



Deposited via The University of Leeds.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/194860/>

Version: Accepted Version

Article:

Dokla, EME, Abutaleb, NS, Milik, SN et al. (2023) SAR investigation and optimization of benzimidazole-based derivatives as antimicrobial agents against Gram-negative bacteria. *European Journal of Medicinal Chemistry*, 247. 115040. ISSN: 0223-5234

<https://doi.org/10.1016/j.ejmech.2022.115040>

© 2023, Elsevier. This manuscript version is made available under the CC-BY-NC-ND 4.0 license <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: <https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

SAR investigation and Optimization of Benzimidazole-based Derivatives as Antimicrobial Agents Against Gram-Negative Bacteria

Eman M. E. Dokla^{a,b,†,*}, Nader S. Abutaleb^{c,d,†}, Sandra N. Milik^{a,c}, Ezzat A. E. A. Kandil^a, Omar M. Qassem^{a,f}, Yehia Elgammal^c, Maha Nasr^g, Martin J. McPhillie^e, Khaled A. M. Abouzid^a, Mohamed N. Seleem^{c,h}, Peter Imming^b, and Mai Adel^{a,*}

^a Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo 11566, Egypt

^b Institute für Pharmazie, Martin-Luther-Universität Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, Halle (Saale) 06120, Germany

^c Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

^d Department of Microbiology and Immunology, Zagazig University, Zagazig 44519, Egypt

^e School of Chemistry, University of Leeds, Leeds LS2 9JT, United Kingdom

^f Purdue University Institute of Drug Discovery, Purdue University, West Lafayette, IN 47907, USA

^g Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo 11566, Egypt

^h Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA

*** Corresponding author**

Email address: emanelawady@pharma.asu.edu.eg (E.M.E. Dokla), mai.adel@pharma.asu.edu.eg (M. Adel)

[†]These authors have equal contributions.

Abstract

Antibiotic-resistant bacteria represent a serious threat to modern medicine and human life. Only a minority of antibacterial agents are active against Gram-negative bacteria. Hence, the development of novel antimicrobial agents will always be a vital need. In an effort to discover new therapeutics against Gram-negative bacteria, we previously reported a structure-activity-relationship (SAR) study on 1,2-disubstituted benzimidazole derivatives. Compound **III** showed a potent activity against *tolC*-mutant *Escherichia coli* with an MIC value of 2 µg/mL, representing a promising lead for further optimization. Building upon this study, herein, 49 novel benzimidazole compounds were synthesized to investigate their antibacterial activity against Gram-negative bacteria. Our design focused on three main goals, to address the low permeability of our compounds and improve their cellular accumulation, to expand the SAR study to the unexplored ring C, and to optimize the lead compound (**III**) by modification of the methanesulfonamide moiety. Compounds (**25a-d**, **25f-h**, **25k**, **25l**, **25p**, **25r**, **25s**, and **26b**) exhibited potent activity against *tolC*-mutant *E. coli* with MIC values ranging from 0.125 to 4 µg/mL, with compound **25d** displaying the highest potency among the tested compounds with an MIC value of 0.125 µg/mL. As its predecessor, **III**, compound **25d** exhibited an excellent safety profile without any significant cytotoxicity to mammalian cells. Time-kill kinetics assay indicated that **25d** exhibited a bacteriostatic activity and significantly reduced *E. coli* JW55031 burden as compared to DMSO. Additionally, combination of **25d** with colistin partially restored its antibacterial activity against Gram-negative bacterial strains (MIC values ranging from 4 to 16 µg/mL against *E. coli* BW25113, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*). Furthermore, formulation of **III** and **25d** as lipidic nanoparticles (nanocapsules) resulted in moderate enhancement of their antibacterial activity against Gram-negative bacterial strains (*A. Baumannii*, *N. gonorrhoeae*) and compound **25d** demonstrated superior activity to the lead compound **III**. These findings establish compound **25d** as a promising candidate for treatment of Gram-negative bacterial infections and emphasize the potential of nano-formulations in overcoming poor cellular accumulation in Gram-negative bacteria where further optimization and investigation are warranted to improve the potency and broaden the spectrum of our compounds.

Keywords: benzimidazole; Gram-negative bacteria; combination therapy; antimicrobial resistance; nanoparticles

1. Introduction

Antimicrobial resistance is a critical health problem globally [1,2] and therefore has been identified by the World Health Organization (WHO) as a major threat to society [3]. According to the Centers for Disease Control and Prevention (CDC), Gram-negative bacteria including *Neisseria gonorrhoeae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Campylobacter*, *Pseudomonas aeruginosa*, and *Salmonella*, represent the majority of the top microbial health threats [4]. These bacteria cause a wide array of serious infections such as pneumonia, urinary tract infections, food poisoning and blood infections [5]. Gram-negative bacteria have developed resistance against most classes of antibiotics including last resort antibiotics such as carbapenems and colistin [6–8]. An example of antibiotic resistance is multidrug-resistant (MDR) and extended spectrum β -lactamases (ESBL) producing *Escherichia coli*, which cause several life-threatening infections [6,9]. Undoubtedly, there is a critical need to develop new classes of antibiotics against Gram-negative bacteria.

Directions to fight infections due to MDR bacteria include the appropriate usage of the available antibiotics and the development of novel anti-microbial agents with new mechanisms of action to combat the emergence of resistance and shorten the duration of therapy [10,11]. However, development of new compounds against Gram-negative bacteria is compromised by the inability of these compounds to penetrate to their site of action due to the presence of the bacterial outer membrane, that prevents these molecules from gaining entry into the bacterial cell, and provides high protection against toxic compounds from the external environment [12,13]. Furthermore, if the small molecules successfully gain access inside the cell, they are subjected to efflux pumps that pump them out of the cells [14].

In an attempt to develop some predictive rules for optimal physicochemical properties of antibacterial compounds, several studies reported extensive analysis of various attributes of antibacterial activity such as cellular accumulation, whole-cell activity, bacterial efflux, and porin penetration which shed light onto the required physicochemical space for antibacterial activity [15–19]. For example, “eNTRY Rules” were proposed as predictive guidelines for small molecules accumulation in *E. coli* [19]. According to these eNTRY rules, a compound is most likely to accumulate in Gram-negative bacterial cells if it contains a non-sterically hindered ionizable

nitrogen (primary amines are preferred), has low three-dimensionality (globularity ≤ 0.25), is relatively rigid (number of rotatable bonds ≤ 5), and is amphiphilic [19,20].

Another promising solution to facilitate the intracellular delivery of antibacterial agents is through carriers, which were mainly developed to improve the ADMET properties of drugs [21,22]. Various kinds of carriers have been developed for hydrophilic and/or hydrophobic drugs such as liposomes, micelles, and nanoparticles [23–25]. Of these, lipid nanocapsules (LNCs) represent a hybrid between polymeric nanoparticles and liposomes and have been used to encapsulate a wide variety of drugs [26–30]. LNCs properties can be modified by varying their ingredients, or incorporating different additives to allow for the preparation of an optimum delivery system for antimicrobials with enhanced pharmacokinetic and pharmacodynamic properties [28,31].

Benzimidazole is a privileged scaffold and an important pharmacophore in medicinal chemistry [32]. Moreover, benzimidazole derivatives are remarkable compounds for their wide scope of activities as antidiabetic, antiulcer, anti-inflammatory, anticancer, antiviral, and antimicrobial agents [33,34]. Numerous studies have established the antimicrobial potential of benzimidazole derivatives against various strains of microorganisms [33,35]. Considering their structural similarity to purine, benzimidazoles' antibacterial activity is attributed to their competition with purines which results in the inhibition of bacterial proteins synthesis and nucleic acids [36]. We previously reported a SAR study on benzimidazole derivatives against Gram-negative bacteria that yielded compound **III** as a promising lead for further optimization [37]. We herein report the optimization, SAR analysis, formulation, and antibacterial screening of 1,2-disubstituted benzimidazole derivatives for potential activities against Gram-negative bacteria. Different strategies were adopted to overcome the problems of low permeability of Gram-negative bacterial cell envelope and efflux pumps, aiming to discover new antibiotics that are effective against Gram-negative bacteria.

2. Results and Discussion

2.1. Design strategy

Previously, we initiated a screening program based on two formerly reported 1,2-disubstituted benzimidazole derivatives (**I** and **II**) with potential inhibitory activity against Gram-

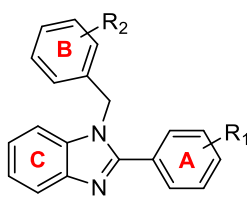
negative bacteria [38]. These derivatives showed moderate activity when tested against *E. coli* JW55031 (*tolC*-mutant strain) with MIC values of 16 and 8 µg/mL respectively (**Figure 1**), while lacking activity against the wild type *E. coli* (*E. coli* BW25113) and other wild-type Gram-negative bacteria [37]. Based on this initial data, we previously synthesized 31 benzimidazole-based derivatives with various substituents at both phenyl rings A & B (**Figure 1**) and evaluated their antibacterial activity. An optimum derivative (**III**) was identified which exhibited better antibacterial activity against *tolC*-mutant *E. coli* strain with an MIC value of 2 µg/mL and combination of **III** with colistin partially restored its activity against a panel of wild-type Gram-negative bacterial strains (MIC values ranging from 8 to 16 µg/mL) [37].

In continuation of our previous work and in an attempt to develop more potent benzimidazole derivatives, we focused our research efforts on three main goals. First, we sought to address the low permeability of the Gram-negative bacterial outer membrane and to improve the cellular accumulation of our compounds. One approach to achieve this was to modify the benzimidazole-based compounds guided by the eNTRY rules [19] to optimize their physicochemical properties for better penetration and accumulation. Preliminary evaluation of compound **III** by submitting its structure to <http://www.entry-way.org/> [19] disclosed favorable value for globularity (0.168) and a rigid structure (rotatable bonds = 5) while indicating the lack of the highly recommended primary amine. Accordingly, synthetic **schemes 1-3** were constructed by introducing basic functionalities (primary amine and amidine), acidic groups (carboxylic and tetrazole), and polar groups (amidoxime and 1,2,4-oxadiazole). These modifications aimed at satisfying the ionizable nitrogen requirement and improving the amphiphilic and polar properties of our compounds while trying not to disrupt the interaction with their cellular target. Another approach to easier passage of the Gram-negative cell envelop was to test the synergistic activity of the optimized derivatives in combination with colistin, a well-established membrane disrupting agent, to attain higher intracellular concentrations. Our third approach was to encapsulate our optimized compound using various lipid nanocapsules and adding different additives to improve the bacterial membrane penetration and cellular accumulation and to evade efflux pumps.

Our second goal was to investigate the SAR of the previously unexplored ring C (**Figure 1**) by varying the substitution pattern as demonstrated in **scheme 3**. Electron donating groups as

methyl and carbamate, and electron withdrawing groups as carboxylate, nitrile, trifluoromethyl, and bromo were chosen as substituents to impart different electronic environment to the molecules.

Our third goal was to optimize the lead compound (**III**) via systematic changes at the methanesulfonamide part (**Scheme 4**) starting by expanding the methyl into cyclopropyl and phenyl moieties, followed by varying the substituents at the newly introduced phenyl ring using sets of small versus bulky, polar versus hydrophobic, and electron donating and electron withdrawing groups at variable positions of the phenyl ring.



Compound	R ₁	R ₂	MIC
I	H	H	16
II	H	4-CH ₃	8
III	3-NHSO ₂ CH ₃	4-CH ₃	2

Figure 1. Chemical structures of previously reported benzimidazole derivatives and their antibacterial activity against *E. coli* JW55031 (*tolC*-mutant). MIC, minimum inhibitory concentration ($\mu\text{g/mL}$).

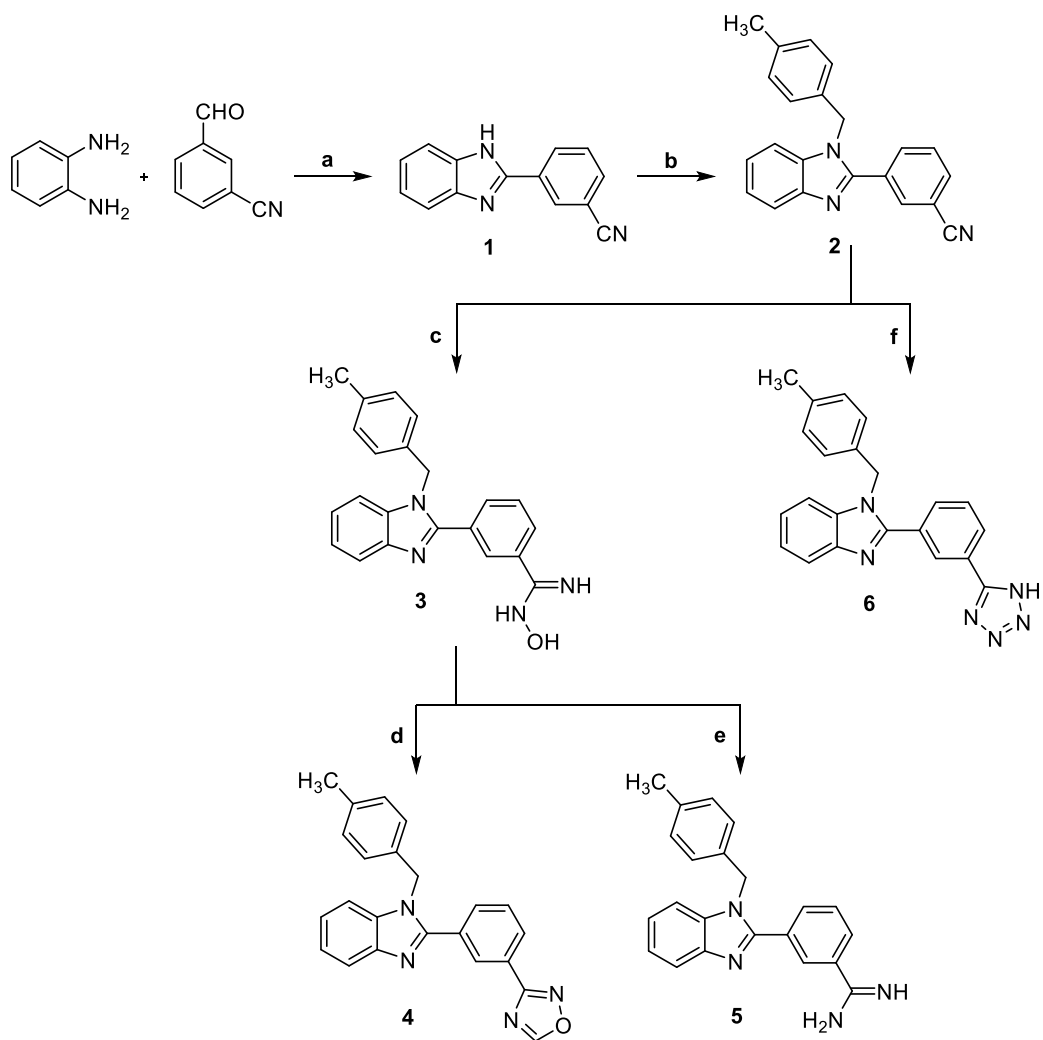
2.2. Chemistry

The designed 1,2-disubstituted benzimidazole derivatives were synthesized as outlined in **schemes 1-4** and as discussed here. In **scheme 1**, *o*-phenylenediamine was cyclized with 3-cyanobenzaldehyde using sodium acetate in ethanol to give 2-(3-cyano phenyl)-1*H*-benzimidazole (**1**) in moderate yield [37]. Further alkylation of **1** with 4-methylbenzyl chloride using cesium carbonate in DMF furnished 1-(4-methylbenzyl)-2-(3-cyanophenyl)-1*H*-benzimidazole (**2**) in fair yield [37]. The cyano derivative (**2**) was refluxed with hydroxylamine solution (50% in water) in ethanol to yield the corresponding iminoxime (**3**) in very good yield [39]. Iminoxime derivative (**3**) was either refluxed with triethyl orthoformate to establish the desired oxadiazole derivative (**4**)-[40] in moderate yield or acylated using acetic acid and acetic anhydride followed by reaction with H₂/Pd to furnish the final amidine derivative (**5**) in moderate yield [41]. Finally, the cyano derivative (**2**) was reacted with sodium azide and ammonium chloride in DMF to give the tetrazole derivative (**6**) in poor yield [42].

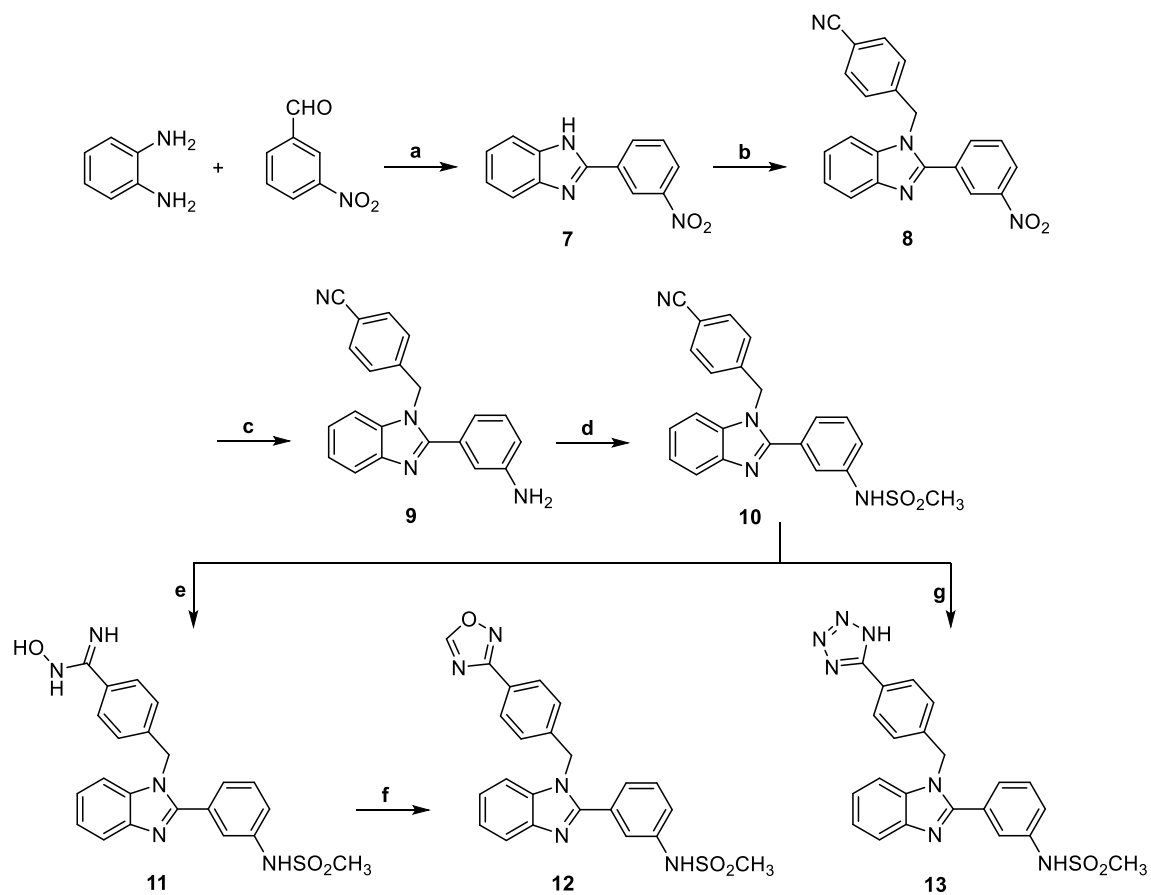
In **scheme 2**, 2-(3-nitro phenyl)-1*H*-benzimidazole (**7**) was prepared in moderate yield by the reaction of *o*-phenylenediamine with 3-nitrobenzaldehyde [37]. Subsequent alkylation of **7** using 4-cyanobenzyl bromide gave intermediate **8** in moderate yield which was further reduced using stannous chloride dihydrate in EtOAc to afford the desired amine derivative (**9**) in moderate yield [37]. Subsequent mesylation of **9** using mesyl chloride in pyridine gave the desired methanesulfonamide intermediate (**10**) in moderate yield [37]. The *p*-cyano group of intermediate **10** was treated in the same manner (**Scheme 1**) to give the desired final compounds (**11-13**) in poor to good yields.

To synthesize the final derivatives **19a-l**, **20a-b**, **21a-c**, and **22a-b** (**Scheme 3**), the appropriate 3-, 4-, or 5-substituted-2-fluoro-1-nitrobenzene (**16a-l**) was reacted with 4-methylbenzylamine and K₂CO₃ in anhydrous DMF to give the desired 4-,5-, or 6-substituted-*N*-(4-methylbenzyl)-2-nitroaniline intermediates (**17a-l**) in good to excellent yields [43]. Subsequent reduction of **17a-l** intermediates using either iron and ammonium chloride [44] when the phenyl was substituted with an electron donating group (i.e., **17a,b,h,i**) or stannous chloride dihydrate [43] when the phenyl was substituted with an electron withdrawing group (i.e., **17c-g,j,k,l**) followed by cyclization using sodium hydroxy(3-nitrophenyl)methanesulfonate in DMF [45] gave the desired benzimidazole intermediates (**18a-l**) in poor to moderate yields. Further reduction of the appropriate nitro derivatives (**18a-l**) using stannous chloride dihydrate in EtOAc and subsequent reaction of the amine intermediates with mesyl chloride in pyridine gave the final compounds (**19a-l**) in fair to good yields [37]. The final amine derivatives (**20a,b**) were obtained in moderate to good yields after hydrolysis of the carbamate derivatives (**19a,b**) by heating in 10% NaOH solution [46], while the final carboxylic acid derivatives (**21a-c**) were synthesized in moderate to very good yields by hydrolyzing the ethyl ester derivatives (**19c-e**) using LiOH.H₂O [47]. Finally, the cyano substituted derivatives (**19f,g**) were reacted with hydroxylamine solution in ethanol to give the corresponding iminoxime derivatives (**22a,b**) in very good to excellent yields [39].

