Research paper

Development of benzimidazole-based derivatives as antimicrobial agents and their synergistic effect with colistin against gram-negative bacteria

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ABSTRACT

Gram-negative bacteria pose a distinctive risk worldwide, especially with the evolution of major resistance to carbapenems, fluoroquinolones and colistin. Therefore, development of new antibacterial agents to target Gram-negative infections is of utmost importance. Using phenotypic screening, we synthesized and tested thirty-one benzimidazole derivatives against *E. coli* JW55031 (TolC mutant strain). Compound **6c** showed potent activity with MIC value of 2 μg/ml, however, it lacked activity against several Gram-negative microbes with intact efflux systems, including *E. coli* BW25113 (wild-type strain). Combination of **6c** with colistin partially restored its antibacterial activity against wild strains (MIC range, 8−16 μg/ml against *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*). **6c** exhibited no cytotoxicity against two mammalian cell lines. Therefore, compound **6c** represents a promising lead for further optimization to overcome Gram-negative resistance alone or in combination therapy.

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1. Introduction

According to the WHO reports, antimicrobial resistance (AMR) is showing an alarming increase worldwide [1]. The CDC (Centers for Disease Control and Prevention) surveillance report in 2013, documented at least two million resistant infections in the United States with around 23,000 related deaths. This burden extends to a healthcare economic cost of around 20 billion dollars [2]. Moreover, despite the lack of national surveillance reports, several individual studies document high rates of antimicrobial resistance among both Gram-positive and Gram-negative infections in Africa [3,4]. Unfortunately, the last class of antibiotics was introduced in 1987. Since then, only derivatives of existing classes were developed [5]. Combined with antibiotics misuse and low interest from pharmaceutical companies to invest in antibiotic development [6], the need to introduce new classes of antibiotics is greater than ever.

Among the CDC top eighteen bacterial and fungal threats, Gram-negative bacteria represents half of these threats (e.g. *E. coli*, *K. pneumoniae*, *A. baumannii*, *Campylobacter*, *P. aeruginosa*, and

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Salmonella) [7]. These microbes are the causative agents of many infections, including but not limited to, pneumonia, urinary tract infections, food poisoning, and blood infections [8]. Many strains of these bacteria have developed resistance against most antibiotics including first-line drugs; carbapenems, fluoroquinolones, and aminoglycosides [9]. Resistance is acquired through several mechanisms that include the overexpression of antibiotic-inactivating enzymes (e.g. extended-spectrum β-lactamases (ESBL)-producing Enterobacteriaceae), increased activity of efflux pumps, and mutations of the outer membrane or target enzymes [9–11]. Further exacerbating the situation, resistance to colistin has recently emerged through various mechanisms including the acquirement of *mcr-1* gene [12–14]. Colistin is the last resort antibiotic as it has broad activity against Gram-negative infections. Given the fact that Gram-negative infections are the most common infections in humans, such resistance constitutes a real threat that should be addressed [6].

Phenotypic screening of compound databases is a promising approach to discover potential leads for antibacterial development. This approach avoids many of the pitfalls of molecular target-centered screening, where antibiotic leads often failed to reach preclinical development stages [15]. Benzimidazole is considered a privileged scaffold having a wide range of biological activities [16]. As antimicrobials, in addition to marketed drugs as thiabendazole and albendazole, several benzimidazole-based derivatives were reported against both Gram-positive and Gram-negative microorganisms [17,18]. Benzimidazole can act as a bioisostere for purine which is crucial for bacterial cell wall, nucleic acid and protein biosynthesis. Furthermore, the benzimidazole drugs in clinical use have an established safety and efficacy profiles [18]. Based on these premises, we selected a 1,2-disubstituted benzimidazole scaffold to initiate a phenotypic antibacterial screening. Our selection was additionally supported by their ease of synthesis and diversification, thus allowing extensive structure activity relationship (SAR) exploration.

