9, 58.4, 52.8, 30.1, 19.7. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{21}\text{H}_{26}\text{N}_5\text{O}_5$, 428.1928; found 428.1931. Anal. Calcd for $\text{C}_{21}\text{H}_{25}\text{N}_5\text{O}_5$: C, 59.01; H, 5.90; N, 16.38. Found: 58.98; H, 6.19; N, 15.80.

5.2.2.5.20. *N*-(1-(tert-Butyl)-6-morpholino-1H-benzodjimidazol-5-yl)-5-nitrofuranyl-2-carboxamide (**50**). Procedure **A** was used. Yellow powder, total yield: 27%, m.p. 225 °C decom. ^1H NMR (300 MHz, DMSO- d_6) δ 10.16 (s, 1H), 8.52 (s, 1H), 8.25 (s, 1H), 7.85 (d, $J = 3.9$ Hz, 1H), 7.72 (s, 1H), 7.57 (d, $J = 3.9$ Hz, 1H), 3.91–3.88 (m, 4H), 2.99–2.96 (m, 4H), 1.70 (s, 9H). ^{13}C NMR (100 MHz, DMSO) δ 153.4, 151.1, 148.0, 142.4, 141.8, 138.1, 129.5, 127.3, 116.4, 113.8, 109.8, 106.8, 66.9, 56.1, 52.8, 28.9. HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{20}\text{H}_{24}\text{N}_5\text{O}_5$, 414.1772; found 414.1771.

5.2.2.5.21. *N*-(1-(tert-Butyl)-6-morpholino-2-propyl-1H-benzodjimidazol-5-yl)-5-nitrofuranyl-2-carboxamide (**51**). Procedure **A** was used. Yellow powder, total yield: 32%, m.p. 193–194 °C. ^1H NMR (400 MHz, DMSO- d_6) δ 10.12 (s, 1H), 8.40 (s, 1H), 7.86 (d, $J = 3.9$ Hz, 1H), 7.67 (s, 1H), 7.57 (d, $J = 3.9$ Hz, 1H), 3.89 (t, $J = 4.2$ Hz, 4H), 3.04 (t, $J = 7.5$ Hz, 2H), 2.96–2.94 (m, 4H), 1.88–1.83 (m, 2H), 1.81 (s, 9H), 1.01 (t, $J = 7.4$ Hz, 3H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{23}\text{H}_{30}\text{N}_5\text{O}_5$, 456.2241; found 456.2243.

5.2.2.5.22. *N*-(1-(tert-Butyl)-6-methoxy-2-methyl-1H-benzodjimidazol-5-yl)-5-nitrofuranyl-2-carboxamide (**52**). Procedure **D** was used. Brown powder, total yield: 24%, m.p. 192–193 °C. ^1H NMR (400 MHz, DMSO- d_6) δ 10.00 (s, 1H), 8.11 (s, 1H), 7.84 (d, $J = 3.9$ Hz, 1H), 7.71 (d, $J = 3.9$ Hz, 1H), 7.49 (s, 1H), 4.02 (s, 3H), 2.94 (s, 3H), 1.88 (s, 9H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{18}\text{H}_{21}\text{N}_4\text{O}_5$, 373.1506; found 373.1503.

5.2.2.5.23. *N*-(1-(3-Ethoxypropyl)-6-(2-morpholinoethoxy)-2-propyl-1H-benzodjimidazol-5-yl)-5-nitrofuranyl-2-carboxamide (**53**). Procedure **D** was used. Brown oil, total yield: 28%. ^1H NMR (400 MHz, DMSO- d_6) δ 9.72 (s, 1H), 7.96 (s, 1H), 7.84 (d, $J = 3.9$ Hz, 1H), 7.58 (d, $J = 3.9$ Hz, 1H), 7.27 (s, 1H), 4.26–4.19 (m, 4H), 3.51 (t, $J = 4.4$ Hz, 4H), 3.41–3.29 (m, 4H), 2.82–2.77 (m, 4H), 2.54 (s, 4H), 1.96–1.93 (m, 2H), 1.85–1.75 (m, 2H), 1.14 (t, $J = 7.0$ Hz, 3H), 1.00 (t, $J = 7.6$ Hz, 3H). HRMS (ESI): m/z ($M + H^+$) calcd for $\text{C}_{26}\text{H}_{36}\text{N}_5\text{O}_7$, 530.2609; found 530.2603.