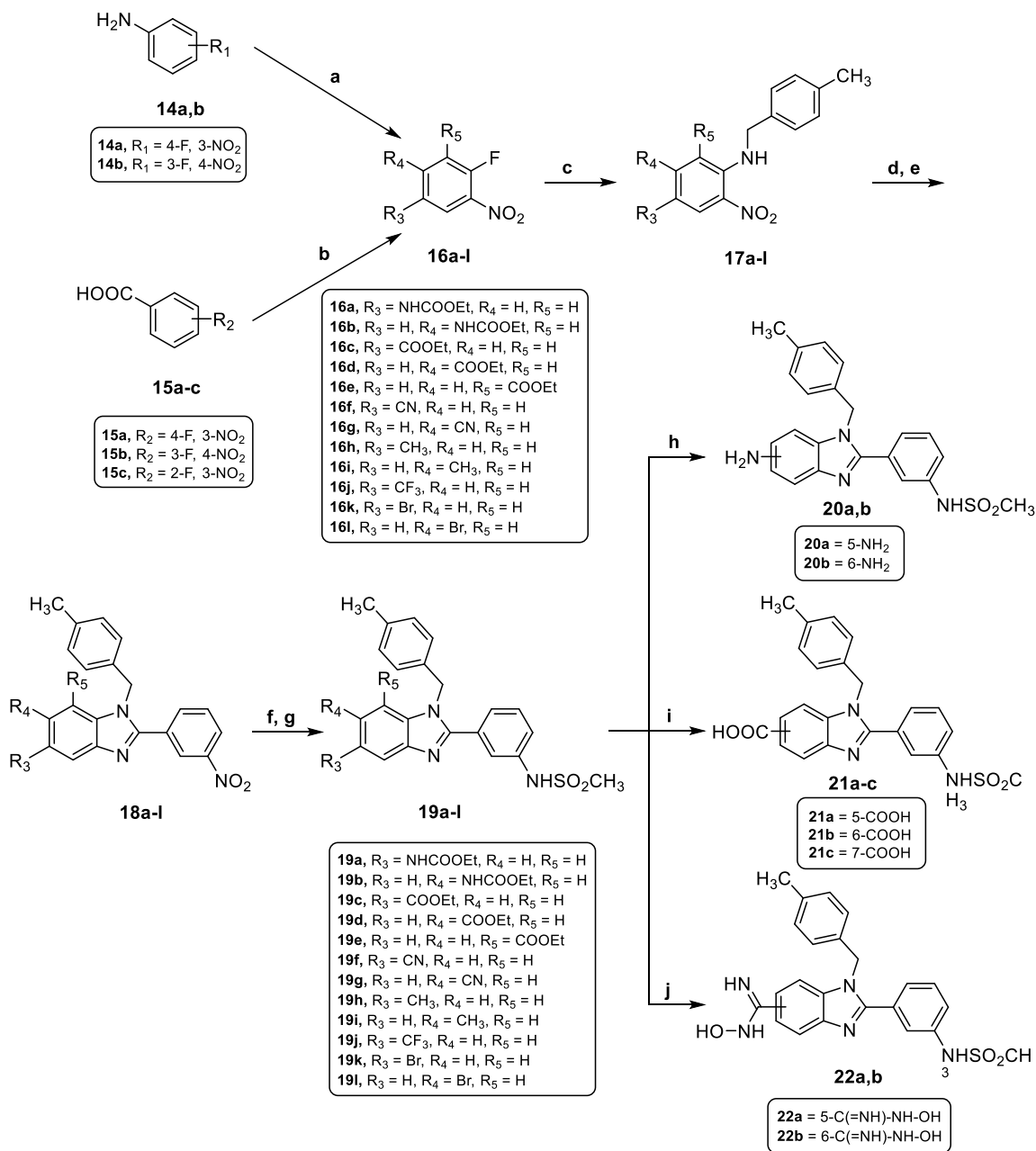
At last, the final compounds (**III**, **25a-u** and **26a,b**) were synthesized in poor to very good yields as we described previously [37].



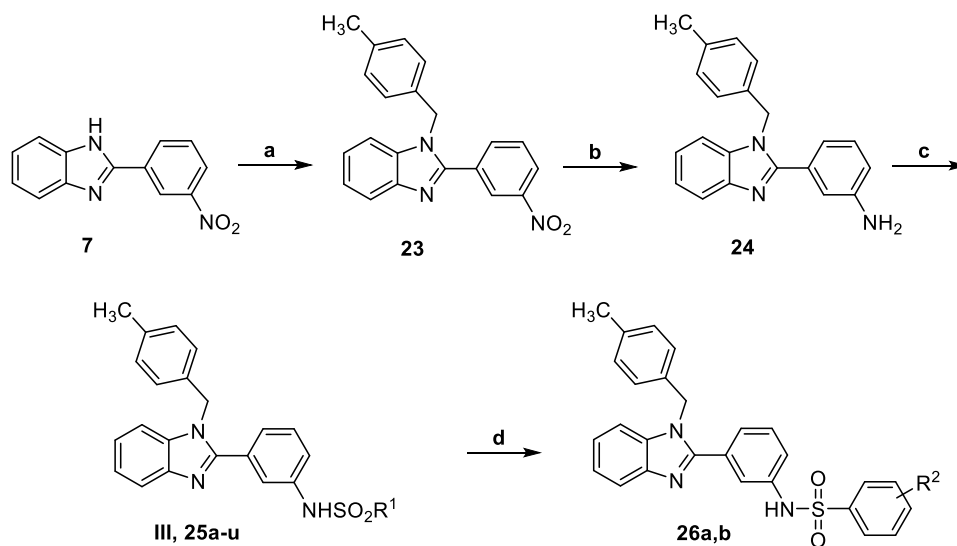
Scheme 1. Reagents and conditions: **(a)** sodium acetate, EtOH, reflux, 48 h, 61% yield; **(b)** 4-methylbenzyl chloride, Cs₂CO₃, DMF, rt, 48 h, 48% yield; **(c)** NH₂OH (50% in water), EtOH, reflux, 4 h, 85% yield; **(d)** triethyl orthoformate, reflux, 24 h, 58% yield; **(e)** (1) Ac₂O, AcOH, 0.5 h, (2) H₂, Pd/C (5%), rt, 2 h, 60% yield; **(f)** NaN₃, NH₄Cl, DMF, 125°C, 24 h, 20% yield.



Scheme 2. Reagents and conditions: **(a)** sodium acetate, EtOH, reflux, 48 h, 68% yield; **(b)** 4-cyanobenzyl bromide, Cs_2CO_3 , DMF, rt, 48 h, 51% yield; **(c)** $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, EtOAc, reflux, overnight, 56% yield; **(d)** $\text{CH}_3\text{SO}_2\text{Cl}$, pyridine, rt, overnight, 50% yield; **(e)** NH_2OH (50% in water), EtOH, reflux, 4 h, 75% yield; **(f)** triethyl orthoformate, reflux, 24 h, 52% yield; **(g)** NaN_3 , NH_4Cl , DMF, 125°C, 24 h, 29% yield.



Scheme 3. Conditions and reagents. **(a)** ClCOOEt, DIPEA, THF, rt, 2 h, 87-92% yield; **(b)** EtOH, H₂SO₄, reflux, 24 h, 87-89% yield; **(c)** 4-methylbenzylamine, K₂CO₃, DMF, 80-90°C, 12 h, 78-95% yield; **(d)** **(1)** Fe, NH₄Cl, EtOH/H₂O, reflux, 6 h (**17a,b,h,i**) or **(2)** SnCl₂·2H₂O, EtOAc, reflux, 16 h (**17c-g,j,k,l**); **(e)** sodium hydroxy(3-nitrophenyl)methanesulfonate, DMF, 130°C, 4 h, 34-59% yield; **(f)** SnCl₂·2H₂O, EtOAc, reflux, 16 h; **(g)** CH₃SO₂Cl, pyridine, rt, 48 h, 43-80% yield; **(h)** **19a,b**, NaOH (10% in water), reflux, 1.5 h, 58-65% yield; **(i)** **19c-e**, LiOH·H₂O, THF/H₂O, rt, 2-4 days, 61-83% yield; **(j)** **19f,g**, NH₂OH (50% in water), EtOH, reflux, 24 h, 87-97% yield.



III , R ¹ = methyl	25k , R ¹ = 4-tolyl
25a , R ¹ = cyclopropyl	25l , R ¹ = 4-methoxyphenyl
25b , R ¹ = phenyl	25m , R ¹ = 4-isopropylphenyl
25c , R ¹ = 4-chlorophenyl	25n , R ¹ = 4-(<i>t</i> -butyl)phenyl
25d , R ¹ = 3-chlorophenyl	25o , R ¹ = 4-acetamidophenyl
25e , R ¹ = 4-bromophenyl	25p , R ¹ = 3,4-dimethoxyphenyl
25f , R ¹ = 3-bromophenyl	25q , R ¹ = 4-nitrophenyl
25g , R ¹ = 3,4-difluorophenyl	25r , R ¹ = 3-nitrophenyl
25h , R ¹ = 4-fluoro-3-chlorophenyl	25s , R ¹ = 2-nitrophenyl
25i , R ¹ = 4-(trifluoromethyl)phenyl	25t , R ¹ = 2-naphthyl
25j , R ¹ = 4-(trifluoromethoxy)phenyl	25u , R ¹ = 4-(1,1'-biphenyl)

26a , R ² = 4-amino
26b , R ² = 3-amino

Scheme 4. Reagents and conditions: (a) 4-methylbenzyl chloride, Cs₂CO₃, DMF, rt, 48 h, 57% yield; (b) SnCl₂·2H₂O, EtOAc, reflux, overnight, 51% yield; (c) RSO₂Cl, pyridine, rt, overnight, 23-76% yield; (d) **25q,r**, SnCl₂·2H₂O, EtOAc, reflux, overnight, 43-53% yield.

2.3. Biological activity

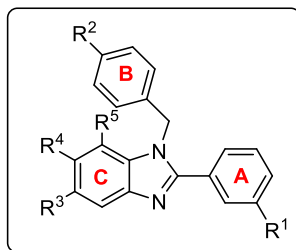
2.3.1. Screening of the synthesized benzimidazoles against *Escherichia coli* and other Gram-negative bacteria

As we previously reported, our lead compound (**III**) is a substrate for the AcrA-AcrB-TolC efflux system [37] which is responsible for the elimination of small molecules and xenobiotics, imparting resistance to *E. coli* against different antibiotics [48]. Mutations in TolC protein impair the efflux mechanism and increase *E. coli* sensitivity to antibiotics [49]. Accordingly, all 49 synthesized benzimidazole derivatives were initially screened alongside the lead compound (**III**) against *E. coli* JW55031 (*tolC*-mutant) strain for their *in vitro* antibacterial activity (**Table 1** and **2**). Linezolid and gentamicin were used as reference antibiotics as both display significant activity against *tolC*-mutant *E. coli*, and while linezolid lacks antibacterial activity against wild-type *E. coli*, gentamicin is active against several *E. coli* wild-type strains [50,51]. Fifteen compounds (**5**,

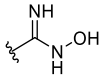

6, 25a-d, 25f-h, 25k, 25l, 25p, 25r, 25s, 26b) exhibited moderate to potent activity against the *E. coli* JW55031 (*tolC*-mutant) strain with MIC values ranging from 0.125 – 32 $\mu\text{g/mL}$. Compounds **5, 6, 25l**, and **25p** showed lower activity than compound **III**, while compounds **25c, 25h, 25k, 25r**, and **26b** demonstrated equipotent activity. Interestingly, compounds **25a, 25b, 25d, 25f, 25g**, and **25s** exhibited superior activity to compound **III** with **25d** manifesting the most potent activity with an MIC value of 0.125 $\mu\text{g/mL}$ representing a 16-fold improved activity in comparison to **III**.

Following this initial assessment, compounds displaying activity against *E. coli* JW55031 were examined against other medically important Gram-negative strains comprising *E. coli* BW25113 (wild-type), *A. baumannii*, and *N. gonorrhoeae*, where these compounds failed to exhibit significant activity (data not shown). Evidently, this lack of activity can be attributed to the presence of intact efflux pump systems, and/or failure to pass the outer membrane.

Table 1. Initial screening of the benzimidazole-based compounds **3-6, 11-13, 19a-l, 20a,b, 21a-c, 22a,b, III** against *tolC*-mutant *E. coli*

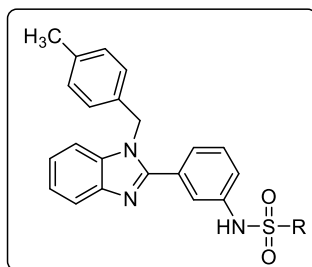


Compounds/ Control antibiotics	R ¹	R ²	R ³	R ⁴	R ⁵	MIC
3		CH ₃	H	H	H	>64
4		CH ₃	H	H	H	>64
5		CH ₃	H	H	H	32
6		CH ₃	H	H	H	8
11	NHSO ₂ CH ₃		H	H	H	>64
12	NHSO ₂ CH ₃		H	H	H	>64
13	NHSO ₂ CH ₃		H	H	H	>64
19a	NHSO ₂ CH ₃	CH ₃	NHCOOEt	H	H	>64
19b	NHSO ₂ CH ₃	CH ₃	H	NHCOOEt	H	>64

19c	NHSO ₂ CH ₃	CH ₃	COOEt	H	H	>64
19d	NHSO ₂ CH ₃	CH ₃	H	COOEt	H	>64
19e	NHSO ₂ CH ₃	CH ₃	H	H	COOEt	>64
19f	NHSO ₂ CH ₃	CH ₃	CN	H	H	>64
19g	NHSO ₂ CH ₃	CH ₃	H	CN	H	>64
19h	NHSO ₂ CH ₃	CH ₃	CH ₃	H	H	>64
19i	NHSO ₂ CH ₃	CH ₃	H	CH ₃	H	>64
19j	NHSO ₂ CH ₃	CH ₃	CF ₃	H	H	>64
19k	NHSO ₂ CH ₃	CH ₃	Br	H	H	>64
19l	NHSO ₂ CH ₃	CH ₃	H	Br	H	>64
20a	NHSO ₂ CH ₃	CH ₃	NH ₂	H	H	>64
20b	NHSO ₂ CH ₃	CH ₃	H	NH ₂	H	>64
21a	NHSO ₂ CH ₃	CH ₃	COOH	H	H	>64
21b	NHSO ₂ CH ₃	CH ₃	H	COOH	H	>64
21c	NHSO ₂ CH ₃	CH ₃	H	H	COOH	>64
22a	NHSO ₂ CH ₃	CH ₃		H	H	>64
22b	NHSO ₂ CH ₃	CH ₃	H		H	>64
III	NHSO ₂ CH ₃	CH ₃	H	H	H	2
Linezolid						8
Gentamicin						0.25

MIC, minimum inhibitory concentration ($\mu\text{g/mL}$)

Table 2. Initial screening of the benzimidazole-based compounds **25a-u**, **26a,b**, **III** against *tolC*-mutant *E. coli*



Compounds/ Control antibiotics	R	MIC
25a	cyclopropyl	1
25b	phenyl	1
25c	4-Cl phenyl	2
25d	3-Cl phenyl	0.125
25e	4-Br phenyl	>64
25f	3-Br phenyl	0.5
25g	3,4-diF phenyl	1
25h	3-Cl,4-F phenyl	2
25i	4-CF ₃ phenyl	>64

25j	4-OCF ₃ phenyl	>64
25k	4-CH ₃ phenyl	2
25l	4-OCH ₃ phenyl	4
25m	4-isopropyl phenyl	>64
25n	4-t-butyl phenyl	>64
25o	4-NHCOCH ₃ phenyl	>64
25p	3,4-diMeO phenyl	4
25q	4-NO ₂ phenyl	>64
25r	3-NO ₂ phenyl	2
25s	2-NO ₂ phenyl	1
25t	2-naphthyl	>64
25u	4-(1,1'-biphenyl)	>64
26a	4-NH ₂ phenyl	>64
26b	3-NH ₂ phenyl	2
III	CH ₃	2
Linezolid		8
Gentamicin		0.25

MIC, minimum inhibitory concentration (μg/mL)

2.3.2. Structure Activity Relationship (SAR)

To achieve antibacterial activity against wild-type Gram-negative bacteria, our first goal was to improve the bacterial membrane penetration and cellular accumulation of our compounds through chemical modifications of the lead compound **III** guided by the eNTRY rules [19]. Introduction of primary amines at ring C (**20a**, **20b**), or an amidine at ring A (**5**) to meet the ionizable amine requirement failed to show activity against wild-type or *tolC*-mutant *E. coli*, except for compound **5** that retained modest activity against *tolC*-mutant *E. coli* (MIC = 32 μg/mL), as we previously reported that the *meta*-position of ring A can tolerate polar substituents. Moreover, when an acidic functionality was introduced to ring A and B as tetrazole (**6**, **13**) or to ring C as carboxylic acid (**21a-c**) to improve the amphiphilic nature of these compounds, they were inactive against both wild-type or *tolC*-mutant *E. coli* except for compound **6** that showed moderate activity with an MIC value of 8 μg/mL against the *tolC*-mutant *E. coli*. Finally, introduction of polar substituents as iminoxime at ring A, B and C (**3**, **11**, **22a**, **22b**) or an oxadiazole moiety at ring A and B (**4**, **12**) to improve the polarity of the compounds rendered them inactive against both *E. coli* strains. In conclusion, all the suggested modifications failed to address the penetration problem in wild-type *E. coli* (data not shown) or yet to improve the activity against the *tolC*-mutant strain (**Table 1**) which might be contributed to the intricate balance between target

activity and cellular penetration and accumulation where increased polarity to achieve cellular accumulation might result in the loss of target activity.

In reference to our previously reported SAR study [37], we concluded that ring A favored polar *meta* substituents with a methanesulfonamide group being an optimum fragment, while ring B preferred a *para* hydrophobic group with a methyl group being optimal. Compound **III** combined these two fragments and showed optimal activity against the *tolC*-mutant *E. coli*. In continuation of our previous work, we focused on investigating the chemical space around the unexplored ring C and the methanesulfonamide moiety. First, we tested ring C with varied substituents at 5-, 6-, and 7-positions comprising polar substituents with hydrogen bond donor/acceptor features and variable sizes (**19a**: 5-NHCOOEt, **19b**: 6-NHCOOEt, **19c**: 5-COOEt, **19d**: 6-COOEt, **19e**: 7-COOEt, **19f**: 5-CN, **19g**: 6-CN, **20a**: 5-NH₂, **20b**: 6-NH₂, **21a**: 5-COOH, **21b**: 6-COOH, **21c**: 7-COOH, **22a**: 5-iminoxime, **22b**: 6-iminoxime) or small hydrophobic (**19h**: 5-CH₃, **19i**: 6-CH₃) versus bulky substituents (**19j**: 5-CF₃, **19k**: 5-Br, **19l**: 6-Br). Interestingly, all the introduced modifications at ring C rendered the compounds inactive against the *tolC*-mutant *E. coli* strain.

Next, we turned to the methanesulfonamide moiety and started with the extension of the methyl group into a bulkier cyclopropyl (**25a**) and a phenyl (**25b**). These derivatives retained antibacterial activity (MIC = 1 µg/mL) which indicated flexible binding requirements at this position. Following, we probed the introduced phenyl with variable substituents at the *ortho*, *meta*, and *para* positions, where 21 derivatives were synthesized and tested for their antibacterial activity. As inferred from **Table 2** and **Figure 2**, substitution at *meta*-position gave better activity than at *para*-position (**25d**: *m*-Cl versus **25c**: *p*-Cl, **25f**: *m*-Br versus **25e**: *p*-Br, **25r**: *m*-NO₂ versus **25q**: *p*-NO₂, **26b**: *m*-NH₂ versus **26a**: *p*-NH₂). *Meta*-position preferred hydrophobic substituents (**25d**: *m*-Cl and **25f**: *m*-Br; MIC values ranged from 0.125 to 0.5 µg/mL) to polar ones (**25r**: *m*-NO₂ and **26b**: *m*-NH₂; MIC value of 2 µg/mL) and *m*-chloro showed optimal activity with an MIC value of 0.125 µg/mL against the mutant *E. coli* strain. Regarding the *para*-position, small groups (**25c**: *p*-Cl, **25k**: *p*-CH₃, **25l**: *p*-OCH₃) were tolerated with comparable activity to compound **III**, while bulky or highly polar derivatives (**25e**: *p*-Br, **25i**: *p*-CF₃, **25j**: *p*-OCF₃, **25m**: *p*-isopropyl, **25n**: *p*-*t*-butyl, **25o**: *p*-NHCOCH₃, **25q**: *p*-NO₂) abolished the activity. Disubstitution at both *meta*- and *para*-positions (**25g**: 3,4-diF, **25h**: 3-Cl,4-F) reduced the activity when compared to the *meta*-

monosubstitution, except for 3,4-diMeO (**25p**) that showed similar activity as its *para* derivative (**25l**). Replacement of the sulfonamide phenyl group with 2-naphthyl (**25t**) or biphenyl (**25u**) resulted in total loss of activity. Finally, *ortho* substitution with a nitro group (**25s**) displayed comparable activity to compound **III**.

In summary, substitution of ring C proved detrimental to antibacterial activity, which indicates stringent structural requirements for this ring as even minor modifications such as introducing a small methyl group rendered the compound totally inactive. On the other hand, the methanesulfonamide moiety allowed greater variation regarding bulkier substituents than methyl and a distinct SAR for the introduced phenyl group. This data support the premise that our benzimidazole-based derivatives exerted their antibacterial activity through a plausible cellular target and not through promiscuous activity as opposed to compounds that display a flat SAR which indicates non-selective growth interaction or no binding to a specific target [52]. However, it is a challenge to direct the structure-activity relationship study based on the *in vivo* activity without knowing the exact molecular targets [53,54].

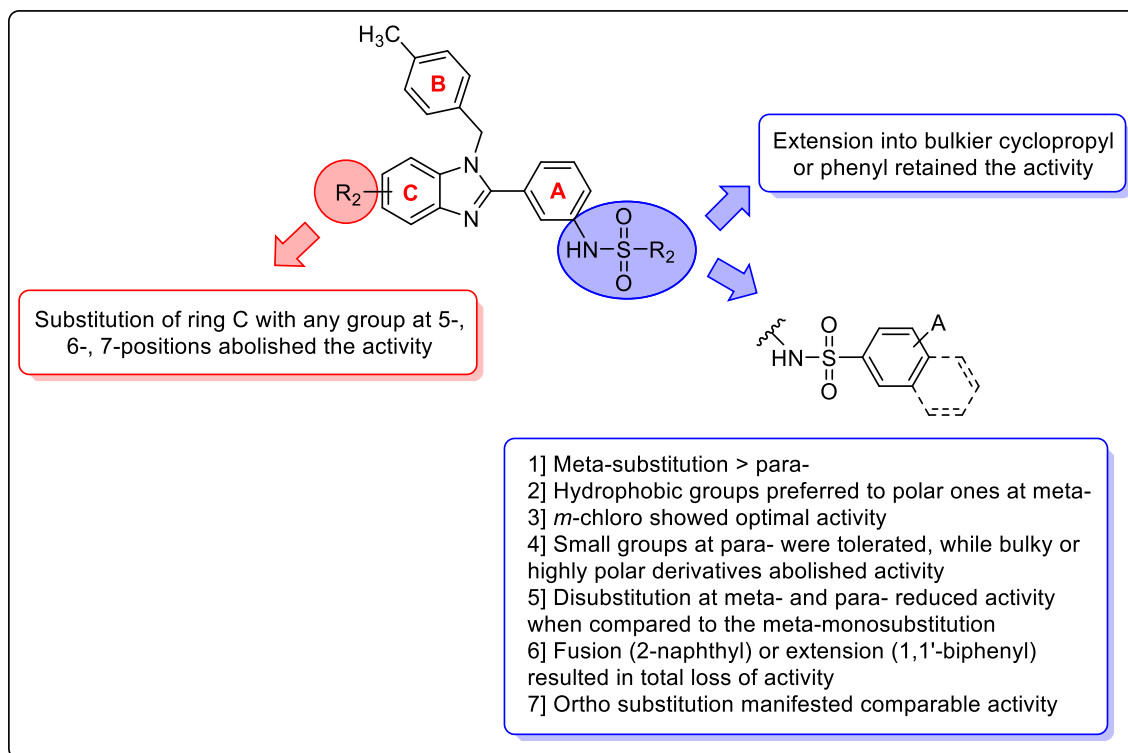


Figure 2. Structure activity relationship of the synthesized benzimidazole derivatives against *tolC*-mutant *E. coli*.