2. Results and discussion

2.1. Design strategy

Two 1,2-disubstituted benzimidazoles (**4a**, **4b**), previously reported as antimicrobial agents [19], were explored for their potential to inhibit Gram-negative bacteria. These compounds showed a modest activity against *E. coli* JW55031 (TolC mutant) (MIC values of 16 and 8 μg/ml respectively). They were inactive against *E. coli* BW25113 (wild type strain) or other Gram-negative bacteria (*Acinetobacter baumannii*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Our initial intent was to explore whether these simple benzimidazole-based small molecules were optimizable to yield more potent derivatives active against the wild-type *E. coli*. We explored the SAR around this structural scaffold by synthesizing thirty-one derivatives with variable substituents at both phenyl rings A & B (Fig. 1 and Scheme 1). We investigated several polar/hydrophobic, HB donor/acceptor or small/bulky substituents to help construct a preliminary SAR proposal for these derivatives.

The presence of an outer membrane in most Gram-negative bacteria constitutes an additional barrier against the penetration of many antibiotics [20]. An old class of antibiotics; polymyxins (e.g. colistin) disrupt the outer membrane of Gram-negative bacteria through interaction with the lipopolysaccharide (LPS) and phospholipid components [21]. As antibiotic synergy is an effective strategy to improve the treatment efficacy and minimize resistance
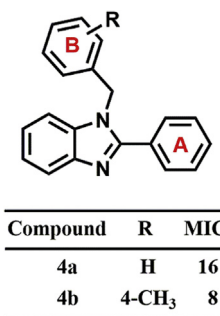

Fig. 1. Chemical structures of the starting benzimidazole derivatives and their antibacterial activity against *E. coli* JW55031 (TolC mutant) (MIC in μg/ml).

Compound	R	MIC
4a	H	16
4b	4-CH$_3$	8

[22], we decided to investigate the synergistic effect of combining our optimized 1,2-benzimidazole derivative with sub-inhibitory concentration of colistin against several Gram-negative bacterial strains. This would confirm whether the presence of the outer membrane confers resistance for Gram-negative bacterial strains against our 1,2-benzimidazole derivatives or not.

2.2. Chemistry

The desired 1,2-disubstituted benzimidazole derivatives were synthesized according to previously reported procedures [23–27] as depicted in Scheme 1. *O*-phenylene diamine was cyclized with the appropriate benzaldehyde using sodium acetate in ethanol to give 2-(substituted phenyl)-1*H*-benzimidazole (**3**) in moderate to good yields. This compound was alkylated with appropriate benzyl/alkyl halides using cesium carbonate in DMF to give the desired 1,2-disubstituted benzimidazole (**4**) in poor to moderate yields. The appropriate 1-substituted-2-(3- or 4-nitrophenyl) benzimidazole (**4**) was reduced using stannous chloride dihydrate in EtOAc to afford the desired amino derivatives (**5**) in moderate yields. Further acylation of the appropriate amine derivatives (**5**) was achieved using acetic anhydride in DCM to give the desired acetamido derivatives (**6a-b**). Mesylation of the appropriate amine derivatives (**5**) using mesityl chloride in pyridine gave the desired methanesulfonamide derivatives (**6c-s**) in poor to moderate yields.

2.3. Biological activity

2.3.1. Initial screening of synthesized benzimidazoles against Escherichia coli

The synthesized 1,2-disubstituted benzimidazole derivatives were initially evaluated for their *in vitro* antibacterial activity against *E. coli* JW55031 (TolC mutant) strain (Table 1). This strain has a deletion in *tolC* gene which is a key component of AcrA-AcrB-TolC efflux system that serves to actively eliminate small molecules and many xenobiotics from accumulating inside *E. coli* [28]. Out of thirty-three synthesized derivatives, seventeen compounds (**4a**, **4b**, **4c**, **4f**, **5a**, **5i**, **5l**, **6c**, **6d**, **6e**, **6h**, **6i**, **6j**, **6k**, **6m**, **6n** and **6r**) exhibited moderate to potent activity against *E. coli* JW55031 strain with MIC values ranging from 2 to 16 μg/ml. Compound **6c** exhibited the most potent activity against the TolC mutant *E. coli* strain with MIC value of 2 μg/ml.