5.2.2.5.24. *N*-(6-(2-Methoxyethoxy)-1-(4-methylpentan-2-yl)-1H-benzodjimidazol-5-yl)uran-2-carboxamide (**54**). Procedure **D** was used. Brown oil, total yield: 18%. ^1H NMR (300 MHz, DMSO- d_6) δ 9.18 (s, 1H), 8.32 (s, 1H), 8.25 (s, 1H), 7.96 (m, 1H), 7.44 (s, 1H), 7.27 (d, $J = 3.3$ Hz, 1H), 6.73 (m, 1H), 4.67–4.64 (m, 1H), 4.30 (t, $J = 4.5$ Hz, 2H), 3.72 (t, $J = 4.5$ Hz, 2H), 3.33 (s, 3H), 2.01–1.93 (m, 2H), 1.70–

1.62 (m, 1H), 1.49 (d, $J = 6.9$ Hz, 3H), 0.89 (d, $J = 6.9$ Hz, 3H), 0.83 (d, $J = 6.3$ Hz, 3H). HRMS (ESI): m/z ($M + H^+$) calcd for $C_{21}H_{27}N_3O_4$, 386.2074; found 386.2077.

5.2.2.5.25. *N*-(1-(4-Methylpentan-2-yl)-6-(2-morpholinoethoxy)-1H-benzo[d]imidazol-5-yl)furan-2-carboxamide (**55**). Procedure **D** was used. Brown powder, total yield: 32%, m.p. 60–62 °C. 1H NMR (300 MHz, DMSO- d_6) δ 9.33 (s, 1H), 8.27 (s, 1H), 8.18 (s, 1H), 7.95 (m, 1H), 7.42 (s, 1H), 7.30 (d, $J = 3.6$ Hz, 1H), 6.73 (dd, $J_1 = 3$ Hz, $J_2 = 1.8$ Hz, 1H), 4.69–4.66 (m, 1H), 4.36–4.33 (m, 2H), 3.59–3.56 (m, 4H), 2.92–2.66 (m, 6H), 2.02–1.94 (m, 2H), 1.70–1.61 (m, 1H), 1.50 (d, $J = 6.6$ Hz, 3H), 0.89 (d, $J = 6.6$ Hz, 3H), 0.83 (d, $J = 6.3$ Hz, 3H). HRMS (ESI): m/z ($M + H^+$) calcd for $C_{24}H_{33}N_4O_4$, 441.2496; found 441.2495.

5.2.2.5.26. *N*-(1-(4-Methylpentan-2-yl)-6-(2-morpholinoethoxy)-1H-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**56**). Procedure **D** was used. Yellow powder, total yield: 26%, m.p. 88–90 °C. 1H NMR (300 MHz, DMSO- d_6) δ 9.70 (s, 1H), 8.28 (s, 1H), 8.08 (s, 1H), 7.85 (d, $J = 3.6$ Hz, 1H), 7.60 (d, $J = 3.6$ Hz, 1H), 7.43 (s, 1H), 4.69–4.65 (m, 1H), 4.29–4.25 (m, 2H), 3.52–3.48 (m, 4H), 2.79–2.76 (m, 2H), 2.48–2.46 (m, 4H), 1.99–1.94 (m, 2H), 1.69–1.64 (m, 1H), 1.50 (d, $J = 6.6$ Hz, 3H), 0.89 (d, $J = 6.6$ Hz, 3H), 0.83 (d, $J = 6.3$ Hz, 3H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 154.3, 151.4, 148.0, 147.5, 141.8, 136.9, 131.2, 121.6, 116.4, 114.0, 113.7, 95.2, 67.5, 66.0, 57.0, 53.5, 49.4, 44.5, 24.6, 22.6, 22.0, 21.5. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{24}H_{32}N_5O_6$, 486.2347; found 486.2349.