2.3.3. *In vitro* cytotoxicity evaluation of the benzimidazole derivative 25d

The lack of toxicity is one of the essential attributes in development of new drugs. In this regard, the most potent benzimidazole derivative **25d** was evaluated for its cytotoxicity against kidney epithelial (Vero) cells to determine its potential *in vitro* cytotoxicity against mammalian cells. The compound exhibited an excellent safety profile and tolerability to Vero cells without any significant cytotoxicity. The compound's CC_{50} (concentration required for reducing the cell viability by 50%) was higher than 128 $\mu\text{g/mL}$ (**Figure 3**). These results are in coincidence with the results of the cytotoxicity for the lead compound **III** which displayed similar cell tolerability against Vero cells [37]. In summary, the newly developed benzimidazole derivative, **25d**, exhibited 16-fold more potent antibacterial activity as compared to the lead compound **III** while retaining the important feature of lack of cytotoxicity to mammalian cells.

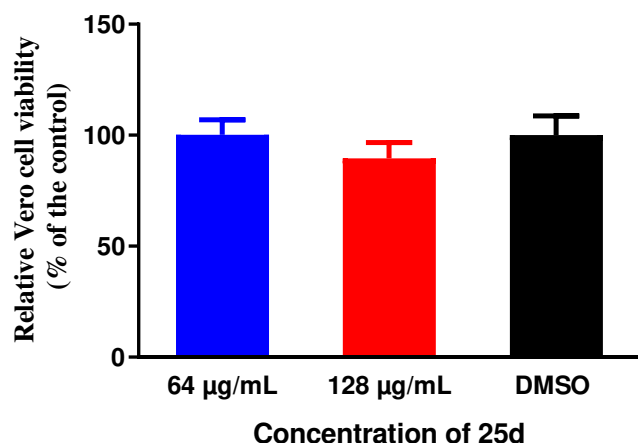


Figure 3. Cytotoxicity assessment of compound **25d** (tested in quadruplicate) against kidney epithelial cells (Vero) using the MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay. Results are presented as percent viable cells relative to DMSO (negative control). The absorbance values represent an average of four samples analyzed for the compound. Error bars represent standard deviation values.

2.3.4. *Time-kill kinetics of 25d*

The benzimidazole derivative **25d** was assessed in a killing kinetics assay to further validate its antibacterial mode of killing (**Figure 4**). The compound exhibited a bacteriostatic activity against *E. coli* JW55031. Yet, **25d** was found to significantly reduce *E. coli* burden as compared to DMSO (negative control). After 24 h, **25d** (10 \times MIC) reduced *E. coli* burden by 0.4-

log₁₀ units. In addition, at the 24-h time point the compound provided around 2.54- and 2.9 log₁₀-reduction in the *E. coli* count as compared to the DMSO-treated control.

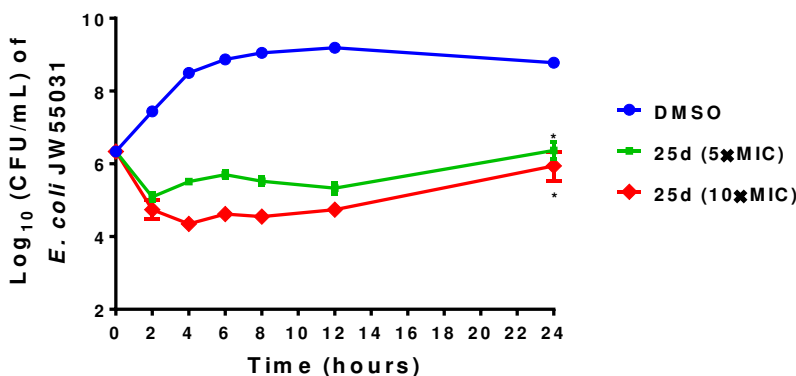


Figure 4. Time-kill assay of **25d** (at 5 × and 10 × MIC) against *E. coli* JW55031. DMSO (vehicle) served as a negative control. The error bars represent standard deviation values. The data were analyzed via a two-way ANOVA with post-hoc Dunnett's test for multiple comparisons. An asterisk (*) indicates a statistically significant difference ($P < 0.05$) between treatment with **25d** compared to DMSO treatment (negative control).

2.3.5. Synergistic activity of **25d** with colistin.

The outer membrane of Gram-negative bacteria is well known to limit the intracellular access of antibiotics into bacterial cells resulting in the overall failure of antibacterial therapy [55]. To test whether the outer membrane was hindering the antibacterial activity of the benzimidazole compounds, compound **25d** was combined with a sub-inhibitory concentration (0.25× MIC) of colistin -a membrane disrupting antibiotic- and tested against four Gram-negative bacterial strains. Introducing a sub-inhibitory concentration of colistin resulted in an increased activity of **25d** against the tested Gram-negative strains with MIC values ranging from 4 to 16 µg/mL. These results suggest that the outer membrane of Gram-negative bacteria contributed to a great extent to the resistance against the presented compounds. Erythromycin and daptomycin were included as control antibiotics. Erythromycin is active against Gram-negative bacteria but its activity is impeded by the outer membrane [50]. Therefore, when combined with colistin, its penetration into the bacterial cells increased leading to an enhancement of activity (MIC = 0.25 - 8 µg/mL). Conversely, daptomycin is active against Gram-positive bacteria only [56] and hence, it could not inhibit Gram-negative bacteria even in the presence of colistin (**Table 3**).

Table 3. MICs (µg/mL) of **25d** and control antibiotics against Gram-negative bacterial isolates in the absence and presence of sub-inhibitory concentrations (0.25 × MIC) of colistin.

Compound/ Control antibiotics	<i>E. coli</i> BW25113 (wild-type strain)		<i>K. pneumoniae</i> ATCC 1706		<i>A. baumannii</i> ATCC 19606		<i>P. aeruginosa</i> ATCC 9027	
	(-) COL	(+) COL	(-) COL	(+) COL	(-) COL	(+) COL	(-) COL	(+) COL
25d	>64	16	>64	8	>64	4	>64	16
Erythromycin	32	2	64	4	8	0.25	>64	8
Daptomycin	>128	>128	>128	>128	>128	>128	>128	>128

COL: Colistin

2.3.6. Formulation studies of **III** and **25d**

Lipidic nanocapsules were prepared for our reference compound (**III**) and the most promising derivative; **25d**, for possible potentiation of their antibacterial activity against resistant strains, based on reports that loading of antibacterials into nanoparticles potentiated their activity [57,58]. Several functional additives were used to prepare the nanocapsules, namely tween 80, stearylamine, cremophor RH 40 and verapamil. Stearylamine is known to impart a positive charge to the nanoparticles, which was reported to enhance the antibacterial activity of antibiotics in both Gram-negative and Gram-positive bacteria [59]. On the other hand, tween 80, cremophor RH 40 and verapamil were reported to exhibit efflux pump inhibitory activity [60–62].

The nanocapsules particle size ranged from 64.3 to 69.5 nm, their polydispersity ranged from 0.41-0.43, and the zeta potential of formulations containing stearylamine ranged from +4.09 to +10.6 mV, and those not containing stearylamine ranged from -3.58 to -6.08 mV. Their encapsulation efficiency for compounds **III** and **25d** ranged from 99.1% to 100.5% owing to their lipophilicity.

As shown in **table 4**, compound **25d** formulations demonstrated better antibacterial activity than **III**. Moreover, comparing formula A2 and formula B2 (both loaded with **25d**) with the blank formulations (Blank A and B), it is obvious that formula B2 exhibited better antibacterial activity (containing both stearylamine and tween 80) as compared to formula A2 (containing tween 80 only). Formula B2 exhibited an MIC value of 16 and 32 $\mu\text{g/mL}$ against *A. Baumannii* and *N. gonorrhoeae*, respectively, whereas the blank formulation (Blank B) did not demonstrate any antibacterial effect on its own on all the bacterial strains tested (**Table 4**). However, formula B2 exhibited lower activity (4 $\mu\text{g/mL}$) than the free **25d** (0.125 $\mu\text{g/mL}$) against *tolC*-mutant *E. coli* and failed to show any activity against *E. coli* BW25113 (wild-type).

Accordingly, formulation B was further optimized. The concentration of stearylamine was increased from 10 mg to 20 mg (formulation C). Blank C displayed modest antibacterial effect against *N. gonorrhoeae*, which may imply a role of the increased intensity of cationic charge on the nanoparticles with the interaction with the cellular membrane of these particular species. This led to one-fold increase in the antibacterial activity of formula C against *N. gonorrhoeae* (MIC = 16 µg/mL). *N. gonorrhoeae* represents an urgent public health threat worldwide that causes more than 600,000 infection cases annually in the U.S. alone [63–65]. Due to the increasing incidence of infections and bacterial resistance to most antibiotics, there is a prompt need to discover new anti-gonococcal therapeutics.

Afterwards, further optimization was performed to assess the effect of other efflux pump inhibitors. Formulations D and E were prepared using cremophor RH 40 and verapamil respectively, while keeping the amount of stearylamine at 10 mg. Blank D prepared using cremophor RH 40 did not mostly display antibacterial action on its own (only weak activity against *N. gonorrhoeae*). When loaded with **25d**, it resulted in 4-fold enhancement of the activity (MIC = 16 µg/mL) against *N. gonorrhoeae*. Similarly, shifting the efflux pump inhibitor type to verapamil led to a slight increase in the antibacterial activity against *N. gonorrhoeae*.

Results of this encapsulation experiment suggest that further optimization of the nanoparticles' formulation is still necessary, in order to reach a formulation which can potentiate the antibacterial effect of **25d** on all tested Gram-negative bacterial strains.

Table 4. MICs (µg/mL) of **III**, **25d** nanoparticulate formulations and their blanks against Gram-negative bacterial isolates.

Formulations ^{a/} Test agents/ Control drugs	<i>E. coli</i> JW55031 (TolC Mutant)	<i>E. coli</i> BW25113 (wild-type)	<i>A. Baumannii</i> ATCC 19606	<i>N. gonorrhoeae</i> CDC 165
Formula A1 (III)	64	>64	>64	>64
Formula A2 (25d)	>64	>64	>64	64
Blank A	>64	>64	>64	>64
Formula B1 (III)	>64	>64	>64	64
Formula B2 (25d)	4	>64	16	32
Blank B	>64	>64	>64	>64
Formula C (25d)	>64	>64	>64	16
Blank C	>64	>64	>64	32
Formula D (25d)	>64	>64	>64	16
Blank D	>64	>64	>64	64

Formula E (25d)	>64	>64	>64	32
Blank E	>64	>64	>64	64
III	2	>64	>64	>64
25d	0.125	>64	>64	>64
Gentamicin	0.25	0.25	16	NT
Azithromycin	NT	NT	NT	1

^aComposition of different nanoparticulate formulations containing either **III** or **25d** is described in the experimental section, **Table A**.

^bNT, not tested.

3. Conclusion

Our previously reported SAR study on a 1,2-disubstituted benzimidazole series, identified compound **III** which exhibited potent activity against *tolC*-mutant *Escherichia coli* with an MIC value of 2 µg/mL while lacking activity against wild-type Gram-negative strains. Combination with colistin partially restored compound **III** antibacterial activity (8-16 µg/mL) against these strains. In continuation to the previous study, we synthesized and screened a total of 49 novel derivatives for their *in vitro* antibacterial activity. SAR investigation of the unexplored ring C indicated the detrimental effect of any substitution on antibacterial activity (compounds **19a-l**, **20a,b**, **21a-c**, and **22a,b**), while extension of the methanesulfonamide moiety of compound **III** gave more active derivatives, as exemplified by compound **25d** which exhibited the most potent activity against *E. coli* JW55031 (*tolC*-mutant) strain with an MIC value of 0.125 µg/mL representing a 16-fold improved activity in comparison to **III**. Time-kill kinetics assay indicated that **25d** possessed bacteriostatic activity. Attempts to overcome low permeability and improve cellular accumulation, by applying the “eNTRY rules” to our structural design failed to address this problem which indicates the intricate balance between maintaining the target activity and improving the cellular accumulation. Conversely, combination of **25d** with sub-inhibitory concentration of colistin or formulation as lipid nanocapsules displayed favorable synergism and partially restored the antibacterial activity against some of the tested wild-type Gram-negative bacterial strains with compound **25d** showing superior activity compared to the lead compound **III**. In addition, compound **25d** displayed an excellent safety profile against mammalian cells. Based on these premises, compound **25d** presents a valid candidate for further structural modification and formulation optimization to improve its penetration and cellular accumulation in Gram-negative bacteria.

4. Experimental

4.1. Chemistry

Chemicals and solvents were purchased from Sigma-Aldrich (Germany) and Alfa Aesar (Germany) and were used as such without further purification. Reactions were followed using analytical thin layer chromatography (TLC), performed on Aluminum silica gel 60 F₂₅₄ TLC plates, purchased from Merck, with visualization under UV light (254 nm). Melting points were measured in capillary tubes using Stuart Scientific SMP1 apparatus and reported uncorrected. FT-IR spectra were measured with a Thermo Scientific Nicolet iS10 spectrometer using a KBr disc. ¹HNMR spectra were determined on an Agilent Technologies VNMRS 400 or a Varian INOVA 500 NMR spectrometer in δ scale (ppm) and J (Hz) and was referred to the deuterated solvent peak (DMSO-*d*₆ δ = 2.5 ppm). ¹³CNMR spectra were determined on either instrument at 101 MHz or 126 MHz and referred to the solvent peak (DMSO-*d*₆ δ = 39.52 ppm). All NMR datasets were acquired at 298 K unless otherwise stated. APCI mass spectrometry was carried out on an Advion Expression compact mass spectrometer. High resolution mass spectrometry (HRMS) was carried out using a Bruker MaXis Impact Time of Flight spectrophotometer, using electrospray ionization (ES+). The purity of the final compounds was assessed by HPLC on an Agilent 1290 Infinity Series equipped with a UV detector and a C₁₈ reverse phase column eluting with two systems: MeOH-H₂O gradient (5-95%) and 0.1% TFA over 30 mins at a flow rate of 1 mL/min or MeCN-H₂O gradient (5-95%) and 0.1% TFA over 5 mins at a flow rate of 0.5 mL/min. MarvinSketch 21.3.0 was used for characterizing chemical structures [66].

4.1.1. General procedure for the synthesis of target compounds 3-6

Intermediates **1** and **2** were synthesized as previously reported [37]. The desired final compounds **3-6** were synthesized according to the following procedures [39–42] and is illustrated as follows.

Step (a): A mixture of *o*-phenylenediamine (5.41 g, 50.0 mmol, 1.00 equiv), 3-cyano benzaldehyde (6.88 g, 52.5 mmol, 1.05 equiv) and sodium acetate (4.10 g, 50.0 mmol, 1.00 equiv) in EtOH (150 mL) was refluxed for 48 h. Upon completion of the reaction as indicated by TLC, the mixture was cooled, evaporated, stirred with cold water and the resulting solid was filtered,

washed with water, and dried to yield the desired 3-(1*H*-benzimidazol-2-yl)benzonitrile (**1**) in 61% yield that was used as such in the next step.

Step (b): To a solution of 3-(1*H*-benzimidazol-2-yl)benzonitrile (**1**) (2.19 g, 10.0 mmol, 1.00 equiv) in DMF (20 mL) was added Cs₂CO₃ (3.91 g, 12.0 mmol, 1.20 equiv) and the suspension was stirred at room temperature for 30 minutes. 4-methylbenzyl chloride (1.55 g, 11.0 mmol, 1.10 equiv) was added and the reaction mixture was stirred at room temperature for 48 h. Upon completion of the reaction as indicated by TLC, the mixture was poured into ice, stirred for 1 hr and the resulting solid was filtered, washed with water, dried, and purified by column chromatography (EtOAc/Hexane 1:4) to yield the desired 3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)benzonitrile (**2**) in 48% yield.

Step (c): To a solution of 3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)benzonitrile (**2**) (1.00 g, 3.10 mmol, 1.00 equiv) in EtOH (50 mL) was added hydroxylamine solution (50% in water, 0.60 mL, 9.30 mmol, 3.00 equiv) and the reaction mixture was heated under reflux for 4 h. The solvent was removed in vacuo and the resulting solid was crystallized from EtOH to afford the target compound (**3**).

4.1.1.1. *N*-hydroxy-3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)benzimidamide (3**).** Crystallized from EtOH. White solid, yield 85%, mp: 106-108 °C. **FT-IR** (ν max, cm⁻¹): 3700-3100 (Iminoxime), 2972, 2917 (CH aliphatic), 1686 (C=N), 1639 (C=C). **¹H NMR (500 MHz, DMSO-*d*₆):** δ 9.76 (s, 1H), 8.11 (s, 1H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.74 (d, *J* = 7.1 Hz, 1H), 7.70 (d, *J* = 7.7 Hz, 1H), 7.54 (t, *J* = 7.7 Hz, 1H), 7.47 (d, *J* = 7.0 Hz, 1H), 7.25 (tt, *J* = 5.9, 6.2 Hz, 2H), 7.08 (d, *J* = 7.7 Hz, 2H), 6.90 (d, *J* = 7.7 Hz, 2H), 5.93 (s, 2H), 5.57 (s, 2H), 2.22 (s, 3H). **¹³C NMR (126 MHz, DMSO-*d*₆):** δ 153.5, 150.8, 143.2, 137.2, 136.3, 134.5, 134.3, 130.6, 129.8 (2C), 129.7, 129.1, 127.2, 126.7, 126.7 (2C), 123.2, 122.7, 119.8, 111.7, 47.8, 21.1. **(ESI-TOF) HRMS *m/z*: [M+H]⁺ calcd for C₂₂H₂₁N₄O: 357.1710; found: 357.1715.**

Step (d): A solution of *N*-hydroxy-3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)benzimidamide (**3**) (0.21 g, 0.59 mmol) in triethyl orthoformate (5 mL) was refluxed for 24 h. The resulting mixture was concentrated in vacuo and the residue was purified by column chromatography to give the desired product (**4**).

4.1.1.2. 3-(3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl)-1,2,4-oxadiazole (4). $R_f = 0.25$ (DCM/MeOH 9.7:0.3). White solid, yield 58%, mp: 116-118 °C. **FT-IR** (ν_{max} , cm^{-1}): 3058, 3020 (CH aromatic), 2917 (CH aliphatic), 1651 (C=N), 1611 (C=C). **^1H NMR (500 MHz, DMSO- d_6)**: δ 9.77 (s, 1H), 8.44 (s, 1H), 8.19 (d, $J = 7.8$ Hz, 1H), 7.95 (d, $J = 7.8$ Hz, 1H), 7.79 - 7.72 (m, 2H), 7.51 (q, $J = 2.9$ Hz, 1H), 7.28 (q, $J = 3.1$ Hz, 2H), 7.09 (d, $J = 7.9$ Hz, 2H), 6.92 (d, $J = 7.9$ Hz, 2H), 5.60 (s, 2H), 2.23 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6)**: δ 168.2, 166.8, 152.6, 143.1, 137.2, 136.5, 134.2, 132.2, 131.7, 130.4, 129.8 (2C), 128.8, 128.3, 127.0, 126.6 (2C), 123.4, 122.9, 119.9, 111.8, 47.9, 21.1. **(ESI-TOF) HRMS m/z : [M+H] $^+$ calcd for $\text{C}_{23}\text{H}_{19}\text{N}_4\text{O}$: 367.1553; found: 367.1553.**

Step (e): To a solution of *N*-hydroxy-3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)benzimidamide (**3**) (0.20 g, 0.56 mmol, 1.00 equiv) in glacial AcOH (3 mL) was added Ac₂O (0.09 g, 80.0 μL , 0.84 mmol, 1.50 equiv) and stirred at room temperature for 0.5 h. Upon completion of the reaction as indicated by TLC, Pd/C (5%, 0.04 g) was added, and the reaction mixture was stirred at room temperature under H₂ using balloon for 2h. Upon completion of the reaction as indicated by TLC, the mixture was filtered through celite, and the filter pad was washed with glacial AcOH (10 mL). The filtrate was evaporated in vacuo, and the resulting residue was dissolved in water, NaOH solution (10%) was added dropwise till a white precipitate was formed. The precipitate was filtered, dried, and purified by flash chromatography to yield the desired product (**5**).

4.1.1.3. 3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)benzimidamide (5). $R_f = 0.45$ (DCM/MeOH 9.5:0.5). White solid, yield 60%, mp: 120-122 °C. **FT-IR** (ν_{max} , cm^{-1}): 3397, 3198 (NH₂), 2930 (CH aliphatic), 1650 (C=N), 1598 (C=C). **^1H NMR (500 MHz, DMSO- d_6)**: δ 8.18 (s, 1H), 7.93 (d, $J = 7.8$ Hz, 1H), 7.77 (d, $J = 7.8$ Hz, 1H), 7.73 (q, $J = 2.9$ Hz, 1H), 7.56 (t, $J = 7.8$ Hz, 1H), 7.47 (q, $J = 2.9$ Hz, 1H), 7.25 (tt, $J = 2.2, 4.6$ Hz, 2H), 7.08 (d, $J = 7.9$ Hz, 2H), 6.90 (d, $J = 8.0$ Hz, 2H), 6.54 (s, 3H), 5.57 (s, 2H), 2.22 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6)**: δ 162.3, 153.4, 143.1, 137.4, 137.2, 136.3, 134.3, 130.6, 130.5, 129.8 (2C), 129.0, 128.4, 127.9, 126.6 (2C), 123.2, 122.7, 119.7, 111.7, 47.8, 21.1. **(ESI-TOF) HRMS m/z : [M+H] $^+$ calcd for $\text{C}_{22}\text{H}_{21}\text{N}_4$: 341.1760; found: 341.1757.**

Step (f): A mixture of 3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)benzimidamide (**2**) (0.40 g, 1.23 mmol, 1.00 equiv), NaN₃ (0.24 g, 3.70 mmol, 3.00 equiv), and NH₄Cl (0.2 g, 3.70 mmol,

3.00 equiv)) in dry DMF (10 mL) was heated at 125°C for 24 h. Upon completion of the reaction as indicated by TLC, the mixture was poured into ice/water and 4N HCl was added dropwise till precipitation. The crude product was filtered, dried, and purified by flash chromatography to give the desired product (**6**).