Next, compounds that showed activity against the *E. coli* TolC mutant strain were further tested against other Gram-negative bacterial pathogens including standard strains of *Escherichia coli* (ATCC 25922), *Acinetobacter baumannii* (ATCC 19606) and *Klebsiella pneumonia* (ATCC BAA-1706) but the compounds were found to be
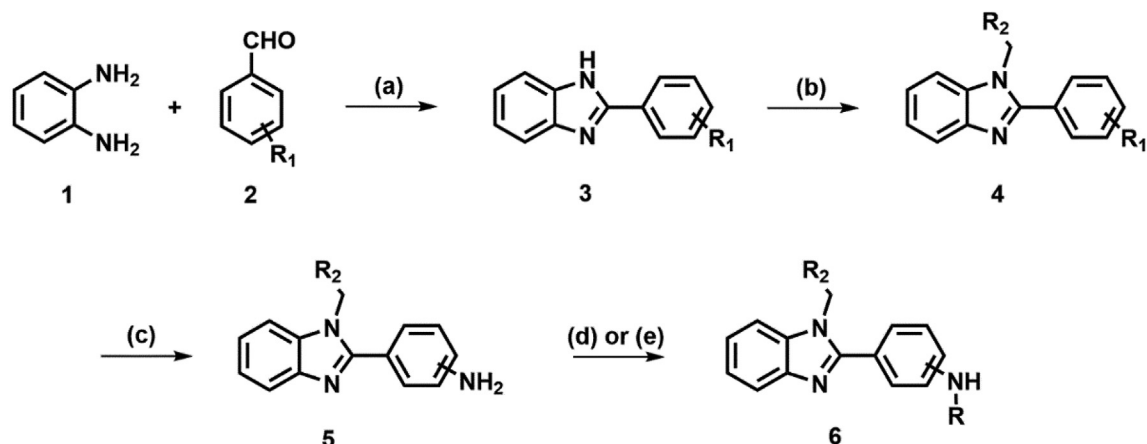
Scheme 1. Reagents and conditions: (**a**) EtOH, Na acetate, reflux, 1−2 days; (**b**) Benzyl/alkyl halide, Cs$_2$CO$_3$, DMF, rt, 48 h; (**c**) SnCl$_2$·2H$_2$O, EtOAc, reflux, overnight; (**d**) Ac$_2$O, DCM, rt, overnight; (**e**) MeSO$_2$Cl, pyridine, rt, overnight.

Table 1
Initial screening (MIC in μg/mL) of the benzimidazole-based compounds and control antibiotics against TolC-mutant *Escherichia coli* JW55031.

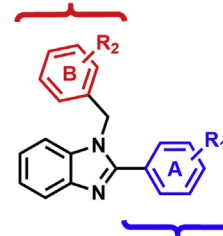| Compounds/Control antibiotics	R$_1$	R$_2$	MIC
4a [19]	H	Phenyl	16
4b [19]	H	4-CH$_3$ phenyl	8
4c [29]	H	4-CF$_3$ phenyl	8
4d	4-MeO	4-CH$_3$ phenyl	>128
4e [30]	4-Cl	4-CH$_3$ phenyl	>128
4f	3-CN	4-CH$_3$ phenyl	16
4g	4-CN	4-CH$_3$ phenyl	>128
4h	3-CF$_3$	4-CH$_3$ phenyl	>128
4i	4-CF$_3$	4-CH$_3$ phenyl	128
4j	4-OCF$_3$	4-CH$_3$ phenyl	>128
4k	3-NO$_2$	4-CH$_3$ phenyl	>128
5a	3-NH$_2$	4-CH$_3$ phenyl	4
5i	3-NH$_2$	4-CF$_3$ phenyl	8
5l	3-NH$_2$	3,4-diCl phenyl	4
6a	3-NHCOCH$_3$	4-CH$_3$ phenyl	>128
6b	3-NHCOCH$_3$	4-Cl phenyl	>128
6c	3-NHSO$_2$CH$_3$	4-CH$_3$ phenyl	2
6d	3-NHSO$_2$CH$_3$	phenyl	16
6e	3-NHSO$_2$CH$_3$	3-CH$_3$ phenyl	16
6f	3-NHSO$_2$CH$_3$	3-F phenyl	64
6g	3-NHSO$_2$CH$_3$	4-F phenyl	32
6h	3-NHSO$_2$CH$_3$	3-Cl phenyl	16
6i	3-NHSO$_2$CH$_3$	4-Cl phenyl	8
6j	3-NHSO$_2$CH$_3$	4-Br phenyl	4
6k	3-NHSO$_2$CH$_3$	4-CF$_3$ phenyl	16
6l	3-NHSO$_2$CH$_3$	4-OCF$_3$ phenyl	>128
6m	3-NHSO$_2$CH$_3$	2,4-diCl phenyl	8
6n	3-NHSO$_2$CH$_3$	3,4-diCl phenyl	8
6o	3-NHSO$_2$CH$_3$	3-CN phenyl	>128
6p	3-NHSO$_2$CH$_3$	4-CN phenyl	64
6q	3-NHSO$_2$CH$_3$	1-naphthyl	>128
6r	3-NHSO$_2$CH$_3$	cyclohexyl	16
6s	4-NHSO$_2$CH$_3$	4-CH$_3$ phenyl	>128
Linezolid			8
Gentamicin			0.5