5.2.2.5.27. *N*-(1-(4-Methylpentan-2-yl)-6-(2-morpholinoethoxy)-2-propyl-1H-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**57**). Procedure **D** was used. Brown powder, total yield: 23%, m.p. 40–42 °C. 1H NMR (300 MHz, DMSO- d_6) δ 9.68 (s, 1H), 7.99 (s, 1H), 7.85 (d, $J = 3.9$ Hz, 1H), 7.59 (d, $J = 3.9$ Hz, 1H), 7.33 (s, 1H), 4.63–4.59 (m, 1H), 4.27 (t, $J = 5.3$ Hz, 2H), 3.49 (t, $J = 4.7$ Hz, 4H), 2.85–2.74 (m, 4H), 2.48–2.46 (m, 4H), 2.17–1.97 (m, 2H), 1.84–1.74 (m, 3H), 1.54 (d, $J = 6.6$ Hz, 3H), 1.00 (t, $J = 7.4$ Hz, 3H), 0.91 (d, $J = 6.3$ Hz, 3H), 0.79 (d, $J = 6.6$ Hz, 3H). HRMS (ESI): m/z ($M + H^+$) calcd for $C_{27}H_{38}N_5O_6$, 528.2817; found 528.2808.

5.2.2.5.28. *N*-(1-(4-Methylpentan-2-yl)-6-(2-(piperidin-1-yl)ethoxy)-2-propyl-1H-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**58**). Procedure **D** was used. Brown oil, total yield: 18%. 1H NMR (400 MHz, DMSO- d_6) δ 9.73 (s, 1H), 8.01 (s, 1H), 7.85 (d, $J = 3.9$ Hz, 1H), 7.60 (d, $J = 3.9$ Hz, 1H), 7.34 (s, 1H), 4.61 (br.s, 1H), 4.27 (t, $J = 2.7$ Hz, 2H), 2.88–2.80 (m, 2H), 2.79–2.75 (m, 2H), 2.51–2.50 (m, 2H), 2.06–1.99 (m, 1H), 1.85–1.73 (m, 4H), 1.54 (d, $J = 6.8$ Hz, 3H), 1.41–1.31 (m, 4H), 1.29–1.23 (m, 4H), 1.00 (t, $J = 7.3$ Hz, 3H), 0.91 (d, $J = 6.6$ Hz, 3H), 0.79 (d, $J = 6.6$ Hz, 3H). HRMS (ESI): m/z ($M + H^+$) calcd for $C_{28}H_{40}N_5O_5$, 526.3024; found 526.3019.

5.2.2.5.29. *N*-(1-(3-Ethoxypropyl)-2-methyl-6-morpholino-1H-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**59**). Procedure **A** was used. Yellow powder, total yield: 21%, m.p. 235–237 °C. 1H NMR (400 MHz, DMSO- d_6) δ 10.11 (s, 1H), 8.41 (s, 1H), 7.85 (d, $J = 3.9$ Hz, 1H), 7.54 (d, $J = 3.9$ Hz, 1H), 7.53 (s, 1H), 4.22 (t, $J = 6.8$ Hz, 2H), 3.89 (t, $J = 4.2$ Hz, 4H), 3.28–3.26 (m, 2H), 3.25 (s, 3H), 2.93 (t, $J = 4.2$ Hz, 4H), 2.51 (s, 3H), 1.97–1.94 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 153.2, 152.5, 151.1, 148.1, 139.1, 137.9, 132.1, 127.0, 116.3, 113.8, 108.7, 103.2, 68.4, 66.8, 58.0, 53.0, 29.1, 21.0, 13.3. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{21}H_{26}N_5O_6$, 444.1878; found 444.1881.