4.1.1.4. 2-(3-(1H-tetrazol-5-yl)phenyl)-1-(4-methylbenzyl)-1H-benzimidazole (6). $R_f = 0.15$ (DCM/MeOH 9.7:0.3). White solid, yield 20%, mp: 124-126 °C. **FT-IR** (ν_{max} , cm^{-1}): 3705 (NH tetrazole), 3448 (NH sulfonamide), 1654 (C=N), 1617 (C=C). **^1H NMR (500 MHz, DMSO- d_6):** δ 8.50 (s, 1H), 8.21 (d, $J = 7.6$ Hz, 1H), 7.90 (d, $J = 7.6$ Hz, 1H), 7.76 (t, $J = 7.7$ Hz, 2H), 7.54 (t, $J = 4.2$ Hz, 1H), 7.29 (t, $J = 3.7$ Hz, 2H), 7.08 (d, $J = 7.7$ Hz, 2H), 6.91 (d, $J = 7.7$ Hz, 2H), 5.63 (s, 2H), 2.21 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6):** δ 155.8, 152.6, 143.1, 137.2, 136.5, 134.2, 131.8, 131.5, 130.4, 129.8 (2C), 128.6, 128.3, 126.6 (2C), 125.7, 123.5, 122.9, 119.8, 111.8, 47.9, 21.1. **(ESI-TOF) HRMS m/z : [M+H] $^+$ calcd for $\text{C}_{22}\text{H}_{19}\text{N}_6$: 367.1665; found: 367.1663. HPLC purity: 98.7%, HPLC t_R : 2.43 min.**

4.1.2. General procedure for the synthesis of target compounds 11-13

Intermediates **7-10** were synthesized as previously reported [37], and the desired final compounds **11-13** were synthesized using the same procedures discussed before for the synthesis of compounds **3-6**.

4.1.2.1. N-hydroxy-4-((2-(3-(methylsulfonamido)phenyl)-1H-benzimidazol-1-yl)methyl)benzimidamide (11). Crystallized from EtOH. White solid, yield 75%, mp: 135-137 °C. **FT-IR** (ν_{max} , cm^{-1}): 3726-3385 (Iminoxime and NH sulfonamide), 2930 (CH aliphatic), 1643 (C=N), 1610 (C=C). **^1H NMR (500 MHz, DMSO- d_6):** δ 9.99 (s, 1H), 9.62 (s, 1H), 7.75 (dd, $J = 2.0, 6.5$ Hz, 1H), 7.64 (s, 1H), 7.58 (d, $J = 8.3$ Hz, 2H), 7.49 (t, $J = 7.8$ Hz, 2H), 7.42 (d, $J = 7.6$ Hz, 1H), 7.35 (t, $J = 4.0$ Hz, 1H), 7.26 (tt, $J = 4.1, 7.4$ Hz, 2H), 6.99 (d, $J = 8.2$ Hz, 2H), 5.76 (s, 2H), 5.61 (s, 2H), 2.99 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6):** δ 153.2, 150.8, 143.1, 139.4, 137.9, 136.4, 133.1, 131.6, 130.3, 126.4 (2C), 126.2 (2C), 124.5, 123.3, 122.8, 121.4, 120.7, 119.8, 111.6, 47.7, 39.9. **(ESI-TOF) HRMS m/z : [M+H] $^+$ calcd for $\text{C}_{22}\text{H}_{22}\text{N}_5\text{O}_3\text{S}$: 436.1438; found: 436.1454.**

4.1.2.2. N-(3-(1-(4-(1,2,4-oxadiazol-3-yl)benzyl)-1H-benzimidazol-2-yl)phenyl)methanesulfonamide (12). $R_f = 0.35$ (DCM/MeOH 9.7:0.3). White solid, yield 52%,

mp: 115-117 °C. **FT-IR** (ν max, cm^{-1}): 3263 (NH sulfonamide), 2923 (CH aliphatic), 1651 (C=N), 1615 (C=C). **^1H NMR (400 MHz, DMSO- d_6)**: δ 9.96 (s, 1H), 9.68 (s, 1H), 7.97 (d, $J = 6.7$ Hz, 2H), 7.77 (d, $J = 7.1$ Hz, 1H), 7.62 (s, 1H), 7.54 – 7.40 (m, 3H), 7.36 (d, $J = 7.8$ Hz, 1H), 7.28 (d, $J = 4.7$ Hz, 2H), 7.20 (d, $J = 6.6$ Hz, 2H), 5.70 (s, 2H), 2.98 (s, 3H).

4.1.2.3. N-(3-(1-(4-(1H-tetrazol-5-yl)benzyl)-1H-benzimidazol-2-yl)phenyl)methanesulfonamide (13). $R_f = 0.14$ (DCM/MeOH 9.7:0.3). White solid, yield 29%, mp: 88-90 °C. **FT-IR** (ν max, cm^{-1}): 3727 (NH tetrazole), 3449 (NH sulfonamide), 2927 (CH aliphatic), 1654 (C=N), 1617 (C=C). **^1H NMR (500 MHz, DMSO- d_6)**: δ 9.99 (s, 1H), 7.96 (d, $J = 8.1$ Hz, 2H), 7.77 (d, $J = 7.0$ Hz, 1H), 7.63 (s, 1H), 7.49 (t, $J = 7.8$ Hz, 2H), 7.43 (d, $J = 7.6$ Hz, 1H), 7.36 (d, $J = 7.7$ Hz, 1H), 7.28 (tt, $J = 6.2, 6.5$ Hz, 2H), 7.21 (d, $J = 8.1$ Hz, 2H), 5.69 (s, 2H), 2.98 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6)**: δ 155.8, 153.2, 143.1, 140.3, 139.3, 136.4, 131.5, 130.3, 127.8 (2C), 127.6 (2C), 124.6, 124.3, 123.4, 122.9, 121.4, 120.6, 119.9, 111.6, 47.8, 39.9. **(ESI-TOF) HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{20}\text{N}_7\text{O}_2\text{S}$: 446.1394; found: 446.1412.**

4.1.3. General procedure for the synthesis of target compounds 19a-l, 20a,b, 21a-c, and 22a,b

The desired final compounds **19a-l**, **20a,b**, **21a-c**, and **22a,b** were synthesized according to the reported procedures [37,39,43–47] and is illustrated as follows.

Step (a): A solution of the appropriate aniline derivative (**14a, b**) (2.26 g, 14.5 mmol, 1.00 equiv) and DIPEA (2.25 g, 3.03 mL, 17.4 mmol, 1.20 equiv) in THF (30 mL) was cooled to 0°C, then ethyl chloroformate (1.65 g, 1.45 mL, 15.2 mmol, 1.05 equiv) was added dropwise and the reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was evaporated, partitioned between EtOAc and water, washed with 2M HCl, dried, and evaporated to give the desired carbamate derivatives (**16a, b**) in 87-92% yield.

Step (b): To a solution of the appropriate benzoic acid derivative (**15a-c**) (1.85 g, 10.0 mmol) in EtOH (40 mL) was added conc. H_2SO_4 (0.5 mL) dropwise and the reaction mixture was refluxed for 24 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was evaporated, partitioned between EtOAc and water, washed with sodium bicarbonate solution (10%), brine, dried, and evaporated to give the desired ethyl benzoate derivatives (**16c-e**) in 87-89% yield.

Step (c): A suspension of the appropriate fluoro derivative (**16a-l**) (8.00 mmol, 1.00 equiv), 4-methylbenzylamine (1.16 g, 1.22 mL, 9.60 mmol, 1.20 equiv), and K₂CO₃ (1.66 g, 12.0 mmol, 1.50 equiv) in anhydrous DMF (10 mL) was heated at 80-90°C for 12 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into ice/water (200 mL), stirred vigorously, and the resulting solid was filtered, washed with n-heptane, and dried to yield the desired derivatives (**17a-l**) in 78-95% yield.

Step (d): (1) A suspension of the appropriate nitro derivative (**17a, b, h, i, k, l**) (6.00 mmol, 1.00 equiv), iron powder (1.00 g, 18.0 mmol, 3.00 equiv), and NH₄Cl (2.90 g, 54.0 mmol, 9.00 equiv) in 70% EtOH/H₂O (60 mL) was refluxed for 6 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was filtered on celite, evaporated, stirred with EtOAc, filtered to remove the insoluble salts, dried and, evaporated to give the corresponding diamine derivative as sticky residue that was used as such into the following step.

Or **(2)** A solution of the appropriate nitro derivative (**17c-g, j**) (6.00 mmol, 1.00 equiv) and SnCl₂·2H₂O (6.80 g, 30.0 mmol, 5.00 equiv) in EtOAc (100 mL) was refluxed for 16 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was cooled, washed with sodium bicarbonate solution (10%), brine, dried, and evaporated to give the corresponding diamine derivative as sticky residue that was used as such into the following step.

Step (e): A solution of the appropriate crude diamine derivative (5.00 mmol, 1.00 equiv) and sodium hydroxy(3-nitrophenyl)methanesulfonate (1.27 g, 5.00 mmol, 1.00 equiv) in DMF (5 mL) was heated at 130°C for 4 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into ice/water (100 mL), stirred vigorously, and the resulting solid was filtered, dried, and purified by column chromatography (EtOAc/heptane) to yield the desired benzimidazole derivatives (**18a-l**) in 34-59% yield.

Step (f): A solution of the appropriate nitro derivative (**18a-l**) (2.00 mmol, 1.00 equiv) and SnCl₂·2H₂O (2.30 g, 10.0 mmol, 5.00 equiv) in EtOAc (50 mL) was refluxed for 16 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was cooled, washed with sodium bicarbonate solution (10%), brine, dried and evaporated to give the corresponding amine derivative that was used as such into the following step.

Step (g): To a solution of the appropriate amine derivative (2 mmol, 1.00 equiv) in pyridine (5 mL) at 0°C was added methanesulfonyl chloride (275 mg, 186 μ L, 2.40 mmol, 1.20 equiv) dropwise and the reaction mixture was stirred at room temperature for 48 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into ice/water (100 mL), stirred vigorously, and the resulting solid was filtered, dried, and purified by column chromatography to yield the desired final products (**19a-l**).

4.1.3.1. Ethyl (1-(4-methylbenzyl)-2-(3-(methylsulfonamido)phenyl)-1H-benzimidazol-5-yl)carbamate (19a). $R_f = 0.30$ (EtOAc/heptane 2:1). White solid, yield 63%, mp: 196-198 °C. **FT-IR** (ν_{max} , cm^{-1}): 3348 (NH sulfonamide), 2986, 2937 (CH aliphatic), 1728 (C=O), 1603 (C=C). **^1H NMR (400 MHz, DMSO- d_6)** δ 9.95 (s, 1H), 9.55 (s, 1H), 7.87 (s, 1H), 7.61 (s, 1H), 7.47 (t, $J = 7.8$ Hz, 1H), 7.40 (d, $J = 7.8$ Hz, 1H), 7.38 – 7.32 (m, 2H), 7.28 (dd, $J = 8.8, 1.4$ Hz, 1H), 7.07 (d, $J = 7.9$ Hz, 2H), 6.86 (d, $J = 8.0$ Hz, 2H), 5.49 (s, 2H), 4.14 (q, $J = 7.1$ Hz, 2H), 2.98 (s, 3H), 2.22 (s, 3H), 1.25 (t, $J = 7.1$ Hz, 3H). **^{13}C NMR (126 MHz, DMSO- d_6)** δ 154.3, 153.4, 143.2, 139.3, 137.1, 134.9, 134.2, 132.3, 131.6, 130.2, 129.7, 129.4 (2C), 126.6 (2C), 126.5, 124.5, 121.3, 120.7, 111.4, 60.5, 47.7, 39.8, 21.0, 15.0. **MS (ESI positive) m/z [M+H] $^+$:** 479.1. **(ESI-TOF) HRMS m/z : [M+H] $^+$ calcd for $\text{C}_{25}\text{H}_{27}\text{N}_4\text{O}_4\text{S}$: 479.1748; found: 479.1766.** **HPLC purity: 98.02%, HPLC t_R : 8.52 min.**

4.1.3.2. Ethyl (1-(4-methylbenzyl)-2-(3-(methylsulfonamido)phenyl)-1H-benzimidazol-6-yl)carbamate (19b). $R_f = 0.35$ (EtOAc/heptane 2:1). Light buff solid, yield 58%, mp: 228-230 °C. **FT-IR** (ν_{max} , cm^{-1}): 3366 (NH carbamate), 3265 (NH sulfonamide), 2969, 2930 (CH aliphatic), 1716 (C=O), 1610 (C=C). **^1H NMR (400 MHz, DMSO- d_6)** δ 9.93 (s, 1H), 9.63 (s, 1H), 7.67 (s, 1H), 7.62 – 7.58 (m, 2H), 7.44 (t, $J = 7.9$ Hz, 1H), 7.36 – 7.30 (m, 2H), 7.21 (dd, $J = 8.7, 1.9$ Hz, 1H), 7.09 (d, $J = 7.9$ Hz, 2H), 6.87 (d, $J = 8.0$ Hz, 2H), 5.42 (s, 2H), 4.08 (q, $J = 7.1$ Hz, 2H), 2.95 (s, 3H), 2.22 (s, 3H), 1.20 (t, $J = 7.1$ Hz, 3H). **^{13}C NMR (126 MHz, DMSO- d_6)** δ 154.1, 152.7, 139.3, 138.8, 137.1, 136.5, 135.5, 134.0, 131.6, 130.2, 129.9, 129.8 (2C), 126.3 (2C), 124.3, 121.2, 120.6, 119.7, 115.1, 60.5, 47.7, 39.8, 21.0, 15.0. **MS (ESI positive) m/z [M+H] $^+$:** 479.0. **(ESI-TOF) HRMS m/z : [M+H] $^+$ calcd for $\text{C}_{25}\text{H}_{27}\text{N}_4\text{O}_4\text{S}$: 479.1748; found: 479.1768.** **HPLC purity: 97.61%, HPLC t_R : 8.53 min.**

4.1.3.3. Ethyl 1-(4-methylbenzyl)-2-(3-(methylsulfonamido)phenyl)-1H-benzimidazole-5-carboxylate (19c). $R_f = 0.25$ (EtOAc/heptane/MeOH 1:1:0.1). White fluffy solid, yield 76%, mp: 187-189 °C. **FT-IR** (ν max, cm^{-1}): 3261 (NH sulfonamide), 2974, 2929 (CH aliphatic), 1706 (C=O), 1610 (C=C). **^1H NMR (400 MHz, DMSO- d_6)** δ 9.99 (s, 1H), 8.33 (d, $J = 1.0$ Hz, 1H), 7.88 (dd, $J = 8.5, 1.6$ Hz, 1H), 7.65 – 7.58 (m, 2H), 7.50 (t, $J = 7.8$ Hz, 1H), 7.43 (dt, $J = 7.7, 1.3$ Hz, 1H), 7.38 (ddd, $J = 8.0, 2.2, 1.2$ Hz, 1H), 7.07 (d, $J = 7.9$ Hz, 2H), 6.86 (d, $J = 8.1$ Hz, 2H), 5.59 (s, 2H), 4.34 (q, $J = 7.1$ Hz, 2H), 2.99 (s, 3H), 2.22 (s, 3H), 1.35 (t, $J = 7.1$ Hz, 3H). **^{13}C NMR (101 MHz, DMSO- d_6)** δ 166.1, 154.7, 142.1, 139.2, 138.9, 136.8, 133.3, 130.6, 129.8, 129.3 (2C), 126.1 (2C), 124.2, 124.1, 123.8, 121.3, 120.9, 120.2, 111.3, 60.5, 47.5, 39.4, 20.6, 14.2. **MS (ESI positive) m/z $[\text{M}+\text{H}]^+$:** 464.1. **(ESI-TOF) HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{26}\text{N}_3\text{O}_4\text{S}$:** 464.1639; **found:** 464.1654. **HPLC purity:** 98.13%, **HPLC t_R :** 10.51 min.

4.1.3.4. Ethyl 1-(4-methylbenzyl)-2-(3-(methylsulfonamido)phenyl)-1H-benzimidazole-6-carboxylate (19d). $R_f = 0.30$ (EtOAc/heptane/MeOH 1:1:0.1). White crystalline solid, yield 72%, mp: 211-213 °C. **FT-IR** (ν max, cm^{-1}): 3448 (NH sulfonamide), 3065 (CH aromatic), 2984, 2930 (CH aliphatic), 1712 (C=O), 1654 (C=N), 1603 (C=C). **^1H NMR (400 MHz, DMSO- d_6)** δ 9.99 (s, 1H), 8.08 (d, $J = 1.0$ Hz, 1H), 7.89 (dd, $J = 8.5, 1.5$ Hz, 1H), 7.82 (d, $J = 8.5$ Hz, 1H), 7.65 (t, $J = 1.7$ Hz, 1H), 7.50 (t, $J = 7.8$ Hz, 1H), 7.44 (dt, $J = 7.7, 1.3$ Hz, 1H), 7.38 (ddd, $J = 8.0, 2.1, 1.3$ Hz, 1H), 7.10 (d, $J = 7.9$ Hz, 2H), 6.88 (d, $J = 8.0$ Hz, 2H), 5.65 (s, 2H), 4.31 (q, $J = 7.1$ Hz, 2H), 2.98 (s, 3H), 2.23 (s, 3H), 1.32 (t, $J = 7.1$ Hz, 3H). **^{13}C NMR (101 MHz, DMSO- d_6)** δ 166.0, 155.6, 146.0, 138.9, 136.8, 135.7, 133.4, 130.5, 129.9, 129.4 (2C), 125.9 (2C), 124.2, 124.1, 123.3, 121.3, 120.2, 119.2, 112.9, 60.6, 47.4, 39.4, 20.6, 14.2. **MS (ESI positive) m/z $[\text{M}+\text{H}]^+$:** 464.1. **(ESI-TOF) HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{26}\text{N}_3\text{O}_4\text{S}$:** 464.1639; **found:** 464.1655. **HPLC purity:** 99.13%, **HPLC t_R :** 11.12 min.

4.1.3.5. Ethyl 1-(4-methylbenzyl)-2-(3-(methylsulfonamido)phenyl)-1H-benzimidazole-7-carboxylate (19e). $R_f = 0.30$ (EtOAc/heptane 1:1). White crystalline solid, yield 67%, mp: 76-78 °C. **FT-IR** (ν max, cm^{-1}): 3251 (NH sulfonamide), 2982, 2926 (CH aliphatic), 1712 (C=O), 1587 (C=C). **^1H NMR (400 MHz, DMSO- d_6)** δ 10.01 (s, 1H), 7.98 (dd, $J = 8.0, 1.1$ Hz, 1H), 7.61 (t, $J = 1.8$ Hz, 1H), 7.57 – 7.50 (m, 2H), 7.47 (dt, $J = 7.7, 1.3$ Hz, 1H), 7.39 (ddd, $J = 8.0, 2.1, 1.2$ Hz, 1H), 7.33 (t, $J = 7.8$ Hz, 1H), 6.98 (d, $J = 8.0$ Hz, 2H), 6.52 (d, $J = 8.1$ Hz, 2H), 5.66 (s, 2H), 4.17 (q, $J = 7.1$ Hz, 2H), 3.01 (s, 3H), 2.17 (s, 3H), 1.16 (t, $J = 7.1$ Hz,

3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 165.6, 155.9, 144.3, 138.9, 136.5, 133.6, 132.1, 130.8, 129.9, 129.1 (2C), 125.6 (2C), 124.9, 124.6, 123.7, 121.8, 121.1, 120.5, 118.1, 61.1, 49.1, 39.4, 20.5, 13.8. MS (ESI positive) m/z $[\text{M}+\text{H}]^+$: 464.1. (ESI-TOF) HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{26}\text{N}_3\text{O}_4\text{S}$: 464.1639; found: 464.1649. HPLC purity: 96.31%, HPLC t_R : 10.22 min.

4.1.3.6. *N*-(3-(5-cyano-1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)methanesulfonamide (**19f**). R_f = 0.20 (EtOAc/heptane 1:1). White solid, yield 80%, mp: 242-244 °C. FT-IR (ν max, cm^{-1}): 3270 (NH sulfonamide), 2922 (CH aliphatic), 2217 (nitrile), 1610 (C=C). ^1H NMR (400 MHz, DMSO- d_6) δ 10.00 (s, 1H), 8.30 (s, 1H), 7.72 – 7.63 (m, 3H), 7.50 (t, J = 7.8 Hz, 1H), 7.45 (d, J = 7.7 Hz, 1H), 7.39 (ddd, J = 8.0, 1.9, 1.1 Hz, 1H), 7.07 (d, J = 8.0 Hz, 2H), 6.86 (d, J = 8.0 Hz, 2H), 5.61 (s, 2H), 2.99 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 155.3, 142.0, 139.0, 138.8, 136.9, 133.1, 130.2, 129.9, 129.3 (2C), 126.2 (2C), 126.1, 124.3, 124.1, 121.4, 120.2, 119.7, 112.7, 104.6, 47.5, 39.4, 20.6. MS (ESI positive) m/z $[\text{M}+\text{H}]^+$: 416.9. (ESI-TOF) HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{21}\text{N}_4\text{O}_2\text{S}$: 417.1380; found: 417.1391. HPLC purity: 98.68%, HPLC t_R : 9.88 min.

4.1.3.7. *N*-(3-(6-cyano-1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)methanesulfonamide (**19g**). R_f = 0.25 (EtOAc/heptane 1:1). White solid, yield 80%, mp: 214-216 °C. FT-IR (ν max, cm^{-1}): 3331 (NH sulfonamide), 3006 (CH aromatic), 2922 (CH aliphatic), 2221 (nitrile), 1610 (C=C). ^1H NMR (400 MHz, DMSO- d_6) δ 10.00 (s, 1H), 8.18 (d, J = 0.9 Hz, 1H), 7.91 (dd, J = 8.4, 0.4 Hz, 1H), 7.67 – 7.64 (m, 2H), 7.53 – 7.45 (m, 2H), 7.39 (ddd, J = 7.8, 2.1, 1.5 Hz, 1H), 7.08 (d, J = 7.9 Hz, 2H), 6.86 (d, J = 8.1 Hz, 2H), 5.63 (s, 2H), 2.99 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 156.0, 145.4, 138.9, 136.9, 135.7, 133.2, 130.2, 129.9, 129.3 (2C), 126.2 (2C), 125.7, 124.1, 121.5, 120.5, 120.2, 119.7, 116.4, 104.5, 47.5, 39.4, 20.6. MS (ESI positive) m/z $[\text{M}+\text{H}]^+$: 416.9. (ESI-TOF) HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{21}\text{N}_4\text{O}_2\text{S}$: 417.1380; found: 417.1386. HPLC purity: 98.54%, HPLC t_R : 9.87 min.