MIC, minimum inhibitory concentration (μg/mL).

their inability to cross the outer membrane present in Gram-negative bacteria.

2.3.2. Structure activity relationship (SAR)

The benzimidazole-based lead compounds (**4a** and **4b**) showed decent antimicrobial activity against TolC-mutant *E. coli* JW55031 (MIC = 16 and 8 μg/mL respectively). To investigate the possibility of optimizing these derivatives, we synthesized thirty-one derivatives by varying the substituents at ring A and B (Fig. 1) or replacing ring B with a naphthyl or cyclohexyl moiety. The structure activity relationship for these derivatives is summarized in Fig. 2.

Starting with ring A, we synthesized a series of polar/hydrophobic or HB donor/acceptor substituents at either the para- or meta-position. Substitution at the para-position abolished antimicrobial activity regardless of the substituent nature (**4d**: 4-MeO, **4e**: 4-Cl, **4g**: 4-CN, **4i**: 4-CF$_3$, **4j**: 4-OCF$_3$ and **6s**: 4-NHSO$_2$CH$_3$). Polar substituents at meta-position particularly those with a HB donor/acceptor feature showed moderate to good activity (**4f**: 3-CN, **5a**, **5i**, and **5l**: 3-NH$_2$, and **6c**, **6d**, **6e**, **6h**, **6i**, **6j**, **6k**, **6m**, **6n**, **6r**: 3-NHSO$_2$CH$_3$), except 3-NHCOCH$_3$ (**6a** and **6b**) and 3-NO$_2$ (**4k**)

Fig. 2. Structure activity relationship of the synthesized benzimidazole derivatives against TolC-mutant *E. coli* JW55031.

inactive (data not shown). This suggests that their antibacterial activity is most likely impeded by the expression of efflux pumps or

derivatives which lacked antimicrobial activity. Additionally, hydrophobic substituent at the meta-position (**4h**: 3-CF$_3$) abolished the activity.

Optimization of ring B followed the same pattern by adding varied polar/hydrophobic and small/bulky substituents at both the para- and meta-position or by replacing the phenyl with a bulkier naphthyl or a less planar cyclohexyl. Substitution at the para-position gave better activity relative to the meta-substituents (**6c**: 4-CH$_3$ versus **6e**:3-CH$_3$, **6g**: 4-F versus **6f**: 3-F, **6i**: 4-Cl versus **6h**: 3-Cl, and **6p**: 4-CN versus **6o**: 3-CN). Hydrophobic substituents at the para-position had superior antimicrobial activity (**6c**: 4-CH$_3$, **6i**: 4-Cl, **6j**: 4-Br, **6m**: 2,4-diCl, and **6n**: 3,4-diCl; MIC values between 2 and 8 μg/ml), while polar substituents showed less/no activity (**6p**: 4-CN and **6l**: 4-OCF$_3$). Replacement of the phenyl (ring B) with a naphthyl moiety gave an inactive derivative mostly owing to the large size of naphthalene, while the cyclohexyl analog showed comparable activity.