5.2.2.5.30. *N*-(2-Cyclohexyl-1-isopropyl-6-morpholino-1H-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**60**). Procedure **A** was used. Yellow powder, total yield: 14%, m.p. 280 °C decom. 1H NMR (300 MHz, DMSO- d_6) δ 10.16 (s, 1H), 8.43 (s, 1H), 7.86 (d, $J = 3.6$ Hz, 1H), 7.61 (s, 1H), 7.57 (d, $J = 3.6$ Hz, 1H), 4.83–4.79 (m, 1H), 3.89–3.87 (m, 4H), 2.96–2.94 (m, 5H), 1.90–1.65 (m, 6H), 1.58 (d, $J = 6.6$ Hz, 6H), 1.50–1.25 (m, 4H). ^{13}C NMR (150 MHz, DMSO- d_6)

δ 158.9, 153.2, 151.0, 148.0, 139.8, 137.4, 129.8, 126.8, 116.3, 113.8, 109.1, 105.3, 66.8, 52.7, 46.5, 35.5, 31.6, 25.5, 25.4, 21.0. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{25}H_{32}N_5O_5$, 482.2398; found 482.2394.

5.2.2.5.31. *N*-(2-Cyclohexyl-1-methyl-6-morpholino-1H-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**61**). Procedure **A** was used. Pale yellow powder, total yield: 18%, m.p. 260 °C decom. 1H NMR (300 MHz, DMSO- d_6) δ 10.13 (s, 1H), 8.43 (s, 1H), 7.86 (d, $J = 3.6$ Hz, 1H), 7.58 (s, 1H), 7.56 (d, $J = 3.6$ Hz, 1H), 3.90 (m, 4H), 3.77 (s, 3H), 2.99–2.93 (m, 5H), 1.94–1.26 (m, 10H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 159.6, 153.2, 151.0, 148.0, 138.9, 137.9, 132.5, 126.8, 116.2, 113.8, 108.8, 103.4, 66.8, 52.9, 35.0, 31.0, 29.4, 25.5, 25.4. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{23}H_{28}N_5O_5$, 454.2085; found 454.2088.

5.2.2.5.32. *N*-(1-(tert-Butyl)-2-cyclohexyl-6-morpholino-1H-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**62**). Procedure **A** was used. Pale yellow powder, total yield: 22%, m.p. 270 °C decom. 1H NMR (300 MHz, DMSO- d_6) δ 10.09 (s, 1H), 8.36 (s, 1H), 7.86 (d, $J = 3.9$ Hz, 1H), 7.65 (s, 1H), 7.57 (d, $J = 3.9$ Hz, 1H), 3.89–3.87 (m, 4H), 3.18–3.15 (m, 1H), 2.93–2.91 (m, 4H), 1.91–1.70 (m, 15H), 1.41–1.34 (m, 4H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 161.0, 153.2, 151.0, 147.9, 139.8, 136.9, 131.1, 126.5, 116.3, 113.8, 108.7, 108.0, 66.8, 58.6, 52.8, 40.0, 33.0, 30.9, 25.8, 25.5. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{26}H_{34}N_5O_5$, 496.2554; found 496.2552.

5.2.2.5.33. *N*-(2-Cyclohexyl-1-isopropyl-6-morpholino-1H-1H-benzo[d]imidazol-5-yl)furan-2-carboxamide (**63**). Procedure **A** was used. White powder, total yield: 26%, m.p. 255–256 °C. 1H NMR (300 MHz, DMSO- d_6) δ 9.77 (s, 1H), 8.44 (s, 1H), 8.01 (s, 1H), 7.54 (s, 1H), 7.26 (d, $J = 3$ Hz, 1H), 6.74–6.73 (m, 1H), 4.82–4.75 (m, 1H), 3.86–3.84 (m, 4H), 2.99–2.93 (m, 5H), 1.90–1.65 (m, 6H), 1.58 (d, $J = 7.2$ Hz, 6H), 1.50–1.25 (m, 4H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 158.5, 154.9, 147.8, 145.6, 139.8, 137.1, 129.3, 127.3, 114.4, 112.5, 108.9, 104.8, 66.9, 52.7, 46.4, 35.5, 31.6, 25.5, 25.4, 21.0. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{25}H_{33}N_4O_3$, 437.2547; found 437.2545.