4.1.3.8. *N*-(3-(5-methyl-1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)methanesulfonamide (**19h**). R_f = 0.30 (EtOAc/heptane 2:1). Light buff solid, yield 50%, mp: > 250 °C (decomp.). ^1H NMR (400 MHz, DMSO- d_6) δ 9.95 (s, 1H), 7.63 – 7.61 (m, 1H), 7.51 (s, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.40 (dd, J = 6.5, 1.3 Hz, 1H), 7.37 – 7.31 (m, 2H), 7.12 – 7.01 (m, 3H), 6.85 (d, J = 8.0 Hz, 2H), 5.50 (s, 2H), 2.98 (s, 3H), 2.42 (s, 3H), 2.22 (s, 3H). ^{13}C

NMR (101 MHz, DMSO-d₆) δ 152.5, 142.9, 138.8, 136.6, 134.0, 133.8, 131.3, 131.3, 129.7, 129.2 (2C), 126.1 (2C), 124.2, 124.0, 120.8, 120.2, 119.0, 110.7, 47.2, 39.3, 21.1, 20.6. **MS (ESI positive)** m/z [M+H]⁺: 406.0. **(ESI-TOF) HRMS m/z : [M+H]⁺ calcd for C₂₃H₂₄N₃O₂S: 406.1584; found: 406.1588. HPLC purity: 97.27%, HPLC t_R : 8.09 min.**

4.1.3.9. N-(3-(6-methyl-1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl)methanesulfonamide (19i). R_f = 0.25 (EtOAc/heptane 2:1). Light buff solid, yield 53%, mp: 254-256 °C. **¹H NMR (400 MHz, DMSO-d₆)** δ 9.94 (s, 1H), 7.64 – 7.57 (m, 2H), 7.46 (t, J = 7.8 Hz, 1H), 7.38 (dt, J = 7.7, 1.2 Hz, 1H), 7.34 (ddd, J = 8.0, 2.2, 1.1 Hz, 1H), 7.26 (s, 1H), 7.08 (d, J = 8.0 Hz, 3H), 6.86 (d, J = 8.0 Hz, 2H), 5.50 (s, 2H), 2.97 (s, 3H), 2.39 (s, 3H), 2.22 (s, 3H). **¹³C NMR (126 MHz, DMSO-d₆)** δ 152.1, 140.7, 138.8, 136.6, 136.2, 133.8, 132.2, 131.3, 129.7, 129.3 (2C), 125.9 (2C), 123.9, 123.8, 120.8, 120.2, 118.9, 110.7, 47.1, 39.3, 21.4, 20.6. **MS (ESI positive)** m/z [M+H]⁺: 406.1. **(ESI-TOF) HRMS m/z : [M+H]⁺ calcd for C₂₃H₂₄N₃O₂S: 406.1584; found: 406.1593. HPLC purity: 97.02%, HPLC t_R : 11.00 min.**

4.1.3.10. N-(3-(1-(4-methylbenzyl)-5-(trifluoromethyl)-1H-benzimidazol-2-yl)phenyl)methanesulfonamide (19j). R_f = 0.35 (EtOAc/heptane 1:2). White solid, yield 54%, mp: 251-253 °C. **¹H NMR (400 MHz, DMSO-d₆)** δ 10.00 (s, 1H), 8.11 (s, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.64 (t, J = 1.7 Hz, 1H), 7.58 (dd, J = 8.6, 1.4 Hz, 1H), 7.51 (t, J = 7.8 Hz, 1H), 7.44 (dt, J = 7.7, 1.2 Hz, 1H), 7.39 (ddd, J = 8.0, 2.1, 1.2 Hz, 1H), 7.08 (d, J = 7.9 Hz, 2H), 6.87 (d, J = 8.0 Hz, 2H), 5.61 (s, 2H), 2.99 (s, 3H), 2.22 (s, 3H). **¹³C NMR (101 MHz, DMSO-d₆)** δ 155.0, 142.0, 139.0, 138.2 (q, ⁴ J_{C-F} = 0.7 Hz), 136.9, 133.2, 130.5, 129.9, 129.3 (2C), 124.9 (q, ¹ J_{C-F} = 272.7 Hz), 126.1 (2C), 124.1, 123.2 (q, ² J_{C-F} = 31.3 Hz), 121.3, 120.2, 119.4 (q, ³ J_{C-F} = 3.6 Hz), 116.7 (q, ³ J_{C-F} = 3.9 Hz), 112.3, 47.5, 39.4, 20.6. **MS (ESI positive)** m/z [M+H]⁺: 460.0. **(ESI-TOF) HRMS m/z : [M+H]⁺ calcd for C₂₃H₂₁F₃N₃O₂S: 460.1301; found: 460.1309. HPLC purity: 98.09%, HPLC t_R : 11.25 min.**

4.1.3.11. N-(3-(5-bromo-1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl)methanesulfonamide (19k). R_f = 0.30 (EtOAc/heptane 1:3). White solid, yield 43%, mp: 276-278 °C. **¹H NMR (400 MHz, DMSO-d₆)** δ 9.97 (s, 1H), 7.94 (d, J = 1.6 Hz, 1H), 7.62 (s, 1H), 7.51 – 7.35 (m, 5H), 7.07 (d, J = 7.9 Hz, 2H), 6.85 (d, J = 8.0 Hz, 2H), 5.55 (s, 2H), 2.98 (s, 3H), 2.22 (s, 3H). **¹³C NMR (101 MHz, DMSO-d₆)** δ 153.9, 143.9, 138.9, 136.8, 135.0, 133.4,

130.6, 129.8, 129.3 (2C), 126.1 (2C), 125.5, 124.0, 121.7, 121.2, 120.2, 114.5, 113.1, 47.4, 39.4, 20.6. **MS (ESI positive) m/z $[M+H]^+$:** 469.9, $[(M+2)+H]^+$: 471.9 (1:1). **(ESI-TOF) HRMS m/z :** $[M+H]^+$ calcd for **C₂₂H₂₁BrN₃O₂S**: 470.0532; **found:** 470.0544. **HPLC purity:** 96.11%, **HPLC t_R :** 10.38 min.

4.1.3.12. ***N*-(3-(6-bromo-1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)methanesulfonamide (19I).** R_f = 0.25 (EtOAc/heptane 1:3). White solid, yield 53%, mp: 255-257 °C. **¹H NMR (500 MHz, DMSO-*d*₆):** δ 9.99 (s, 1H), 7.78 (d, J = 1.7 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.63 (t, J = 1.8 Hz, 1H), 7.49 (t, J = 7.9 Hz, 1H), 7.43 (dt, J = 2.5, 4.5 Hz, 1H), 7.40 (dd, J = 1.9, 8.6 Hz, 1H), 7.37 (dq, J = 1.1, 3.4 Hz, 1H), 7.09 (d, J = 7.9 Hz, 2H), 6.86 (d, J = 8.0 Hz, 2H), 5.57 (s, 2H), 2.99 (s, 3H), 2.23 (s, 3H). **¹³C NMR (126 MHz, DMSO-*d*₆):** δ 154.1, 142.1, 139.4, 137.7, 137.3, 133.9, 131.2, 130.3, 129.8 (2C), 126.6 (2C), 125.8, 124.5, 121.6, 121.5, 120.6, 115.6, 114.5, 47.7, 39.9, 21.1. **(ESI-TOF) HRMS m/z :** $[M+H]^+$ calcd for **C₂₂H₂₁BrN₃O₂S**: 470.0532; **found:** 470.0545.

Step (h): A solution of the appropriate carbamate derivative (**19a, b**) (0.60 g, 1.25 mmol) in NaOH solution (10% in water, 10 mL) was refluxed for 1.5 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was cooled, acidified to pH 7-8 using 2M HCl. The resulting solid was extracted with EtOAc, dried, evaporated, and purified by column chromatography to yield the desired final products (**20a, b**).

4.1.3.13. ***N*-(3-(5-amino-1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)methanesulfonamide (20a).** R_f = 0.35 (EtOAc/heptane/MeOH 2:1:0.05). Light brown solid, yield 65%, mp: 264-266 °C. **FT-IR (ν max, cm^{-1}):** 3462, 3372 (NH₂), 3068 (CH aromatic), 2937 (CH aliphatic), 1630 (C=N). **¹H NMR (400 MHz, DMSO-*d*₆)** δ 9.90 (s, 1H), 7.57 (s, 1H), 7.45 – 7.35 (m, 2H), 7.30 (t, J = 8.3 Hz, 2H), 7.11 (d, J = 7.8 Hz, 2H), 6.89 (d, J = 7.8 Hz, 2H), 6.58 (dd, J = 8.5, 1.6 Hz, 1H), 6.42 (s, 1H), 5.35 (s, 2H), 5.02 (s, 2H), 2.96 (s, 3H), 2.25 (s, 3H). **¹³C NMR (101 MHz, DMSO-*d*₆)** δ 149.8, 145.4, 138.7, 137.3, 136.5, 134.7, 133.9, 131.6, 129.6, 129.3 (2C), 125.8 (2C), 123.5, 120.3, 119.9, 119.5, 112.0, 93.6, 47.1, 39.3, 20.6. **MS (ESI positive) m/z $[M+H]^+$:** 406.9. **(ESI-TOF) HRMS m/z :** $[M+H]^+$ calcd for **C₂₂H₂₃N₄O₂S**: 407.1536; **found:** 407.1540. **HPLC purity:** 97.26%, **HPLC t_R :** 8.74 min.

4.1.3.14. N-(3-(6-amino-1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl)methanesulfonamide. (20b). $R_f = 0.40$ (EtOAc/heptane/MeOH 3:1:0.1). Light brown solid, yield 58%, mp: > 242 °C (decomp.). **FT-IR** (ν max, cm^{-1}): 3462, 3371 (NH₂), 2928 (CH aliphatic), 1607 (C=C). **¹H NMR (400 MHz, DMSO-*d*₆)** δ 9.91 (s, 1H), 7.58 (t, $J = 1.8$ Hz, 1H), 7.45 – 7.35 (m, 2H), 7.33 – 7.26 (m, 2H), 7.11 (d, $J = 7.9$ Hz, 2H), 6.90 (d, $J = 8.1$ Hz, 2H), 6.58 (dd, $J = 8.5, 2.0$ Hz, 1H), 6.43 (d, $J = 1.9$ Hz, 1H), 5.35 (s, 2H), 5.03 (s, 2H), 2.96 (s, 3H), 2.25 (s, 3H). **¹³C NMR (101 MHz, DMSO-*d*₆)** δ 149.8, 145.4, 138.8, 137.3, 136.5, 134.7, 133.9, 131.6, 129.6, 129.3 (2C), 125.8 (2C), 123.5, 120.3, 119.9, 119.5, 112.0, 93.6, 47.1, 39.3, 20.6. **MS (ESI positive)** m/z [M+H]⁺: 406.9. **(ESI-TOF) HRMS** m/z : [M+H]⁺ calcd for **C₂₂H₂₃N₄O₂S**: 407.1536; **found**: 407.1543. **HPLC purity**: 99.05%, **HPLC *t*_R**: 8.80 min.

Step (i): A solution of the appropriate ester derivative (**19c-e**) (0.46 g, 1.00 mmol, 1.00 equiv) and LiOH.H₂O (0.21 g, 5.00 mmol, 5.00 equiv) in THF/H₂O (1:1, 20 mL) was stirred at room temperature for 2-4 days. Upon completion of the reaction as indicated by TLC, the reaction mixture was evaporated, dissolved in water, neutralized with 2M HCl and the resulting solid was filtered, washed with water, dried, and purified by column chromatography to give the desired final products (**21a-c**).

4.1.3.15. 1-(4-methylbenzyl)-2-(3-(methylsulfonamido)phenyl)-1H-benzimidazole-5-carboxylic acid (21a). $R_f = 0.20$ (EtOAc). White solid, yield 68%, mp: > 300 °C (decomp.). **FT-IR** (ν max, cm^{-1}): 3700-3300 (OH broad of COOH), 3211 (NH sulfonamide), 2925 (CH aliphatic), 1664 (C=O), 1615 (C=C). **¹H NMR (400 MHz, DMSO-*d*₆)** δ 12.77 (s, 1H), 9.98 (s, 1H), 8.31 (s, 1H), 7.87 (dd, $J = 8.5, 1.3$ Hz, 1H), 7.63 (s, 1H), 7.57 (d, $J = 8.6$ Hz, 1H), 7.50 (t, $J = 7.8$ Hz, 1H), 7.44 (d, $J = 7.7$ Hz, 1H), 7.38 (d, $J = 7.7$ Hz, 1H), 7.08 (d, $J = 7.9$ Hz, 2H), 6.87 (d, $J = 8.0$ Hz, 2H), 5.58 (s, 2H), 2.99 (s, 3H), 2.22 (s, 3H). **¹³C NMR (101 MHz, DMSO-*d*₆)** δ 167.7, 154.5, 142.1, 139.0, 138.9, 136.8, 133.4, 130.7, 129.8, 129.3 (2C), 126.1 (2C), 125.1, 124.1, 124.0, 121.2, 121.0, 120.3, 111.1, 47.5, 39.4, 20.6. **MS (ESI positive)** m/z [M+H]⁺: 435.9. **(ESI-TOF) HRMS** m/z : [M+H]⁺ calcd for **C₂₃H₂₂N₃O₄S**: 436.1326; **found**: 436.1332.

4.1.3.16. 1-(4-methylbenzyl)-2-(3-(methylsulfonamido)phenyl)-1H-benzimidazole-6-carboxylic acid (21b). $R_f = 0.30$ (EtOAc). White crystalline solid, yield 83%, mp: 246-248 °C. **FT-IR** (ν max, cm^{-1}): 3700-3300 (OH broad peak of COOH), 3019 (CH aromatic), 2932 (CH

aliphatic), 1689 (C=O), 1619 (C=C). ¹H NMR (500 MHz, DMSO-d₆) δ 12.82 (s, 1H), 9.99 (s, 1H), 8.06 (d, *J* = 0.9 Hz, 1H), 7.88 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.82 – 7.79 (m, 1H), 7.66 (t, *J* = 1.7 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 1H), 7.44 (dt, *J* = 7.7, 1.2 Hz, 1H), 7.38 (ddd, *J* = 8.0, 2.2, 1.1 Hz, 1H), 7.10 (d, *J* = 7.9 Hz, 2H), 6.89 (d, *J* = 8.0 Hz, 2H), 5.63 (s, 2H), 2.99 (s, 3H), 2.23 (s, 3H). ¹³C NMR (101 MHz, DMSO-d₆) δ 167.6, 155.4, 145.8, 138.9, 136.8, 135.6, 133.5, 130.6, 129.9, 129.4 (2C), 125.9 (2C), 125.1, 124.1, 123.6, 121.3, 120.2, 119.0, 113.1, 47.4, 39.4, 20.6. MS (ESI positive) *m/z* [M+H]⁺: 435.9. (ESI-TOF) HRMS *m/z*: [M+H]⁺ calcd for C₂₃H₂₂N₃O₄S: 436.1326; found: 436.1336.

4.1.3.17. 1-(4-methylbenzyl)-2-(3-(methylsulfonamido)phenyl)-1*H*-benzimidazole-7-carboxylic acid (21c). R_f = 0.15 (EtOAc). White solid, yield 61%, mp: 253-255 °C. FT-IR (ν max, cm⁻¹): 3700-3100 (OH of COOH), 3333 (NH sulfonamide), 2937 (CH aliphatic), 1686 (C=O). ¹H NMR (400 MHz, DMSO-d₆) δ 13.16 (s, 1H), 9.99 (s, 1H), 7.95 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.66 – 7.58 (m, 2H), 7.55 – 7.45 (m, 2H), 7.39 (ddd, *J* = 7.9, 2.1, 1.3 Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 6.96 (d, *J* = 7.9 Hz, 2H), 6.53 (d, *J* = 8.0 Hz, 2H), 5.78 (s, 2H), 3.01 (s, 3H), 2.16 (s, 3H). ¹³C NMR (101 MHz, DMSO-d₆) δ 167.4, 155.9, 144.4, 138.9, 136.4, 134.0, 132.6, 131.0, 129.9, 129.1 (2C), 125.8 (2C), 125.5, 124.7, 123.6, 121.8, 121.1, 120.6, 119.0, 49.1, 39.4, 20.5. MS (ESI positive) *m/z* [M+H]⁺: 436.0. (ESI-TOF) HRMS *m/z*: [M+H]⁺ calcd for C₂₃H₂₂N₃O₄S: 436.1326; found: 436.1335. HPLC purity: 97.72%, HPLC *t*_R: 7.96 min.

Step (j): To a solution of the appropriate nitrile derivative (**19f, g**) (0.08 g, 0.20 mmol, 1.00 equiv) in EtOH (5 mL) was added NH₂OH solution (50% in water, 40.0 μL, 0.60 mmol, 3.00 equiv) and the reaction mixture was refluxed for 24 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was evaporated, stirred with water, filtered, dried, and crystallized from EtOH to give the desired final products (**22a, b**).

4.1.3.18. *N*-hydroxy-1-(4-methylbenzyl)-2-(3-(methylsulfonamido)phenyl)-1*H*-benzimidazole-5-carboximidamide (22a). Crystallized from EtOH. White solid, yield 87%, mp: 235-237 °C. FT-IR (ν max, cm⁻¹): 3507, 3403 (Iminoxime), 3082 (CH aromatic), 2922 (CH aliphatic), 1685 (C=N). ¹H NMR (400 MHz, DMSO-d₆) δ 9.97 (s, 1H), 9.55 (s, 1H), 8.03 (d, *J* = 1.0 Hz, 1H), 7.64 – 7.59 (m, 2H), 7.51 – 7.39 (m, 3H), 7.38 – 7.34 (m, 1H), 7.08 (d, *J* = 7.9 Hz, 2H), 6.87 (d, *J* = 8.0 Hz, 2H), 5.83 (s, 2H), 5.55 (s, 2H), 2.99 (s, 3H), 2.22 (s, 3H). ¹³C NMR (101

MHz, DMSO-d₆) δ 153.3, 151.3, 142.3, 138.9, 136.7, 136.3, 133.6, 131.0, 129.8, 129.3 (2C), 128.0, 126.1 (2C), 124.0, 121.0, 120.7, 120.2, 116.4, 110.7, 47.3, 39.4, 20.6. **MS (ESI positive)** m/z [M+H]⁺: 450.0. **(ESI-TOF) HRMS** m/z : [M+H]⁺ calcd for **C₂₃H₂₄N₅O₃S**: 450.1594; **found**: 450.1609. **HPLC purity**: 98.14%, **HPLC t_R**: 6.91 min.

4.1.3.19. N-hydroxy-1-(4-methylbenzyl)-2-(3-(methylsulfonamido)phenyl)-1H-benzimidazole-6-carboximidamide (22b). Crystallized from EtOH. White solid, yield 97%, mp: 210-212 °C. **FT-IR** (ν max, cm⁻¹): 3488, 3372 (Iminoxime), 2925 (CH aliphatic), 1643 (C=N). **¹H NMR (400 MHz, DMSO-d₆)** δ 9.96 (s, 1H), 9.57 (s, 1H), 7.80 (s, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.67 – 7.62 (m, 2H), 7.48 (t, J = 7.8 Hz, 1H), 7.43 – 7.34 (m, 2H), 7.09 (d, J = 7.9 Hz, 2H), 6.88 (d, J = 7.9 Hz, 2H), 5.81 (s, 2H), 5.55 (s, 2H), 2.98 (s, 3H), 2.23 (s, 3H). **¹³C NMR (101 MHz, DMSO-d₆)** δ 153.5, 151.1, 143.1, 138.9, 136.7, 135.8, 133.6, 131.0, 129.8, 129.3 (2C), 128.2, 126.0 (2C), 123.9, 121.0, 120.2, 120.1, 118.7, 108.2, 47.3, 39.4, 20.6. **MS (ESI positive)** m/z [M+H]⁺: 450.0. **(ESI-TOF) HRMS** m/z : [M+H]⁺ calcd for **C₂₃H₂₄N₅O₃S**: 450.1594; **found**: 450.1609. **HPLC purity**: 97.57%, **HPLC t_R**: 6.46 min.

4.1.4. General procedure for the synthesis of reference compound III and target compounds 25a-u and 26a,b

The reference compound **III**, and the desired final compounds **25a-u** and **26a,b** were synthesized as described previously [37].

4.1.4.1. N-(3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl)methanesulfonamide (III) [37]. R_f = 0.3 (DCM/MeOH 9.8:0.2). Off-white solid, yield 47%, mp: 246-248 °C. **FT-IR** (ν max, cm⁻¹): 3447 (NH sulfonamide), 3056, 3019 (CH aromatic), 2952, 2925 (CH aliphatic), 1651 (C=N), 1607 (C=C). **¹H NMR (500 MHz, DMSO-d₆)**: δ 9.99 (s, 1H), 7.74 (q, J = 2.9 Hz, 1H), 7.64 (s, 1H), 7.51 - 7.45 (m, 2H), 7.42 (d, J = 7.7 Hz, 1H), 7.36 (q, J = 3.0 Hz, 1H), 7.27 - 7.23 (m, 2H), 7.08 (d, J = 7.9 Hz, 2H), 6.88 (d, J = 8.0 Hz, 2H), 5.55 (s, 2H), 2.99 (s, 3H), 2.23 (s, 3H). **¹³C NMR-APT (126 MHz, DMSO-d₆)**: δ 153.1, 143.1, 139.3, 137.2, 136.4, 134.2, 131.7, 130.3, 129.8 (2C), 126.6 (2C), 124.5, 123.3, 122.7, 121.4, 120.7, 119.8, 111.7, 47.7, 40.3, 21.1. **(ESI-TOF) HRMS** m/z : [M+H]⁺ calcd for **C₂₂H₂₂N₃O₂S**: 392.1427; **found**: 392.1438. **HPLC purity**: 99.5%, **HPLC t_R**: 2.49 min.

4.1.4.2. *N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)cyclopropanesulfonamide (**25a**). $R_f = 0.25$ (DCM/MeOH 9.8:0.2). Yellow solid, yield 35%, mp: 238-240 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 9.97 (s, 1H), 7.73 (q, $J = 2.9$ Hz, 1H), 7.68 (s, 1H), 7.48 (q, $J = 5.2$ Hz, 2H), 7.44 (d, $J = 7.7$ Hz, 1H), 7.40 (d, $J = 7.9$ Hz, 1H), 7.29 - 7.23 (m, 2H), 7.08 (d, $J = 7.9$ Hz, 2H), 6.88 (d, $J = 7.9$ Hz, 2H), 5.55 (s, 2H), 2.63 - 2.56 (m, 1H), 2.22 (s, 3H), 0.91 (t, $J = 4.0$ Hz, 4H). ¹³C NMR (126 MHz, DMSO-*d*₆): δ 153.1, 143.0, 139.3, 137.2, 136.4, 134.2, 131.5, 130.2, 129.8 (2C), 126.6 (2C), 124.6, 123.3, 122.7, 122.0, 121.4, 119.8, 111.7, 47.7, 30.1, 21.1, 5.4 (2C). (ESI-TOF) HRMS m/z : [M+H]⁺ calcd for C₂₄H₂₄N₃O₂S: 418.1583; found: 418.1585. HPLC purity: 96.4%, HPLC t_R : 2.66 min.