2.3.3. Evaluation of the effect of the efflux pump on the antibacterial activity of benzimidazole compounds against Escherichia coli

Next, we investigated if the presence of efflux pumps might be the reason behind the lack of antibacterial activity of the benzimidazole compounds against Gram-negative bacteria. Benzimidazole compounds that showed substantial activity against TolC−mutant *E. coli* JW55031 (MIC ≤ 16 μg/ml) were tested against the wild-type strain (*E. coli* BW 25113). TolC−mutant *E. coli* possesses a deletion in *TolC* gene which is a key component of the AcrA-AcrB-TolC efflux system that is involved in resistance to different antibiotics such as tetracycline, chloramphenicol, ampicillin, nalidixic acid, linezolid, erythromycin and rifampicin [28]. Conversely, *E. coli* BW 25113 does not have a deletion in this gene and its AcrA-AcrB-TolC efflux system is active. None of the tested compounds showed activity against *E. coli* BW25113 (wild type) (MIC > 128 μg/ml, data not shown). In addition, linezolid, which is known to be a substrate of the AcrAB-TolC efflux pump [31], was, as expected, inactive against the wild-type *E. coli* while it showed a modest activity against the TolC-mutant one (MIC = 8 μg/mL) (Table 1). This suggests that the compounds' lack of antibacterial activity against *E. coli* may be due to their high efflux from the bacterial cell.

2.3.4. Synergetic activity of 6c with colistin

The Gram-negative bacterial outer membrane prevents numerous antibiotics from gaining entry into the bacterial cell to achieve a sufficient concentration capable of inhibiting the bacterial growth [31]. To examine if the outer membrane was impeding the antibacterial activity of the benzimidazole compounds, compound **6c** was combined with a sub-inhibitory concentration (0.25x MIC) of colistin (a membrane disrupting antibiotic) and tested against different Gram-negative bacterial strains. As shown in

Table 2, the inclusion of the permeabilizing agent colistin, at sub-inhibitory concentration resulted in an increased activity of **6c** against the tested Gram-negative strains with MIC values ranging from 8 to 16 μg/ml. Such observation suggests that the outer membrane of Gram-negative bacteria contributes − in part − to the resistance against the presented compounds. To compare, erythromycin, linezolid and daptomycin were tested likewise. Erythromycin is active against Gram-negative bacteria but is impeded by the outer membrane [32]. Therefore, when combined with a permeabilizing agent, like colistin, its penetration into the bacterial cells was ameliorated leading to an enhanced potency. On the other hand, linezolid and daptomycin are effective against Gram-positive bacteria only. Thus, even in the presence of colistin, they could not inhibit Gram-negative bacteria.

2.3.5. In vitro cytotoxicity analysis of benzimidazole derivative 6c

Ensuring safety and lack of toxicity are essential themes in drug development. In this regard, the most potent benzimidazole derivative **6c** was evaluated for its cytotoxicity using two types of cell lines; human colorectal (Caco-2) and monkey kidney epithelial (Vero) cells to detect their potential *in vitro* cytotoxicity. This compound exhibited an excellent safety profile without a significant cytotoxicity against the two tested cell lines. The experimental CC$_{50}$ (compound's concentration required for the reduction of cell viability by 50%) for both Caco-2 and Vero cell lines were higher than 128 μg/mL (Fig. 3).

3. Conclusion

We report the development of a benzimidazole derivative **6c** with potent activity against TolC−mutant *E. coli*. Compound **6c** lacks antibacterial activity against wild-type Gram-negative bacteria apparently due to lack of permeability through the outer membrane and the high efflux by the bacterial efflux pump systems. However, co-treatment with colistin partially restored its activity against Gram-negative bacteria, emphasizing the implication of the outer membrane in imparting microbial resistance to these benzimidazole derivatives. Compound **6c** represents a potential lead with an excellent safety profile and further structural explorations are warranted to establish this series as novel antimicrobial agents to overcome Gram-negative bacterial resistance.