5.2.2.5.34. *N*-(1-(tert-Butyl)-6-morpholino-2-(pyridin-4-yl)-1H-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**64**). Procedure **A** was used. Yellow powder, total yield: 19%, m.p. 260 °C decom. 1H NMR (300 MHz, DMSO- d_6) δ 10.19 (s, 1H), 8.70 (d, $J = 6.0$ Hz, 2H), 8.51 (s, 1H), 7.87 (d, $J = 3.9$ Hz, 1H), 7.80 (s, 1H), 7.60–7.58 (m, 3H), 3.94–3.92 (m, 4H), 3.02–3.00 (m, 4H), 1.59 (s, 9H). HRMS (ESI): m/z ($M + H^+$) calcd for $C_{25}H_{27}N_6O_5$, 491.2037; found 491.2034.

5.2.2.5.35. *N*-(1-(tert-Butyl)-2-cyclohexyl-6-thiomorpholino-1H-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**65**). Procedure **C** was used. Yellow powder, total yield: 21%, m.p. 257–259 °C. 1H NMR (300 MHz, DMSO- d_6) δ 10.03 (s, 1H), 8.36 (s, 1H), 7.86 (d, $J = 3.6$ Hz, 1H), 7.64 (s, 1H), 7.58 (d, $J = 3.6$ Hz, 1H), 3.21–3.18 (m, 1H), 3.14–3.12 (m, 4H), 2.93–2.91 (m, 4H), 1.92–1.67 (m, 15H), 1.46–1.27 (m, 4H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 161.1, 153.3, 151.1, 147.9, 139.8, 138.2, 131.2, 126.5, 116.4, 113.8, 108.7, 108.6, 58.7, 54.7, 40.1, 33.1, 30.1, 28.2, 25.9, 25.6. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{26}H_{34}N_5O_4S$, 512.2326; found 512.2332.

5.2.2.5.36. *N*-(6-(2-Methoxyethoxy)-1-(4-methylpentan-2-yl)-2-propyl-1H-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**66**). Procedure **D** was used. Yellow powder, total yield: 25%, m.p. 78–80 °C. 1H NMR (400 MHz, DMSO- d_6) δ 9.62 (s, 1H), 8.08 (s, 1H), 7.84 (d, $J = 3.9$ Hz, 1H), 7.59 (d, $J = 3.9$ Hz, 1H), 7.40 (s, 1H), 4.62–4.61 (m, 1H), 4.29 (t, $J = 4.4$ Hz, 2H), 3.71 (t, $J = 4.4$ Hz, 2H), 3.30 (s, 3H), 2.91–2.78 (m, 2H), 2.04–1.97 (m, 1H), 1.85–1.77 (m, 3H), 1.54 (d, $J = 6.8$ Hz, 3H), 1.34–1.24 (m, 1H), 1.00 (t, $J = 7.3$ Hz, 3H), 0.91 (d, $J = 6.6$ Hz, 3H), 0.79 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 158.4, 154.2, 151.4, 147.9, 146.8, 146.4, 122.3, 118.4, 116.4, 113.6, 113.1, 111.9, 70.4, 70.1, 58.4, 50.0, 42.9, 29.0, 24.9, 22.4, 20.6, 19.6, 13.7. HRMS (ESI): m/z ($M + H^+$) calcd for $C_{24}H_{33}N_4O_6$, 473.2395; found 473.2398.