4.1.4.3. *N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (**25b**). $R_f = 0.25$ (EtOAc/hexane 1:2). Buff solid, yield 48%, mp: 200-202 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 10.56 (s, 1H), 7.75 (q, $J = 2.9$ Hz, 2H), 7.72 - 7.69 (m, 1H), 7.62 - 7.57 (m, 1H), 7.55 (t, $J = 1.7$ Hz, 1H), 7.52 (q, $J = 5.1$ Hz, 2H), 7.45 - 7.41 (m, 1H), 7.39 (t, $J = 7.7$ Hz, 1H), 7.37 - 7.35 (m, 1H), 7.28 - 7.21 (m, 3H), 7.05 (d, $J = 7.9$ Hz, 2H), 6.79 (d, $J = 8.0$ Hz, 2H), 5.43 (s, 2H), 2.23 (s, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆): δ 152.9, 143.0, 139.7, 138.6, 137.2, 136.2, 134.0, 133.5, 131.5, 130.2, 129.8 (2C), 129.7 (2C), 127.1 (2C), 126.6 (2C), 125.0, 123.2, 122.7, 121.7, 121.0, 119.8, 111.7, 47.6, 21.1. (ESI-TOF) HRMS m/z : [M+H]⁺ calcd for C₂₇H₂₄N₃O₂S: 454.1583; found: 454.1585. HPLC purity: 98.2%, HPLC t_R : 2.91 min.

4.1.4.4. 4-chloro-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (**25c**). $R_f = 0.25$ (DCM/MeOH 9.8:0.2). White solid, yield 32%, mp: 227-229 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 10.65 (s, 1H), 7.75 (dt, $J = 2.9, 3.4$ Hz, 2H), 7.73 - 7.70 (m, 1H), 7.60 (dt, $J = 2.9, 3.4$ Hz, 2H), 7.55 (t, $J = 1.2$ Hz, 1H), 7.46 - 7.43 (m, 1H), 7.41 (t, $J = 4.7$ Hz, 2H), 7.27 (dt, $J = 2.4, 4.6$ Hz, 1H), 7.24 (tt, $J = 2.1, 4.6$ Hz, 2H), 7.05 (d, $J = 7.9$ Hz, 2H), 6.79 (d, $J = 8.1$ Hz, 2H), 5.44 (s, 2H), 2.23 (s, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆): δ 152.8, 143.0, 138.5, 138.4, 138.3, 137.2, 136.2, 134.0, 131.6, 130.4, 130.0 (2C), 129.7 (2C), 129.0 (2C), 126.6 (2C), 125.3, 123.3, 122.8, 121.9, 121.2, 119.8, 111.7, 47.6, 21.1. (ESI-TOF) HRMS m/z : [M+H]⁺ calcd for C₂₇H₂₃ClN₃O₂S: 488.1194; found: 488.1192. HPLC purity: 98.8%, HPLC t_R : 3.14 min.

4.1.4.5. 3-chloro-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (**25d**). $R_f = 0.30$ (EtOAc/hexane 1:2). White solid, yield 58%,

mp: 176-178 °C. **FT-IR** (ν max, cm^{-1}): 3448 (NH sulfonamide), 3058 (CH aromatic), 2925 (CH aliphatic), 1685 (C=N), 1604 (C=C). **^1H NMR (500 MHz, DMSO- d_6)**: δ 10.66 (s, 1H), 7.77 (t, J = 1.8 Hz, 1H), 7.73 - 7.67 (m, 3H), 7.58 - 7.54 (m, 2H), 7.46 - 7.43 (m, 1H), 7.42 (t, J = 4.7 Hz, 2H), 7.29 - 7.26 (m, 1H), 7.26 - 7.22 (m, 2H), 7.05 (d, J = 7.9 Hz, 2H), 6.80 (d, J = 8.0 Hz, 2H), 5.45 (s, 2H), 2.22 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6)**: δ 152.8, 143.0, 141.5, 138.1, 137.2, 136.3, 134.4, 134.0, 133.6, 131.9, 131.6, 130.4, 129.7 (2C), 126.7, 126.6 (2C), 125.8, 125.5, 123.3, 122.8, 122.1, 121.5, 119.8, 111.7, 47.6, 21.1. **(ESI-TOF) HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{23}\text{ClN}_3\text{O}_2\text{S}$: 488.1194; found: 488.1204. HPLC purity: 99.7%, HPLC t_R : 3.14 min.**

4.1.4.6. 4-bromo-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (25e). R_f = 0.35 (EtOAc/hexane 1:2). Off-white solid, yield 32%, mp: 213-215 °C. **^1H NMR (500 MHz, DMSO- d_6)**: δ 10.66 (s, 1H), 7.75 (dd, J = 2.0, 6.7 Hz, 2H), 7.71 (ddd, J = 2.8, 2.8, 5.2 Hz, 1H), 7.67 (dd, J = 2.0, 6.7 Hz, 2H), 7.56 (t, J = 1.2 Hz, 1H), 7.44 (ddd, J = 2.3, 2.3, 5.3 Hz, 1H), 7.41 (t, J = 4.5 Hz, 2H), 7.27 (dt, J = 2.4, 4.6 Hz, 1H), 7.24 (tt, J = 2.2, 4.6 Hz, 2H), 7.05 (d, J = 7.9 Hz, 2H), 6.79 (d, J = 8.1 Hz, 2H), 5.44 (s, 2H), 2.23 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6)**: δ 152.8, 143.0, 139.0, 138.3, 137.2, 136.2, 134.0, 132.9 (2C), 131.6, 130.4, 129.7 (2C), 129.1 (2C), 127.5, 126.6 (2C), 125.3, 123.3, 122.8, 121.8, 121.1, 119.8, 111.7, 47.6, 21.1. **(ESI-TOF) HRMS m/z : $[2\text{M}+\text{H}]^+$ calcd for $\text{C}_{54}\text{H}_{45}\text{Br}_2\text{N}_6\text{O}_4\text{S}_2$: 1063.1305; found: 1063.1323.**

4.1.4.7. 3-bromo-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (25f). R_f = 0.35 (DCM/MeOH 9.8:0.2). White solid, yield 33%, mp: 98-100 °C. **^1H NMR (500 MHz, DMSO- d_6)**: δ 10.65 (s, 1H), 7.91 (s, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.72 (s, 2H), 7.56 (s, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.41 (d, J = 8.6 Hz, 3H), 7.26 (t, J = 8.8 Hz, 3H), 7.05 (d, J = 7.4 Hz, 2H), 6.80 (d, J = 7.4 Hz, 2H), 5.45 (s, 2H), 2.23 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6)**: δ 152.8, 143.0, 141.6, 138.1, 137.2, 136.4, 136.3, 134.0, 132.1, 131.6, 130.4, 129.7 (2C), 129.5, 126.6 (2C), 126.1, 125.5, 123.3, 122.8, 122.7, 122.1, 121.5, 119.8, 111.7, 47.6, 21.1. **(ESI-TOF) HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{23}\text{BrN}_3\text{O}_2\text{S}$: 532.0688; found: 532.0686. HPLC purity: 98.5%, HPLC t_R : 3.18 min.**

4.1.4.8. 3,4-difluoro-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (25g). R_f = 0.40 (DCM/MeOH 9.8:0.2). White solid, yield 33%, mp: 128-130 °C. **^1H NMR (500 MHz, DMSO- d_6)**: δ 10.67 (s, 1H), 7.80 (t, J = 8.1 Hz, 1H), 7.71

(d, $J = 6.7$ Hz, 1H), 7.61 (d, $J = 3.4$ Hz, 2H), 7.55 (s, 1H), 7.44 (t, $J = 7.5$ Hz, 3H), 7.29 (t, $J = 6.1$ Hz, 1H), 7.22 (t, $J = 7.2$ Hz, 2H), 7.05 (d, $J = 7.7$ Hz, 2H), 6.79 (d, $J = 7.7$ Hz, 2H), 5.45 (s, 2H), 2.22 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6):** δ 152.8, 152.6 (dd, $^1J_{\text{C-F}}$, $^2J_{\text{C-F}} = 253.3, 12.5$ Hz), 149.7 (dd, $^1J_{\text{C-F}}$, $^2J_{\text{C-F}} = 253.3, 13.5$ Hz), 143.0, 138.1, 137.2, 136.9 (q, $J_{\text{C-F}} = 3.9$ Hz), 136.3, 134.0, 131.6, 130.4, 129.7 (2C), 126.5 (2C), 125.5, 125.1 (q, $J_{\text{C-F}} = 3.8$ Hz), 123.3, 122.8, 122.2, 121.5, 119.8, 119.4 (d, $J_{\text{C-F}} = 18.6$ Hz), 117.0 (d, $J_{\text{C-F}} = 19.9$ Hz), 111.7, 47.6, 21.0. **(ESI-TOF) HRMS m/z: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{22}\text{F}_2\text{N}_3\text{O}_2\text{S}$: 490.1395; found: 490.1391. HPLC purity: 96.2%, HPLC t_{R} : 3.09 min.**

4.1.4.9. 3-chloro-4-fluoro-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (25h). $R_{\text{f}} = 0.35$ (EtOAc/hexane 1:2). White solid, yield 40%, mp: 154-156 °C. **^1H NMR (500 MHz, DMSO- d_6):** δ 10.66 (s, 1H), 7.94 (q, $J = 3.0$ Hz, 1H), 7.76 - 7.73 (m, 1H), 7.73 - 7.70 (m, 1H), 7.59 (t, $J = 8.9$ Hz, 1H), 7.56 (q, $J = 1.2$ Hz, 1H), 7.46 - 7.43 (m, 3H), 7.30 - 7.27 (m, 1H), 7.26 - 7.22 (m, 2H), 7.04 (d, $J = 7.8$ Hz, 2H), 6.79 (d, $J = 8.1$ Hz, 2H), 5.45 (s, 2H), 2.22 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6):** δ 161.1 (d, $^1J_{\text{C-F}} = 255.4$ Hz), 152.8, 143.0, 138.0, 137.19, 137.16 (d, $^4J_{\text{C-F}} = 3.8$ Hz), 136.3, 134.0, 131.7, 130.4, 129.7 (2C), 129.67, 128.6 (d, $^3J_{\text{C-F}} = 8.9$ Hz), 126.6 (2C), 125.6, 123.3, 122.8, 122.3, 121.6, 121.4 (d, $^2J_{\text{C-F}} = 18.8$ Hz), 119.8, 118.7 (d, $^2J_{\text{C-F}} = 22.4$ Hz), 111.7, 47.6, 21.1. **(ESI-TOF) HRMS m/z: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{22}\text{ClFN}_3\text{O}_2\text{S}$: 506.1099; found: 506.1105. HPLC purity: 99.3%, HPLC t_{R} : 3.20 min.**

4.1.4.10. *N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)-4-(trifluoromethyl)benzenesulfonamide (25i). $R_{\text{f}} = 0.5$ (DCM/MeOH 9.9:0.1). White solid, yield 30%, mp: 173-175 °C. **^1H NMR (500 MHz, DMSO- d_6):** δ 10.82 (s, 1H), 7.97 (d, $J = 8.4$ Hz, 2H), 7.93 (d, $J = 8.5$ Hz, 2H), 7.71 (dd, $J = 2.4, 6.3$ Hz, 1H), 7.59 (s, 1H), 7.45 (dd, $J = 2.5, 6.3$ Hz, 1H), 7.42 (d, $J = 6.9$ Hz, 2H), 7.28 (dt, $J = 2.3, 4.5$ Hz, 1H), 7.24 (tt, $J = 2.1, 4.6$ Hz, 2H), 7.04 (d, $J = 7.9$ Hz, 2H), 6.78 (d, $J = 8.0$ Hz, 2H), 5.45 (s, 2H), 2.21 (s, 3H). **^{13}C NMR (126 MHz, DMSO- d_6):** δ 152.8, 143.7, 143.0, 138.1, 137.2, 136.3, 134.0, 133.1 (q, $^2J_{\text{C-F}} = 32.5$ Hz), 131.7, 130.4, 129.7 (2C), 128.1 (2C), 127.1 (2C, q, $^3J_{\text{C-F}} = 3.8$ Hz), 126.5 (2C), 125.4, 123.8 (q, $^1J_{\text{C-F}} = 274.2$ Hz), 123.3, 122.8, 121.9, 121.2, 119.8, 111.7, 47.6, 21.0. **(ESI-TOF) HRMS m/z: $[\text{2M}+\text{H}]^+$ calcd for $\text{C}_{56}\text{H}_{45}\text{F}_6\text{N}_6\text{O}_4\text{S}_2$: 1043.2842; found: 1043.2882.**

4.1.4.11. ***N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)-4-(trifluoromethoxy)benzenesulfonamide (25j).** $R_f = 0.50$ (EtOAc/hexane 1:2). White solid, yield 38%, mp: 164-166 °C. **¹H NMR (500 MHz, DMSO-*d*₆):** δ 10.70 (s, 1H), 7.89 (d, $J = 8.6$ Hz, 2H), 7.71 (d, $J = 6.8$ Hz, 1H), 7.58 (s, 1H), 7.52 (d, $J = 8.2$ Hz, 2H), 7.43 (m, 3H), 7.25 (m, 3H), 7.05 (d, $J = 7.6$ Hz, 2H), 6.79 (d, $J = 7.6$ Hz, 2H), 5.45 (s, 2H), 2.22 (s, 3H). **¹³C NMR (126 MHz, DMSO-*d*₆):** δ 152.8, 151.6, 143.0, 138.6, 138.3, 137.2, 136.2, 134.1, 131.6, 130.4, 129.79 (2C), 129.76, 129.7 (2C), 126.6 (2C), 125.3, 123.3, 122.8, 121.9 (2C), 121.2, 120.2 (q, $^1J_{C-F} = 260$ Hz), 119.8, 111.7, 47.6, 21.0. **(ESI-TOF) HRMS *m/z*: [2M+H]⁺ calcd for C₅₆H₄₅F₆N₆O₆S₂: 1075.2741; found: 1075.2776.**

4.1.4.12. **4-methyl-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (25k).** $R_f = 0.25$ (DCM/MeOH 9.8:0.2). White solid, yield 47%, mp: 207-209 °C. **¹H NMR (500 MHz, DMSO-*d*₆):** δ 10.50 (s, 1H), 7.70 (q, $J = 2.8$ Hz, 1H), 7.64 (d, $J = 8.2$ Hz, 2H), 7.55 (s, 1H), 7.44 (q, $J = 2.9$ Hz, 1H), 7.39 (t, $J = 7.8$ Hz, 1H), 7.35 (d, $J = 7.7$ Hz, 1H), 7.31 (d, $J = 8.1$ Hz, 2H), 7.27 (d, $J = 7.4$ Hz, 1H), 7.25 - 7.21 (m, 2H), 7.05 (d, $J = 7.9$ Hz, 2H), 6.79 (d, $J = 7.9$ Hz, 2H), 5.42 (s, 2H), 2.29 (s, 3H), 2.23 (s, 3H). **¹³C NMR (126 MHz, DMSO-*d*₆):** δ 152.9, 143.9, 143.0, 138.8, 137.2, 136.9, 136.2, 134.0, 131.5, 130.2 (2C), 129.7 (2C), 127.2 (2C), 126.6 (2C), 124.9, 123.2, 122.7, 121.5, 120.8, 119.8, 111.7, 47.6, 21.4, 21.1. **(ESI-TOF) HRMS *m/z*: [M+H]⁺ calcd for C₂₈H₂₆N₃O₂S: 468.1740; found: 468.1741. HPLC purity: 98.1%, HPLC *t_R*: 3.04 min.**

4.1.4.13. **4-methoxy-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (25l).** $R_f = 0.25$ (DCM/MeOH 9.8:0.2). White solid, yield 70%, mp: 206-208 °C. **¹H NMR (500 MHz, DMSO-*d*₆):** δ 10.43 (s, 1H), 7.72 - 7.68 (m, 3H), 7.55 (t, $J = 1.8$ Hz, 1H), 7.45 - 7.42 (m, 1H), 7.39 (t, $J = 7.8$ Hz, 1H), 7.35 (dt, $J = 2.5, 4.5$ Hz, 1H), 7.26 (ddd, $J = 1.2, 2.2, 7.9$ Hz, 1H), 7.23 (tt, $J = 2.2, 4.6$ Hz, 2H), 7.05 (d, $J = 7.9$ Hz, 2H), 7.02 (dd, $J = 2.1, 6.9$ Hz, 2H), 6.79 (d, $J = 8.1$ Hz, 2H), 5.43 (s, 2H), 3.76 (s, 3H), 2.22 (s, 3H). **¹³C NMR (126 MHz, DMSO-*d*₆):** δ 163.0, 153.0, 143.0, 138.9, 137.2, 136.2, 134.0, 131.5, 131.3, 130.2, 129.7 (2C), 129.3 (2C), 126.6 (2C), 124.8, 123.2, 122.7, 121.4, 120.7, 119.8, 114.9 (2C), 111.7, 56.1, 47.6, 21.1. **(ESI-TOF) HRMS *m/z*: [M+H]⁺ calcd for C₂₈H₂₆N₃O₃S: 484.1689; found: 484.1696. HPLC purity: 99.1%, HPLC *t_R*: 2.94 min.**

4.1.4.14. 4-isopropyl-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (25m). $R_f = 0.45$ (DCM/MeOH 9.8:0.2). White solid, yield 55%, mp: 126-128 °C. $^1\text{H NMR}$ (500 MHz, DMSO- d_6): δ 10.53 (s, 1H), 7.70 (d, $J = 8.2$ Hz, 3H), 7.58 (s, 1H), 7.45 (t, $J = 4.1$ Hz, 1H), 7.39 (t, $J = 7.1$ Hz, 3H), 7.34 (d, $J = 7.5$ Hz, 1H), 7.28 (d, $J = 8.1$ Hz, 1H), 7.22 (q, $J = 4.9$ Hz, 2H), 7.05 (d, $J = 7.8$ Hz, 2H), 6.79 (d, $J = 7.8$ Hz, 2H), 5.43 (s, 2H), 2.89 (dq, $J = 6.8, 6.8$ Hz, 1H), 2.22 (s, 3H), 1.13 (d, $J = 6.9$ Hz, 6H). $^{13}\text{C NMR}$ (126 MHz, DMSO- d_6): δ 154.3, 153.0, 143.0, 138.8, 137.4, 137.2, 136.2, 134.1, 131.5, 130.3, 129.7 (2C), 127.7 (2C), 127.3 (2C), 126.7 (2C), 124.8, 123.2, 122.7, 121.3, 120.5, 119.8, 111.7, 47.6, 33.8, 23.8, 21.1. (ESI-TOF) HRMS m/z : $[2M+H]^+$ calcd for $\text{C}_{60}\text{H}_{59}\text{N}_6\text{O}_4\text{S}_2$: 991.4034; found: 991.4073.

4.1.4.15. 4-(*tert*-butyl)-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (25n). $R_f = 0.30$ (DCM/MeOH 9.9:0.1). White solid, yield 53%, mp: 110-112 °C. $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 10.57 (s, 1H), 7.75 – 7.64 (m, 3H), 7.58 (s, 1H), 7.53 (d, $J = 8.1$ Hz, 2H), 7.47 – 7.20 (m, 6H), 7.04 (d, $J = 7.3$ Hz, 2H), 6.78 (d, $J = 7.3$ Hz, 2H), 5.42 (s, 2H), 2.21 (s, 3H), 1.21 (s, 9H).

4.1.4.16. *N*-(4-(*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)sulfamoyl)phenyl)acetamide (25o). $R_f = 0.40$ (DCM/MeOH 9.5:0.5). White solid, yield 76%, mp: 156-158 °C. $^1\text{H NMR}$ (500 MHz, DMSO- d_6): δ 10.45 (s, 1H), 10.30 (s, 1H), 7.72 - 7.66 (m, 5H), 7.55 (d, $J = 1.6$ Hz, 1H), 7.44 (q, $J = 2.9$ Hz, 1H), 7.39 (t, $J = 7.7$ Hz, 1H), 7.36 (q, $J = 2.6$ Hz, 1H), 7.27 - 7.21 (m, 3H), 7.03 (d, $J = 7.9$ Hz, 2H), 6.78 (d, $J = 8.0$ Hz, 2H), 5.43 (s, 2H), 2.22 (s, 3H), 2.06 (s, 3H). $^{13}\text{C NMR}$ (126 MHz, DMSO- d_6): δ 169.5, 153.0, 143.7, 143.0, 138.8, 137.2, 136.2, 134.0, 133.2, 131.5, 130.2, 129.7 (2C), 128.4 (2C), 126.6 (2C), 124.8, 123.2, 122.7, 121.6, 120.9, 119.8, 119.0 (2C), 111.7, 47.6, 24.6, 21.0. (ESI-TOF) HRMS m/z : $[M+H]^+$ calcd for $\text{C}_{29}\text{H}_{27}\text{N}_4\text{O}_3\text{S}$: 511.1798; found: 511.1820.