5.2.2.5.37. *N*-(1-Cyclobutyl-2-methyl-6-morpholino-1*H*-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**67**). Procedure **A** was used. Yellow powder, total yield: 28%, m.p. 280 °C decom. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.12 (s, 1H), 8.40 (s, 1H), 7.84 (d, *J* = 3.9 Hz, 1H), 7.61 (s, 1H), 7.54 (d, *J* = 3.9 Hz, 1H), 5.02–4.93 (m, 1H), 3.89 (t, *J* = 4.3 Hz, 4H), 2.95 (t, *J* = 4.3 Hz, 4H), 2.81–2.71 (m, 2H), 2.52 (s, 3H), 2.51–2.49 (m, 2H), 2.02–1.90 (m, 1H), 1.89–1.84 (m, 1H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ 153.1, 152.2, 150.9, 147.8, 139.2, 137.4, 131.1, 126.8, 116.1, 113.6, 108.6, 104.3, 66.6, 52.5, 49.0, 28.7, 14.6, 14.4. HRMS (ESI): *m/z* (M + H⁺) calcd for C₂₁H₂₄N₅O₅, 426.1772; found 426.1769.

5.2.2.5.38. *N*-(1-(*tert*-Butyl)-2-methyl-6-thiomorpholino-1*H*-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**68**). Procedure **C** was used. Pale yellow powder, total yield: 23%, m.p. 259–260 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.04 (s, 1H), 8.34 (s, 1H), 7.84 (d, *J* = 3.9 Hz, 1H), 7.61 (s, 1H), 7.54 (d, *J* = 3.9 Hz, 1H), 3.16–3.14 (m, 4H), 2.91–2.89 (m, 4H), 2.70 (s, 3H), 1.77 (s, 9H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ 153.2, 152.3, 151.0, 147.9, 139.7, 138.1, 131.8, 126.5, 116.3, 113.7, 108.4, 108.2, 58.3, 54.6, 30.1, 28.1, 19.6. HRMS (ESI): *m/z* (M + H⁺) calcd for C₂₁H₂₆N₅O₄S, 444.1700; found 444.1702.

5.2.2.5.39. *N*-(1-Isopropyl-2-methyl-6-thiomorpholino-1*H*-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**69**). Procedure **C** was used. Yellow powder, total yield: 31%, m.p. 229–231 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.08 (s, 1H), 8.38 (s, 1H), 7.84 (d, *J* = 3.9 Hz, 1H), 7.61 (s, 1H), 7.55 (d, *J* = 3.9 Hz, 1H), 4.76–4.71 (m, 1H), 3.17–3.15 (m, 4H), 2.92–2.90 (m, 4H), 2.53 (s, 3H), 1.55 (d, *J* = 6.9 Hz, 6H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ 153.2, 151.9, 151.1, 147.9, 139.7, 138.6, 130.3, 126.7, 116.2, 113.7, 108.7, 105.3, 54.6, 47.2, 28.1, 20.8, 14.5. HRMS (ESI): *m/z* (M + H⁺) calcd for C₂₀H₂₄N₅O₄S, 430.1544; found 430.1541.

5.2.2.5.40. *N*-(1-Isopentyl-2-methyl-6-morpholino-1*H*-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**70**). Procedure **A** was used. Yellow powder, total yield: 24%, m.p. 271–272 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.13 (s, 1H), 8.41 (s, 1H), 7.85 (d, *J* = 3.9 Hz, 1H), 7.56 (d, *J* = 3.9 Hz, 1H), 7.55 (s, 1H), 4.17 (t, *J* = 7.5 Hz, 2H), 3.89 (t, *J* = 4.1 Hz, 4H), 2.94 (t, *J* = 4.1 Hz, 4H), 2.52 (s, 3H), 1.68–1.55 (m, 3H), 0.97 (d, *J* = 6.3 Hz, 6H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ 153.3, 152.3, 151.1, 148.1, 139.1, 137.9, 131.9, 126.9, 116.3, 113.9, 108.7, 103.3, 66.9, 52.9, 41.4, 38.1, 25.4, 22.3, 13.5. HRMS (ESI): *m/z* (M + H⁺) calcd for C₂₂H₂₈N₅O₅, 442.2085; found 442.2087.