4.1.4.17. 3,4-dimethoxy-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (25p). $R_f = 0.20$ (DCM/MeOH 9.8:0.2). White solid, yield 47%, mp: 120-122 °C. $^1\text{H NMR}$ (500 MHz, DMSO- d_6): δ 10.37 (s, 1H), 7.72 - 7.69 (m, 1H), 7.59 (t, $J = 1.7$ Hz, 1H), 7.46 - 7.43 (m, 1H), 7.40 (t, $J = 7.8$ Hz, 1H), 7.35 (q, $J = 2.6$ Hz, 1H), 7.32 (dd, $J = 2.2, 8.5$ Hz, 1H), 7.30 (d, $J = 2.1$ Hz, 1H), 7.27 (dq, $J = 1.2, 3.3$ Hz, 1H), 7.23 (tt, $J = 2.2, 4.6$ Hz, 2H), 7.04 (d, $J = 7.9$ Hz, 2H), 7.01 (d, $J = 8.5$ Hz, 1H), 6.78 (d, $J = 8.0$ Hz, 2H), 5.43 (s, 2H), 3.75 (s, 6H), 2.22 (s, 3H). $^{13}\text{C NMR-APT}$ (126 MHz, DMSO- d_6): δ 153.0, 152.7, 149.1, 143.0, 138.9,

137.2, 136.2, 134.0, 131.4, 131.1, 130.2, 129.7 (2C), 126.6 (2C), 124.8, 123.2, 122.7, 121.7, 121.0, 120.9, 119.7, 111.8, 111.5, 109.8, 56.2, 56.2, 47.6, 21.0. **(ESI-TOF) HRMS m/z: [M+H]⁺ calcd for C₂₉H₂₈N₃O₄S: 514.1795; found: 514.1798. HPLC purity: 99.8%, HPLC t_R: 2.83 min.**

4.1.4.18. N-(3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl)-4-nitrobenzenesulfonamide (25q). R_f = 0.20 (EtOAc/hexane 1:2). Off-white solid, yield 60%, mp: 178-180 °C. **FT-IR (ν max, cm⁻¹):** 3588 (NH sulfonamide), 2967, 2920 (CH aliphatic), 1676 (C=N), 1617 (C=C), 1529 and 1348 (NO₂). **¹H NMR (500 MHz, DMSO-d₆):** δ 10.88 (s, 1H), 8.34 (dd, *J* = 2.1, 6.9 Hz, 2H), 7.99 (dd, *J* = 2.1, 6.9 Hz, 2H), 7.70 (ddd, *J* = 1.8, 1.8, 6.1 Hz, 1H), 7.56 (t, *J* = 1.2 Hz, 1H), 7.43 (ddd, *J* = 2.1, 3.8, 7.5 Hz, 3H), 7.30 (ddd, *J* = 2.7, 4.5, 6.8 Hz, 1H), 7.23 (tt, *J* = 2.3, 4.6 Hz, 2H), 7.03 (d, *J* = 7.9 Hz, 2H), 6.78 (d, *J* = 8.1 Hz, 2H), 5.44 (s, 2H), 2.22 (s, 3H). **¹³C NMR (126 MHz, DMSO-d₆):** δ 152.7, 150.3, 145.1, 143.0, 137.9, 137.2, 136.2, 134.0, 131.7, 130.5, 129.7 (2C), 128.7 (2C), 126.5 (2C), 125.7, 125.2 (2C), 123.3, 122.8, 122.3, 121.5, 119.8, 111.7, 47.6, 21.0. **(ESI-TOF) HRMS m/z: [2M+H]⁺ calcd for C₅₄H₄₅N₈O₈S₂: 997.2796; found: 997.2829.**

4.1.4.19. N-(3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl)-3-nitrobenzenesulfonamide (25r). R_f = 0.30 (EtOAc/hexane 1:2). White solid, yield 57%, mp: 184-185 °C. **FT-IR (ν max, cm⁻¹):** 3566 (NH sulfonamide), 3061 (CH aromatic), 2920 (CH aliphatic), 1651 (C=N), 1607 (C=C), 1526 and 1350 (NO₂). **¹H NMR (500 MHz, DMSO-d₆):** δ 10.82 (s, 1H), 8.51 (t, *J* = 2.0 Hz, 1H), 8.45 - 8.42 (m, 1H), 8.12 - 8.09 (m, 1H), 7.83 (t, *J* = 8.1 Hz, 1H), 7.71 - 7.69 (m, 1H), 7.55 (d, *J* = 1.1 Hz, 1H), 7.45 - 7.41 (m, 3H), 7.31 - 7.28 (m, 1H), 7.26 - 7.21 (m, 2H), 7.03 (d, *J* = 7.9 Hz, 2H), 6.79 (d, *J* = 8.0 Hz, 2H), 5.43 (s, 2H), 2.22 (s, 3H). **¹³C NMR-APT (126 MHz, DMSO-d₆):** δ 152.7, 148.4, 143.0, 141.2, 137.8, 137.2, 136.2, 134.0, 133, 131.9, 131.7, 130.5, 129.7 (2C), 128.2, 126.5 (2C), 125.8, 123.3, 122.8, 122.6, 121.9, 121.9, 119.8, 111.7, 47.6, 21.1. **(ESI-TOF) HRMS m/z: [M+H]⁺ calcd for C₂₇H₂₃N₄O₄S: 499.1434; found: 499.1436. HPLC purity: 98.2%, HPLC t_R: 3.02 min.**

4.1.4.20. N-(3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl)-2-nitrobenzenesulfonamide (25s). R_f = 0.30 (EtOAc/hexane 1:2). White solid, yield 42%, mp: 198-200 °C. **FT-IR (ν max, cm⁻¹):** 3566 (NH sulfonamide), 1651 (C=N), 1614 (C=C), 1541 and 1395 (NO₂). **¹H NMR (500 MHz, DMSO-d₆):** δ 10.97 (s, 1H), 7.97 (ddd, *J* = 1.3, 7.9, 9.2 Hz, 2H), 7.84 (td, *J* = 3.9, 9.2 Hz, 1H), 7.78 (td, *J* = 3.8, 9.0 Hz, 1H), 7.73 - 7.70 (m, 1H), 7.58 (t, *J* = 1.2 Hz,

1H), 7.46 - 7.43 (m, 3H), 7.31 (dt, $J = 2.4, 4.7$ Hz, 1H), 7.26 - 7.22 (m, 2H), 7.04 (d, $J = 7.8$ Hz, 2H), 6.80 (d, $J = 8.1$ Hz, 2H), 5.44 (s, 2H), 2.22 (s, 3H). ^{13}C NMR-APT (126 MHz, DMSO- d_6): δ 152.8, 148.3, 143.0, 137.6, 137.2, 136.2, 135.3, 134.0, 133.1, 131.7, 130.4, 130.3, 129.7 (2C), 126.6 (2C), 125.7, 125.3, 123.3, 122.8, 122.2, 121.6, 119.8, 111.8, 47.7, 21.1. (ESI-TOF) HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{23}\text{N}_4\text{O}_4\text{S}$: 499.1434; found: 499.1445. HPLC purity: 98.9%, HPLC t_R : 2.97 min.

4.1.4.21. *N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)naphthalene-2-sulfonamide (**25t**). $R_f = 0.25$ (DCM/MeOH 9.8:0.2). Off-white solid, yield 58%, mp: 98-100 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 10.68 (s, 1H), 8.46 (d, $J = 1.6$ Hz, 1H), 8.07 (dd, $J = 5.8, 8.1$ Hz, 2H), 7.98 (d, $J = 8.1$ Hz, 1H), 7.78 (dd, $J = 1.9, 8.7$ Hz, 1H), 7.68 (tt, $J = 2.1, 5.8$ Hz, 2H), 7.63 (td, $J = 3.8, 8.7$ Hz, 1H), 7.60 (t, $J = 1.7$ Hz, 1H), 7.41 (ddd, $J = 2.2, 2.2, 5.3$ Hz, 1H), 7.36 (t, $J = 7.8$ Hz, 1H), 7.30 (tt, $J = 2.3, 6.5$ Hz, 2H), 7.22 (tt, $J = 2.3, 4.6$ Hz, 2H), 6.91 (d, $J = 7.9$ Hz, 2H), 6.67 (d, $J = 8.1$ Hz, 2H), 5.36 (s, 2H), 2.16 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 152.9, 143.0, 138.6, 137.1, 136.7, 136.2, 134.8, 134.0, 132.0, 131.5, 130.2, 130.1, 129.7, 129.6 (2C), 129.5, 128.5, 128.3, 128.2, 126.5 (2C), 125.0, 123.2, 122.7, 122.3, 121.6, 121.0, 119.8, 111.7, 47.5, 21.0. (ESI-TOF) HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{31}\text{H}_{26}\text{N}_3\text{O}_2\text{S}$: 504.1740; found: 504.1762.

4.1.4.22. *N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)-[1,1'-biphenyl]-4-sulfonamide (**25u**). $R_f = 0.30$ (DCM/MeOH 9.8:0.2). White solid, yield 23%, mp: 116-118 °C. ^1H NMR (400 MHz, DMSO- d_6) δ 10.63 (s, 1H), 7.92 - 7.75 (m, 4H), 7.74 - 7.62 (m, 3H), 7.59 (s, 1H), 7.52 - 7.36 (m, 6H), 7.31 (d, $J = 6.1$ Hz, 1H), 7.26 - 7.16 (m, 2H), 6.97 (d, $J = 6.1$ Hz, 2H), 6.74 (d, $J = 6.3$ Hz, 2H), 5.42 (s, 2H), 2.13 (s, 3H).

4.1.4.23. **4-amino-*N*-(3-(1-(4-methylbenzyl)-1*H*-benzimidazol-2-yl)phenyl)benzenesulfonamide (**26a**)**. $R_f = 0.25$ (EtOAc/hexane 1:1). Off-white solid, yield 43%, mp: 172-174 °C. FT-IR (ν max, cm^{-1}): 3364, 3246 (NH_2), 2969, 2935 (CH aliphatic), 1697 (C=N), 1595 (C=C). ^1H NMR (500 MHz, DMSO- d_6): δ 10.14 (s, 1H), 7.71 (dd, $J = 2.0, 6.5$ Hz, 1H), 7.55 (s, 1H), 7.43 (dd, $J = 2.1, 6.6$ Hz, 1H), 7.39 (t, $J = 6.4$ Hz, 2H), 7.35 (d, $J = 7.9$ Hz, 1H), 7.30 (d, $J = 7.7$ Hz, 1H), 7.26 - 7.21 (m, 3H), 7.06 (d, $J = 7.9$ Hz, 2H), 6.81 (d, $J = 8.0$ Hz, 2H), 6.53 (d, $J = 8.8$ Hz, 2H), 6.01 (s, 2H), 5.45 (s, 2H), 2.23 (s, 3H). ^{13}C NMR (126 MHz, DMSO- d_6): δ 153.5, 153.2, 143.1, 139.4, 137.2, 136.2, 134.1, 131.3, 130.0, 129.7 (2C), 129.2 (2C), 126.6

(2C), 124.5, 124.2, 123.2, 122.7, 121.0, 120.3, 119.8, 113.1 (2C), 111.7, 47.6, 21.1. **(ESI-TOF) HRMS m/z: [2M+H]⁺ calcd for C₅₄H₄₉N₈O₄S₂: 937.3312; found: 937.3335.**

4.1.4.24. 3-amino-N-(3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl)benzenesulfonamide (26b). R_f = 0.40 (EtOAc/hexane 1:1). Off-white solid, yield 53%, mp: 116-118 °C. **FT-IR** (ν max, cm⁻¹): 3379, 3226 (NH₂), 3061 (CH aromatic), 2964, 2927 (CH aliphatic), 1697 (C=N), 1598 (C=C). **¹H NMR (500 MHz, DMSO-d₆):** δ 10.43 (s, 1H), 7.72 - 7.69 (m, 1H), 7.55 (t, *J* = 1.8 Hz, 1H), 7.44 - 7.41 (m, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.31 (dt, *J* = 2.5, 4.5 Hz, 1H), 7.27 - 7.21 (m, 3H), 7.11 (t, *J* = 7.9 Hz, 1H), 7.05 (d, *J* = 7.9 Hz, 2H), 7.01 (t, *J* = 2.0 Hz, 1H), 6.85 (dq, *J* = 0.9, 3.2 Hz, 1H), 6.80 (d, *J* = 8.1 Hz, 2H), 6.71 (ddd, *J* = 0.8, 2.3, 8.1 Hz, 1H), 5.61 (s, 2H), 5.44 (s, 2H), 2.22 (s, 3H). **¹³C NMR-APT (126 MHz, DMSO-d₆):** δ 153.1, 149.9, 143.0, 140.5, 139.1, 137.1, 136.2, 134.1, 131.4, 130.1, 129.7 (2C), 126.6 (2C), 124.4, 123.2, 122.7, 121.2, 120.6, 119.8, 118.2, 113.7, 111.7, 111.5, 47.6, 21.1. **(ESI-TOF) HRMS m/z: [M+H]⁺ calcd for C₂₇H₂₅N₄O₂S: 469.1692; found: 469.1693. HPLC purity: 96.8%, HPLC t_R: 2.62 min.**

4.2. Biology

4.2.1. Determination of MICs against Gram-negative bacterial strains

The antibacterial activity of the benzimidazole compounds was evaluated using the broth microdilution method as outlined previously [67–70]. Briefly, a 0.5 McFarland standard solution from each strain was prepared and diluted in cation-adjusted Mueller-Hinton broth to reach a concentration of $\sim 5 \times 10^5$ CFU/mL. Serial dilutions of test agents were incubated with bacteria before recording the MICs. The antibacterial activity of the compounds against *N. gonorrhoeae* was evaluated as described in previous reports [71–76].

4.2.2. In vitro cytotoxicity evaluation of the benzimidazole derivative 25d

Compound **25d** was assayed for its potential cytotoxicity against kidney fibroblast (Vero) cells as described previously [77–82]. Briefly, **25d** was incubated with Vero cells for 24 h. DMSO, at a concentration equal to that in drug-treated wells, served as a negative control. Then, cells were incubated with MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) reagent for 3 h before measuring absorbance values (OD₄₉₀).

4.2.3. Time-kill kinetics assay of 25d

To determine the mode of the killing of **25d**, a standard time-kill kinetics assay was performed against *E. coli* JW55031 as described previously [68,72,83–86]. *E. coli* was grown in tryptone soy broth (TSB) to logarithmic phase and further diluted to reach an initial inoculum of $\sim 10^6$ CFU/mL. **25d** was then added (at 5 \times and 10 \times MIC in triplicates), and further incubated at 37 °C for 24 h. Bacteria exposed to DMSO (solvent of compounds) served as a negative control. An aliquot from each treatment was collected after the corresponding times of incubation and subsequently serially diluted and plated onto tryptone soy agar plates. Plates were incubated for 18-20 h at 37 °C before viable CFU/mL was determined.

4.3. Formulation studies of **III** and **25d** derivatives

4.3.1. Materials

Solutol HS 15, tween 80, stearylamine, verapamil and cremophor RH 40 were purchased from Sigma Aldrich, Germany. Labrafac Lipophile WL 1349 was kindly obtained as gift from Gattefosse' company, France. Phospholipid (Epikuron 200) was kindly obtained as gift from Cargill company, Germany.

4.3.2 Preparation of nanoparticles

Lipidic nanoparticles (nanocapsules) for compounds **III** and **25d** were prepared using the phase inversion temperature method, as shown in **Table A** [87,88]. Twenty mg of either compounds, in addition to the functional additives (if present) were dispersed in 1 mL Labrafac Lipophile, then mixed with Solutol[®] HS15. Distilled water (3 mL) and phospholipid (110 mg), in addition to the mixture were heated under magnetic stirring up to 85 °C for the formation of the w/o emulsion. The emulsion was then cooled to 55 °C, accompanied by phase inversion to o/w emulsion. This cycle was repeated twice before adding a certain amount of distilled water at 4 °C. The nanoparticulate dispersion was then stirred for 10 min before further analysis. The final weight of the formulation components was set to 10 grams, yielding a concentration of 2 mg/mL of either **III** or **25d**. As shown in **Table A**, some formulations (Blank A, B, C, D, and E) served as control formulations, not containing any drugs. The nanoparticles were characterized for their particle size, polydispersity, zeta potential using the Zetasizer device (Malvern, UK) after dilution 1:100 with deionized water. The encapsulation efficiency of the loaded selected molecules **III** and **25d** was measured using UV spectrophotometer (Shimadzu, Japan) at 254 nm.

Table A. Composition of different nanoparticulate formulations containing either **III** or **25d**.

Formula code	Compound loaded	Amount of Solutol (mg)	Functional additive present	Amount of functional additive (mg)
Formula A1 (III)	III	900	Tween 80	100
Formula A2 (25d)	25d	900	Tween 80	100
Blank A	--	900	Tween 80	100
Formula B1 (III)	III	900	Tween 80	100
			Stearylamine	10
Formula B2 (25d)	25d	900	Tween 80	100
			Stearylamine	10
Blank B	--	900	Tween 80	100
			Stearylamine	10
Formula C (25d)	25d	1000	Stearylamine	20
Blank C	--	1000	Stearylamine	20
Formula D (25d)	25d	900	Cremophor RH 40	100
			Stearylamine	10
Blank D	--	900	Cremophor RH 40	100
			Stearylamine	10
Formula E (25d)	25d	900	Verapamil	100
			Stearylamine	10
Blank E	--	900	Verapamil	100
			Stearylamine	10

Acknowledgment

This work was supported by a grant from the Science, Technology, and Innovation Funding Authority STDF RS youth project No. 39383 (M.A.). E.M.E.D. has been awarded a postdoctoral fellowship at Martin-Luther-Universität, Germany funded by the Ministry of Higher Education of the Arab Republic of Egypt. S.N.M. has been awarded a PhD scholarship at University of Leeds funded by the Newton-Mosharafa Fund jointly offered by the British Council and the Ministry of Higher Education of the Arab Republic of Egypt. We thank Dr Jeanine Williams (School of Chemistry, University of Leeds) for conducting the HPLC purity determination assays.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/xxxxxxx>.

References

- [1] A. Bryce, A.D. Hay, I.F. Lane, H. V. Thornton, M. Wootton, C. Costelloe, Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by *Escherichia coli* and association with routine use of antibiotics in primary care: systematic

- review and meta-analysis, *BMJ*. 352 :i939 (2016). doi:10.1136/bmj.i939.
- [2] Bhoomika, S. Shakya, A. Patyal, N.E. Gade, Occurrence and characteristics of extended-spectrum β -lactamases producing *Escherichia coli* in foods of animal origin and human clinical samples in Chhattisgarh, India, *Vet. World*. 9 (2016) 996. doi:10.14202/vetworld.2016.996-1000.
- [3] Antimicrobial resistance, (n.d.). <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance> (accessed April 16, 2022).
- [4] Centers for Disease Control and Prevention: Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: Centers for Disease Control and Prevention, (n.d.). <https://www.cdc.gov/drugresistance/biggest-threats.html> (accessed July 25, 2022).
- [5] E. Medina, D.H. Pieper, Tackling Threats and Future Problems of Multidrug-Resistant Bacteria, *Curr. Top. Microbiol. Immunol.* 398 (2016) 3–33. doi:10.1007/82_2016_492.
- [6] M. Exner, S. Bhattacharya, B. Christiansen, J. Gebel, P. Goroncy-Bermes, P. Hartemann, P. Heeg, C. Ilchner, A. Kramer, E. Larson, W. Merkens, M. Mielke, P. Oltmanns, B. Ross, M. Rotter, R.M. Schmithausen, H.-G. Sonntag, M. Trautmann, Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria?, *GMS Hyg. Infect. Control*. 12 (2017) Doc05. doi:10.3205/dgkh000290.
- [7] H.M. Zowawi, P.N.A. Harris, M.J. Roberts, P.A. Tambyah, M.A. Schembri, M.D. Pezzani, D.A. Williamson, D.L. Paterson, The emerging threat of multidrug-resistant Gram-negative bacteria in urology, *Nat. Rev. Urol.* 12 (2015) 570–584. doi:10.1038/nrurol.2015.199.
- [8] Z. Aghapour, P. Gholizadeh, K. Ganbarov, A.Z. Bialvaei, S.S. Mahmood, A. Tanomand, M. Yousefi, M. Asgharzadeh, B. Yousefi, H.S. Kafil, Molecular mechanisms related to colistin resistance in Enterobacteriaceae, *Infect. Drug Resist.* 12 (2019) 965–975. doi:10.2147/IDR.S199844.
- [9] A. Pormohammad, R. Pouriran, H. Azimi, M. Goudarzi, Prevalence of integron classes in Gram-negative clinical isolated bacteria in Iran: a systematic review and meta-analysis, *Iran. J. Basic Med. Sci.* 22 (2019) 118. doi:10.22038/ijbms.2018.32052.7697.
- [10] M. Bassetti, J. Garau, Current and future perspectives in the treatment of multidrug-resistant

- Gram-negative infections, *J. Antimicrob. Chemother.* 76 (2021) iv23–iv37. doi:10.1093/jac/dkab352.
- [11] M. Terreni, M. Taccani, M. Pregnolato, New Antibiotics for Multidrug-Resistant Bacterial Strains: Latest Research Developments and Future Perspectives, *Molecules*. 26 (2021) 2671. doi:10.3390/molecules26092671.
- [12] J.P. Ebejer, M.H. Charlton, P.W. Finn, Are the physicochemical properties of antibacterial compounds really different from other drugs?, *J. Cheminform.* 8 (2016) 1–9. doi:10.1186/s13321-016-0143-5.
- [13] H.I. Zgurskaya, C.A. López, S. Gnanakaran, Permeability Barrier of Gram-Negative Cell Envelopes and Approaches To Bypass It, *ACS Infect. Dis.* 1 (2015) 512–522. doi:10.1021/acsinfecdis.5b00097.
- [14] L.L. Silver, Challenges of antibacterial discovery, *Clin. Microbiol. Rev.* 24 (2011) 71–109. doi:10.1128/CMR.00030-10.
- [15] R. O’Shea, H.E. Moser, Physicochemical properties of antibacterial compounds: Implications for drug discovery, *J. Med. Chem.* 51 (2008) 2871–2878. doi:10.1021/jm700967e.
- [16] D.G. Brown, T.L. May-Dracka, M.M. Gagnon, R. Tommasi, Trends and exceptions of physical properties on antibacterial activity for Gram-positive and Gram-negative pathogens, *J. Med. Chem.* 57 (2014) 10144–10161. doi:10.1021/jm501552x.
- [17] T.D. Davis, C.J. Gerry, D.S. Tan, General platform for systematic quantitative evaluation of small-molecule permeability in bacteria, *ACS Chem. Biol.* 9 (2014) 2535–2544. doi:10.1021/cb5003015.
- [18] G. Mugumbate, J.P. Overington, The relationship between target-class and the physicochemical properties of antibacterial drugs, *Bioorg. Med. Chem.* 23 (2015) 5218–5224. doi:10.1016/j.bmc.2015.04.063.
- [19] M.F. Richter, B.S. Drown, A.P. Riley, A. Garcia, T. Shirai, R.L. Svec, P.J. Hergenrother, Predictive compound accumulation rules yield a broad-spectrum antibiotic, *Nature*. 545 (2017) 299–304. doi:10.1038/nature22308.

- [20] M.F. Richter, P.J. Hergenrother, The challenge of converting Gram-positive-only compounds into broad-spectrum antibiotics, *Ann. N. Y. Acad. Sci.* 1435 (2019) 18–38. doi:10.1111/nyas.13598.
- [21] M. Skwarczynski, S. Bashiri, Y. Yuan, Z.M. Ziora, O. Nabil, K. Masuda, M. Khongkow, N. Rimsueb, H. Cabral, U. Ruktanonchai, M.A.T. Blaskovich, I. Toth, Antimicrobial Activity Enhancers: Towards Smart Delivery of Antimicrobial Agents, *Antibiot. (Basel, Switzerland)*. 11 (2022) 412. doi:10.3390/antibiotics11030412.
- [22] S. Shin, S. Kwon, Y. Yeo, Meta-Analysis of Drug Delivery Approaches for Treating Intracellular Infections, *Pharm. Res.* 1 (2022) 1–30. doi:10.1007/s11095-022-03188-z.
- [23] Z. Drulis-Kawa, A. Dorotkiewicz-Jach, Liposomes as delivery systems for antibiotics, *Int. J. Pharm.* 387 (2010) 187–198. doi:10.1016/j.ijpharm.2009.11.033.
- [24] D.Y. Wang, H.C. van der Mei, Y. Ren, H.J. Busscher, L. Shi, Lipid-Based Antimicrobial Delivery-Systems for the Treatment of Bacterial Infections, *Front. Chem.* 7 (2020) 872. doi:10.3389/fchem.2019.00872.
- [25] N. Abed, P. Couvreur, Nanocarriers for antibiotics: A promising solution to treat intracellular bacterial infections, *Int. J. Antimicrob. Agents.* 43 (2014) 485–496. doi:10.1016/j.ijantimicag.2014.02.009.
- [26] N.T. Huynh, C. Passirani, P. Saulnier, J.P. Benoit, Lipid nanocapsules: A new platform for nanomedicine, *Int. J. Pharm.* 379 (2009) 201–209. doi:10.1016/j.ijpharm.2009.04.026.
- [27] P. Sánchez-Moreno, J.L. Ortega-Vinuesa, A. Martín-Rodríguez, H. Boulaiz, J.A. Marchal-Corrales, J.M. Peula-García, Characterization of Different Functionalized Lipidic Nanocapsules as Potential Drug Carriers, *Int. J. Mol. Sci.* 13 (2012) 2405. doi:10.3390/ijms13022405.
- [28] H. Nehme, P. Saulnier, A.A. Ramadan, V. Cassisa, C. Guillet, M. Eveillard, A. Umerska, Antibacterial activity of antipsychotic agents, their association with lipid nanocapsules and its impact on the properties of the nanocarriers and on antibacterial activity, *PLoS One*. 13 (2018) e0189950. doi:10.1371/journal.pone.0189950.
- [29] N. Dabholkar, T. Waghule, V. Krishna Rapalli, S. Gorantla, A. Alexander, R. Narayan Saha,

- G. Singhvi, Lipid shell lipid nanocapsules as smart generation lipid nanocarriers, *J. Mol. Liq.* 339 (2021) 117145. doi:10.1016/j.molliq.2021.117145.
- [30] S. Deng, M.R. Gigliobianco, R. Censi, P. Di Martino, Polymeric Nanocapsules as Nanotechnological Alternative for Drug Delivery System: Current Status, Challenges and Opportunities, *Nanomater.* (Basel, Switzerland). 10 (2020) 847. doi:10.3390/nano10050847.
- [31] M.E. van Gent, M. Ali, P.H. Nibbering, S.N. Kłodzińska, Current Advances in Lipid and Polymeric Antimicrobial Peptide Delivery Systems and Coatings for the Prevention and Treatment of Bacterial Infections, *Pharm.* 2021, Vol. 13, Page 1840. 13 (2021) 1840. doi:10.3390/pharmceutics13111840.
- [32] Z. Ates-Alagoz, Antimicrobial Activities of 1-*H*-Benzimidazole-based Molecules, *Curr. Top. Med. Chem.* 16 (2016) 2953–2962. doi:10.2174/1568026616666160506130226.
- [33] S. Tahlan, S. Kumar, B. Narasimhan, Antimicrobial potential of 1*H*-benzo[*d*]imidazole scaffold: a review, *BMC Chem.* 13 (2019). doi:10.1186/s13065-019-0521-y.
- [34] S.R. Brishty, M.J. Hossain, M.U. Khandaker, M.R.I. Faruque, H. Osman, S.M.A. Rahman, A Comprehensive Account on Recent Progress in Pharmacological Activities of Benzimidazole Derivatives, *Front. Pharmacol.* 12 (2021) 2863. doi:10.3389/fphar.2021.762807.
- [35] D. Song, S. Ma, Recent Development of Benzimidazole-Containing Antibacterial Agents, *ChemMedChem.* 11 (2016) 646–659. doi:10.1002/cmdc.201600041.
- [36] Y. Bansal, M. Kaur, G. Bansal, Antimicrobial Potential of Benzimidazole Derived Molecules, *Mini Rev. Med. Chem.* 19 (2019) 624–646. doi:10.2174/1389557517666171101104024.
- [37] E.M.E. Dokla, N.S. Abutaleb, S.N. Milik, D. Li, K. El-Baz, M.-A.W. Shalaby, R. Al-Karaki, M. Nasr, C.D. Klein, K.A.M. Abouzid, M.N. Seleem, Development of benzimidazole-based derivatives as antimicrobial agents and their synergistic effect with colistin against gram-negative bacteria., *Eur. J. Med. Chem.* 186 (2020) 111850. doi:10.1016/j.ejmech.2019.111850.

- [38] P. Bandyopadhyay, M. Sathe, S. Ponmariappan, A. Sharma, P. Sharma, A.K. Srivastava, M.P. Kaushik, Exploration of *in vitro* time point quantitative evaluation of newly synthesized benzimidazole and benzothiazole derivatives as potential antibacterial agents, *Bioorg. Med. Chem. Lett.* 21 (2011) 7306–7309. doi:10.1016/j.bmcl.2011.10.034.
- [39] E.M.E. Dokla, C.S. Fang, K.A.M. Abouzid, C.S. Chen, 1,2,4-Oxadiazole derivatives targeting EGFR and c-Met degradation in TKI resistant NSCLC, *Eur. J. Med. Chem.* 182 (2019) 111607. doi:10.1016/j.ejmech.2019.111607.
- [40] R.D. Haugwitz, A.J. Martinez, J. Venslavsky, R.G. Angel, B. V. Maurer, G.A. Jacobs, V.L. Narayanan, L.R. Cruthers, J. Szanto, Antiparasitic agents. 6. Synthesis and anthelmintic activities of novel isothiocyanatophenyl-1,2,4-oxadiazoles, *J. Med. Chem.* 28 (2002) 1234–1241. doi:10.1021/JM00147A019.
- [41] B.D. Judkins, D.G. Allen, T.A. Cook, B. Evans, T.E. Sardharwala, A versatile synthesis of amidines from nitriles via amidoximes, *Synth. Commun.* 26 (1996) 4351–4367. doi:10.1080/00397919608003838.
- [42] E.M.E. Dokla, C.-S. Fang, P.-T. Lai, S.K. Kulp, R.A.T. Serya, N.S.M. Ismail, K.A.M. Abouzid, C.-S. Chen, Development of potent adenosine monophosphate activated protein kinase (AMPK) activators, *ChemMedChem.* 10 (2015). doi:10.1002/cmdc.201500371.
- [43] E. M. E. Dokla, A. Kamal Abdel-Aziz, S.N. Milik, M.J. McPhillie, S. Minucci, K. A. M. Abouzid, Discovery of a Benzimidazole-based Dual FLT3/TrKA Inhibitor Targeting Acute Myeloid Leukemia, *Bioorg. Med. Chem.* (2021) 116596. doi:https://doi.org/10.1016/j.bmc.2021.116596.
- [44] J. Yang, G. Su, Y. Ren, Y. Chen, Synthesis of 3,4-diaminobenzoyl derivatives as factor Xa inhibitors, *Eur. J. Med. Chem.* 101 (2015) 41–51. doi:10.1016/j.ejmech.2015.06.012.
- [45] K.Y. Yeong, M.I.H.N. Azizi, N. Berdigaliyev, W.N. Chen, W.L. Lee, A.N. Shirazi, K. Parang, Sirtuin inhibition and anti-cancer activities of ethyl 2-benzimidazole-5-carboxylate derivatives, *Medchemcomm.* 10 (2019) 2140–2145. doi:10.1039/c9md00323a.
- [46] H. Sahoo, A. Mandal, J. Selvakumar, M. Baidya, Remote C–H Selenylation of 8-Amidoquinolines via Copper-Catalyzed Radical Cross-Coupling, *European J. Org. Chem.*

- 2016 (2016) 4321–4327. doi:10.1002/ejoc.201600772.
- [47] M. Scheiner, D. Dolles, S. Gunesch, M. Hoffmann, M. Nabissi, O. Marinelli, M. Naldi, M. Bartolini, S. Petralla, E. Poeta, B. Monti, C. Falkeis, M. Vieth, H. Hübner, P. Gmeiner, R. Maitra, T. Maurice, M. Decker, Dual-Acting Cholinesterase-Human Cannabinoid Receptor 2 Ligands Show Pronounced Neuroprotection *in Vitro* and Overadditive and Disease-Modifying Neuroprotective Effects *in Vivo*, *J. Med. Chem.* 62 (2019) 9078–9102. doi:10.1021/acs.jmedchem.9b00623.
- [48] H. Okusu, D. Ma, H. Nikaido, AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-antibiotic-resistance (Mar) mutants, *J. Bacteriol.* 178 (1996) 306–8. doi:10.1128/jb.178.1.306-308.1996.
- [49] R.L. Marshall, V.N. Bavro, Mutations in the TolC Periplasmic Domain Affect Substrate Specificity of the AcrAB-TolC Pump, *Front. Mol. Biosci.* 7 (2020) 166. doi:10.3389/fmolb.2020.00166.
- [50] A. Schumacher, R. Trittler, J.A. Bohnert, K. Kümmerer, J.-M. Pagès, W. V. Kern, Intracellular accumulation of linezolid in *Escherichia coli*, *Citrobacter freundii* and *Enterobacter aerogenes*: role of enhanced efflux pump activity and inactivation, *J. Antimicrob. Chemother.* 59 (2007) 1261–1264. doi:10.1093/jac/dkl380.
- [51] N. Chowdhury, S. Suhani, A. Purkaystha, M.K. Begum, T. Raihan, M.J. Alam, K. Islam, A.K. Azad, Identification of *AcrAB-TolC* Efflux Pump Genes and Detection of Mutation in Efflux Repressor *AcrR* from Omeprazole Responsive Multidrug-Resistant *Escherichia coli* Isolates Causing Urinary Tract Infections., *Microbiol. Insights.* 12 (2019) 1178636119889629. doi:10.1177/1178636119889629.
- [52] I. Rothenaigner, K. Hadian, Brief Guide: Experimental Strategies for High-Quality Hit Selection from Small-Molecule Screening Campaigns, *SLAS Discov.* 26 (2021) 851–854. doi:10.1177/24725552211008862.
- [53] J.G. Moffat, F. Vincent, J.A. Lee, J. Eder, M. Prunotto, Opportunities and challenges in phenotypic drug discovery: an industry perspective, *Nat. Rev. Drug Discov.* 16 (2017) 531–543. doi:10.1038/nrd.2017.111.

- [54] G.-Q. Chen, Y. Xu, S.-M. Shen, J. Zhang, Phenotype and target-based chemical biology investigations in cancers, *Natl. Sci. Rev.* 6 (2018) 1111–1127. doi:10.1093/nsr/nwy124.
- [55] I. Ghai, S. Ghai, Understanding antibiotic resistance via outer membrane permeability, *Infect. Drug Resist.* 11 (2018) 523–530. doi:10.2147/ird.s156995.
- [56] C.P. Randall, K.R. Mariner, I. Chopra, A.J. O’Neill, The target of daptomycin is absent from *Escherichia coli* and other gram-negative pathogens., *Antimicrob. Agents Chemother.* 57 (2013) 637–639. doi:10.1128/AAC.02005-12.
- [57] L. Wang, C. Hu, L. Shao, The antimicrobial activity of nanoparticles: present situation and prospects for the future, *Int. J. Nanomedicine.* 12 (2017) 1227–1249. doi:10.2147/ijn.s121956.
- [58] M.A. Mohamed, M. Nasr, W.F. Elkhatib, W.N. Eltayeb, *In Vitro* Evaluation of Antimicrobial Activity and Cytotoxicity of Different Nanobiotics Targeting Multidrug Resistant and Biofilm Forming Staphylococci, *Biomed Res. Int.* 2018 (2018) 7658238. doi:10.1155/2018/7658238.
- [59] Z. Drulis-Kawa, J. Gubernator, A. Dorotkiewicz-Jach, W. Doroszkiewicz, A. Kozubek, A comparison of the *in vitro* antimicrobial activity of liposomes containing meropenem and gentamicin, *Cell. Mol. Biol. Lett.* 11 (2006) 360–375. doi:10.2478/S11658-006-0030-6.
- [60] K.N. Adams, J.D. Szumowski, L. Ramakrishnan, Verapamil, and Its Metabolite Norverapamil, Inhibit Macrophage-induced, Bacterial Efflux Pump-mediated Tolerance to Multiple Anti-tubercular Drugs, *J. Infect. Dis.* 210 (2014) 456–466. doi:10.1093/infdis/jiu095.
- [61] Y. Tayrouz, R. Ding, J. Burhenne, K.-D. Riedel, J. Weiss, T. Hoppe-Tichy, W.E. Haefeli, G. Mikus, Pharmacokinetic and pharmaceutic interaction between digoxin and Cremophor RH40, *Clin. Pharmacol. Ther.* 73 (2003) 397–405. doi:10.1016/S0009-9236(03)00059-6.
- [62] N. Figura, R. Marcolongo, G. Cavallo, A. Santucci, G. Collodel, A. Spreafico, E. Moretti, Polysorbate 80 and *Helicobacter pylori*: a microbiological and ultrastructural study, *BMC Microbiol.* 12 (2012) 217. doi:10.1186/1471-2180-12-217.
- [63] N.S. Abutaleb, A.E.M. Elhassanny, M.N. Seleem, *In vivo* efficacy of acetazolamide in a

- mouse model of *Neisseria gonorrhoeae* infection., *Microb. Pathog.* 164 (2022) 105454. doi:10.1016/j.micpath.2022.105454.
- [64] M. Alhashimi, A. Mayhoub, M.N. Seleem, Repurposing salicylamide for combating multidrug-resistant *Neisseria gonorrhoeae*., *Antimicrob. Agents Chemother.* 63 (2019). doi:10.1128/AAC.01225-19.
- [65] A. Elkashif, M.N. Seleem, Investigation of auranofin and gold-containing analogues antibacterial activity against multidrug-resistant *Neisseria gonorrhoeae*., *Sci. Rep.* 10 (2020) 5602. doi:10.1038/s41598-020-62696-3.
- [66] B. Cherinka, B.H. Andrews, J. Sánchez-Gallego, J. Brownstein, M. Argudo-Fernández, M. Blanton, K. Bundy, A. Jones, K. Masters, D.R. Law, K. Rowlands, A.-M. Weijmans, K. Westfall, R. Yan, Marvin: A Tool Kit for Streamlined Access and Visualization of the SDSS-IV MaNGA Data Set, *Astron. J.* 158 (2019) 74. doi:10.3847/1538-3881/ab2634.
- [67] CLSI, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition*, CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.
- [68] S.A. Yuk, H. Kim, N.S. Abutaleb, A.M. Dieterly, M.S. Taha, M.D. Tsifansky, L.T. Lyle, M.N. Seleem, Y. Yeo, Nanocapsules modify membrane interaction of polymyxin B to enable safe systemic therapy of Gram-negative sepsis., *Sci. Adv.* 7 (2021). doi:10.1126/sciadv.abj1577.
- [69] I.G. Shahin, N.S. Abutaleb, M. Alhashimi, A.E. Kassab, K.O. Mohamed, A.T. Taher, M.N. Seleem, A.S. Mayhoub, Evaluation of *N*-phenyl-2-aminothiazoles for treatment of multi-drug resistant and intracellular *Staphylococcus aureus* infections., *Eur. J. Med. Chem.* 202 (2020) 112497. doi:10.1016/j.ejmech.2020.112497.
- [70] A. Hammad, N.S. Abutaleb, M. Elsebaei, A.B. Norvil, M. Alswah, A.O. Ali, J.A. Abdel-Aleem, A. Alattar, S. Bayoumi, H. Gowher, M.N. Seleem, A.S. Mayhoub, From Phenylthiazoles to Phenylpyrazoles: Broadening the Antibacterial Spectrum towards Carbapenem-Resistant Bacteria, *J. Med. Chem.* (2019) acs.jmedchem.9b00720. doi:10.1021/acs.jmedchem.9b00720.

- [71] A.E.M. Elhassanny, N.S. Abutaleb, M.N. Seleem, Auranofin exerts antibacterial activity against *Neisseria gonorrhoeae* in a female mouse model of genital tract infection., PLoS One. 17 (2022) e0266764. doi:10.1371/journal.pone.0266764.
- [72] N.S. Abutaleb, A.E.M. Elhassanny, A. Nocentini, C.S. Hewitt, A. Elkashif, B.R. Cooper, C.T. Supuran, M.N. Seleem, D.P. Flaherty, Repurposing FDA-approved sulphonamide carbonic anhydrase inhibitors for treatment of *Neisseria gonorrhoeae*., J. Enzyme Inhib. Med. Chem. 37 (2022) 51–61. doi:10.1080/14756366.2021.1991336.
- [73] S. Giovannuzzi, N.S. Abutaleb, C.S. Hewitt, F. Carta, A. Nocentini, M.N. Seleem, D.P. Flaherty, C.T. Supuran, Dithiocarbamates effectively inhibit the α -carbonic anhydrase from *Neisseria gonorrhoeae*., J. Enzyme Inhib. Med. Chem. 37 (2022) 1–8. doi:10.1080/14756366.2021.1988945.
- [74] C.S. Hewitt, N.S. Abutaleb, A.E.M. Elhassanny, A. Nocentini, X. Cao, D.P. Amos, M.S. Youse, K.J. Holly, A.K. Marapaka, W. An, J. Kaur, A.D. Krabill, A. Elkashif, Y. Elgammal, A.L. Graboski, C.T. Supuran, M.N. Seleem, D.P. Flaherty, Structure-Activity Relationship Studies of Acetazolamide-Based Carbonic Anhydrase Inhibitors with Activity against *Neisseria gonorrhoeae*., ACS Infect. Dis. 7 (2021) 1969–1984. doi:10.1021/acsinfecdis.1c00055.
- [75] Y.J. Seong, M. Alhashimi, A. Mayhoub, H. Mohammad, M.N. Seleem, Repurposing Fenamic Acid Drugs To Combat Multidrug-Resistant *Neisseria gonorrhoeae*., Antimicrob. Agents Chemother. 64 (2020). doi:10.1128/AAC.02206-19.
- [76] G.A. Naclerio, N.S. Abutaleb, M. Alhashimi, M.N. Seleem, H.O. Sintim, N-(1,3,4-Oxadiazol-2-yl)Benzamides as Antibacterial Agents against *Neisseria gonorrhoeae*., Int. J. Mol. Sci. 22 (2021). doi:10.3390/ijms22052427.
- [77] A. Kotb, N.S. Abutaleb, M.A. Seleem, M. Hagra, H. Mohammad, A. Bayoumi, A. Ghiaty, M.N. Seleem, A.S. Mayhoub, Phenylthiazoles with tert-Butyl side chain: Metabolically stable with anti-biofilm activity, Eur. J. Med. Chem. 151 (2018) 110–120. doi:10.1016/j.ejmech.2018.03.044.
- [78] M. ElAwamy, H. Mohammad, A. Hussien, N.S. Abutaleb, M. Hagra, R.A.T. Serya, A.T.

- Taher, K.A. Abouzid, M.N. Seleem, A.S. Mayhoub, Alkoxyphenylthiazoles with broad-spectrum activity against multidrug-resistant gram-positive bacterial pathogens, *Eur. J. Med. Chem.* 152 (2018) 318–328. doi:10.1016/j.ejmech.2018.04.049.
- [79] M. Hagra, N.S. Abutaleb, A.M. Sayed, E.A. Salama, M.N. Seleem, A.S. Mayhoub, Evaluation of bisphenylthiazoles as a promising class for combating multidrug-resistant fungal infections., *PLoS One*. 16 (2021) e0258465. doi:10.1371/journal.pone.0258465.
- [80] C.W. Karanja, N. Naganna, N.S. Abutaleb, N. Dayal, K.I. Onyedibe, U. Aryal, M.N. Seleem, H.O. Sintim, Isoquinoline Antimicrobial Agent: Activity against Intracellular Bacteria and Effect on Global Bacterial Proteome., *Molecules*. 27 (2022). doi:10.3390/molecules27165085.
- [81] M.M. Elsebaie, H.T. Nour El-Din, N.S. Abutaleb, A.A. Abuelkhir, H.-W. Liang, A.S. Attia, M.N. Seleem, A.S. Mayhoub, Exploring the structure-activity relationships of diphenylurea as an antibacterial scaffold active against methicillin- and vancomycin-resistant *Staphylococcus aureus*, *Eur. J. Med. Chem.* 234 (2022) 114204. doi:https://doi.org/10.1016/j.ejmech.2022.114204.
- [82] A.M. Sayed, N.S. Abutaleb, A. Kotb, H.G. Ezzat, M.N. Seleem, A.S. Mayhoub, M.M. Elsebaie, Arylpyrazole as selective anti-enterococci; synthesis and biological evaluation of novel derivatives for their antimicrobial efficacy, *J. Heterocycl. Chem.* 1 (2022). doi:https://doi.org/10.1002/jhet.4570.
- [83] H.J. Hamann, N.S. Abutaleb, R. Pal, M.N. Seleem, P.V. Ramachandran, β,γ -Diaryl α -methylene- γ -butyrolactones as potent antibacterials against methicillin-resistant *Staphylococcus aureus*., *Bioorg. Chem.* 104 (2020) 104183. doi:10.1016/j.bioorg.2020.104183.
- [84] N.S. Abutaleb, M.N. Seleem, Repurposing the Antiamoebic Drug Diiodohydroxyquinoline for Treatment of *Clostridioides difficile* Infections., *Antimicrob. Agents Chemother.* 64 (2020). doi:10.1128/AAC.02115-19.
- [85] A. Mancy, N.S. Abutaleb, M.M. Elsebaei, A.Y. Saad, A. Kotb, A.O. Ali, J.A. Abdel-Aleem, H. Mohammad, M.N. Seleem, A.S. Mayhoub, Balancing Physicochemical Properties of

- Phenylthiazole Compounds with Antibacterial Potency by Modifying the Lipophilic Side Chain., *ACS Infect. Dis.* 6 (2020) 80–90. doi:10.1021/acsinfecdis.9b00211.
- [86] M. Hagra, N.S. Abutaleb, N.M. Elhosseiny, T.M. Abdelghany, M. Omara, M.M. Elsebaei, M. Alhashimi, A.B. Norvil, M.I. Gutay, H. Gowher, A.S. Attia, M.N. Seleem, A.S. Mayhoub, Development of Biphenylthiazoles Exhibiting Improved Pharmacokinetics and Potent Activity Against Intracellular *Staphylococcus aureus.*, *ACS Infect. Dis.* 6 (2020) 2887–2900. doi:10.1021/acsinfecdis.0c00137.
- [87] R. Al-Karaki, A. Awadallah, M. Saleh, M. Nasr, Novel oleuropein nanocapsular formulation: preparation, characterization and anticolon cancer activity, *J. Appl. Pharm.* 11 (2019) 1–8. doi:10.21065/19204159/11.01.
- [88] B. Heurtault, P. Saulnier, B. Pech, J.E. Proust, J.P. Benoit, A Novel Phase Inversion-Based Process for the Preparation of Lipid Nanocarriers, *Pharm. Res.* 2002 196. 19 (2002) 875–880. doi:10.1023/A:1016121319668.