5.2.2.5.41. *N*-(1-Isopropyl-6-morpholino-1*H*-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**71**). Procedure **A** was used. Yellow powder, total yield: 27%, m.p. 285–286 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.13 (s, 1H), 8.51 (s, 1H), 8.33 (s, 1H), 7.85 (d, *J* = 3.9 Hz, 1H), 7.68 (s, 1H), 7.56 (d, *J* = 3.9 Hz, 1H), 4.80–4.73 (m, 1H), 3.89 (t, *J* = 4.2 Hz, 4H), 2.94 (t, *J* = 4.2 Hz, 4H), 1.53 (d, *J* = 6.7 Hz, 6H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ 153.3, 151.1, 147.9, 142.1, 140.2, 138.6, 130.1, 127.3, 116.3, 113.8, 109.7, 104.1, 66.8, 52.8, 46.8, 22.2. HRMS (ESI): *m/z* (M + H⁺) calcd for C₁₉H₂₂N₅O₅, 400.1615; found 400.1614.

5.2.2.5.42. *N*-(1-Methyl-6-morpholino-1*H*-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**72**). Procedure **A** was used. Yellow powder, total yield: 23%, m.p. 285 °C decom. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.12 (s, 1H), 8.52 (s, 1H), 8.16 (s, 1H), 7.85 (d, *J* = 3.8 Hz, 1H), 7.64 (s, 1H), 7.57 (d, *J* = 3.8 Hz, 1H), 3.89–3.87 (m, 4H), 3.83 (s, 3H), 2.94–2.92 (m, 4H). HRMS (ESI): *m/z* (M + H⁺) calcd for C₁₇H₁₈N₅O₅, 372.1302; found 372.1304.

5.2.2.5.43. *N*-(1,2-Dimethyl-6-morpholino-1*H*-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**73**). Procedure **A** was used. Yellow powder, total yield: 28%, m.p. >280 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.11 (s, 1H), 8.40 (s, 1H), 7.83 (d, *J* = 3.8 Hz, 1H), 7.55 (s, 1H), 7.53 (d, *J* = 3.8 Hz, 1H), 3.88 (t, *J* = 4.3 Hz, 4H), 3.72 (s, 3H), 2.92 (t, *J* = 4.3 Hz, 4H), 2.50 (s, 3H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ 153.2, 152.9, 151.1, 148.1, 138.9, 137.8, 132.7, 126.9, 116.3, 113.9,

108.6, 103.3, 66.8, 52.9, 29.7, 13.4. HRMS (ESI): *m/z* (M + H⁺) calcd for C₁₈H₂₀N₅O₅, 385.1459; found 386.1458.

5.2.2.5.44. *N*-(1-Isopropyl-2-methyl-6-morpholino-1*H*-benzo[d]imidazol-5-yl)-5-nitrofur-2-carboxamide (**74**). Procedure **A** was used. Yellow powder, total yield: 25%, m.p. 271–272 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.14 (s, 1H), 8.40 (s, 1H), 7.84 (d, *J* = 3.9 Hz, 1H), 7.60 (s, 1H), 7.54 (d, *J* = 3.9 Hz, 1H), 4.78–4.69 (m, 1H), 3.89–3.87 (m, 4H), 2.94–2.92 (m, 4H), 2.53 (s, 3H), 1.55 (d, *J* = 7.2 Hz, 6H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ 153.2, 151.8, 151.0, 148.0, 139.7, 137.4, 130.3, 126.8, 116.2, 113.8, 108.7, 104.8, 66.8, 52.7, 47.2, 20.8, 14.5. HRMS (ESI): *m/z* (M + H⁺) calcd for C₂₀H₂₄N₅O₅, 414.1772; found 414.1773.

5.2.3. General procedure for the synthesis of compounds **76**–**78**

Reaction of compound **31** (1 mmol) with KSCN (4 mmol) and Br₂ (1 mmol) was carried out in AcOH (20 mL) at room temperature overnight. The mixture was evaporated *in vacuo*, and the intermediate **75** was obtained. Compound **75** (1 mmol) in AcOH and H₂O (1:1) (20 mL) was added dropwise into the solution of NaNO₂ (45 equiv) in H₂O (20 mL). The reaction mixture was stirred at room temperature for 5–8 h, neutralized with 1 M NaOH, extracted with CH₂Cl₂, washed with H₂O, evaporated *in vacuo*, and purified with column chromatography to give the expected compound **76**.

Reaction of intermediate **75** (1 mmol) with acyl chloride (1.2 mmol) and Et₃N (1.2 mmol) was carried out in CH₂Cl₂ at room temperature for 3–5 h. After evaporation of the solvent, the residue was purified by column chromatography to give compound **77** and **78**.

5.2.3.1. 4-(2-((7-Methyl-6-(4-methylpentan-2-yl)-2-nitro-6*H*-imidazo[4',5':3,4]benzo[1,2-*d*]thiazol-4-yl)oxy)ethyl)morpholine (**76**). Brown powder, total yield: 8%, m.p. 205–207 °C. HRMS (ESI): *m/z* (M + H⁺) calcd for C₂₂H₃₀N₅O₃S, 446.2220; found 446.2211.

5.2.3.2. *N*-(4-Morpholino-7-propyl-6-(2,2,2-trifluoroethyl)-6*H*-imidazo[4',5':3,4]benzo[1,2-*d*]thiazol-2-yl)-5-nitrofur-2-carboxamide (**77**). Orange powder, total yield: 18%, m.p. >270 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.26 (s, 1H), 7.93 (d, *J* = 4.0 Hz, 1H), 7.84 (d, *J* = 4.0 Hz, 1H), 7.27 (s, 1H), 5.36 (q, *J* = 8.9 Hz, 2H), 3.86 (t, *J* = 4.3 Hz, 4H), 3.32–3.31 (m, 4H), 2.86 (t, *J* = 7.5, 2H), 1.91–1.82 (m, 2H), 1.02 (t, *J* = 7.4 Hz, 3H). HRMS (ESI): *m/z* (M + H⁺) calcd for C₂₂H₂₂F₃N₆O₅S, 539.1319; found 539.1316.

5.2.3.3. *N*-(6-(*tert*-Butyl)-4-(2-methoxyethoxy)-7-methyl-6*H*-imidazo[4',5':3,4]benzo[1,2-*d*]thiazol-2-yl)-5-nitrofur-2-carboxamide (**78**). Yellow powder, total yield: 15%, m.p. 237–239 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.59 (s, 1H), 7.92 (d, *J* = 4.2 Hz, 1H), 7.83 (d, *J* = 4.2 Hz, 1H), 7.30 (s, 1H), 4.34 (t, *J* = 4.5 Hz, 2H), 3.76 (t, *J* = 4.5 Hz, 2H), 3.34 (s, 3H), 2.74 (s, 3H), 1.82 (s, 9H). HRMS (ESI): *m/z* (M + H⁺) calcd for C₂₁H₂₄N₅O₆S, 474.1442; found 474.1445.

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Abbreviations

TB	tuberculosis
<i>Mtb</i>	<i>M. tuberculosis</i>
NR- <i>Mtb</i>	non-replicating <i>Mtb</i>
R- <i>Mtb</i>	replicating <i>Mtb</i>
DOS	directly observed therapy short-course
DIPEA	diisopropylethylamine
DFDNB	1,5-difluoro-2,4-dinitrobenzene
RIF	rifampicin
PZA	pyrazinamide
INH	isoniazid
EMB	ethambutol
LD ₅₀	concentration lethal to 50% of mammalian cells
MIC	minimal inhibitory concentration
ALDH	human aldehyde dehydrogenase
SI	selectivity index.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2014.01.039>.

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