4. Experimental

4.1. Chemistry

Chemicals were purchased from Sigma-Aldrich (Germany), Alfa Aesar (Germany), Oakwood Chemical (USA), and Loba Chemie (India), and were used as such without further purification. Solvents used for column chromatography were redistilled prior to use on BUCHI Rotavapor. Flash column chromatography was performed

Table 2
Minimum Inhibitory Concentration (MIC in μg/ml) of **6c** and control antibiotics (Linezolid, Erythromycin, and Daptomycin) against five Gram-negative bacterial isolates (*Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii and Pseudomonas aeruginosa*) in the absence and presence of sub-inhibitory concentrations (0.25 × MIC) of colistin.

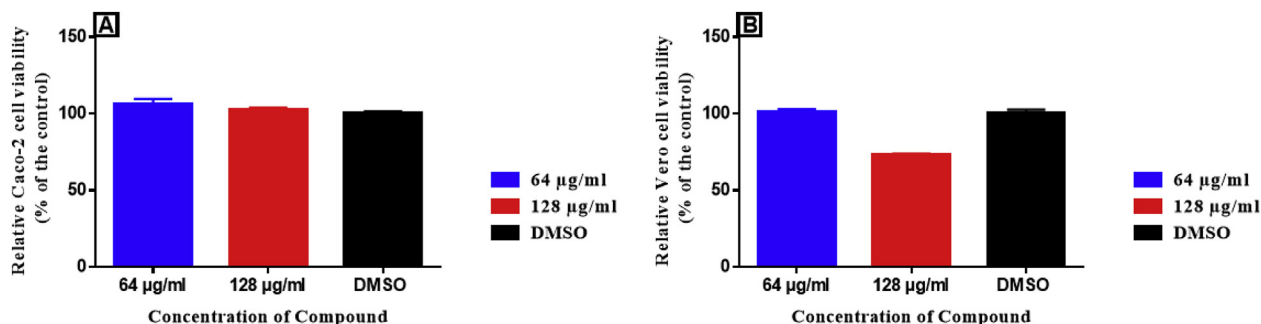| Compounds/Control antibiotics	*E. coli* ATCC 25922		*E. coli* BW25113 (wild-type strain)		*K. pneumoniae* ATCC 1706		*A. baumannii* ATCC 19606		*P. aeruginosa* ATCC 15442	
	(−) COL	(+) COL	(−) COL	(+) COL	(−) COL	(+) COL	(−) COL	(+) COL	(−) COL	(+) COL
6c	>128	16	>128	16	>128	8	>128	8	>128	16
Linezolid	128	128	128	128	>128	>128	>128	>128	>128	>128
Erythromycin	16	1	16	1	64	2	16	0.5	64	2
Daptomycin	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128

COL: Colistin.

Fig. 3. Analyzing the toxicity of compound **6c** (tested in triplicate) against: **A)** human colorectal adenocarcinoma cells (Caco-2), and **B)** monkey 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 three samples analyzed for the compound. Error bars represent standard deviation values.

using silica gel (230−400 mesh particle size) purchased from Sigma-Aldrich. Reactions were followed using analytical thin layer chromatography (TLC), performed on Aluminum silica gel 60 F_{254} TLC plates, purchased from Merck, with visualization under UV light (254 nm). ^{1}HNMR spectra were determined on a Varian NMR instrument at 300 MHz in δ scale (ppm) and J (Hz) and referred to the deuterated solvent peak (DMSO-d_6 $\delta = 2.5$ ppm). ^{13}CNMR spectra were determined on the same instrument at 75 MHz and referred to the solvent peak (DMSO-d_6 $\delta = 39.52$ ppm). High Resolution Electrospray ionization (HR-ESI) mass spectra were obtained using Bruker micrOTOF-Q II instrument. The purity of the final compounds was assessed by HPLC on a Jasco HPLC system with a UV detector (254 nm) on an RP-18 column (ReproSil-Pur-ODS-3, Dr. Maisch GmbH, Germany, 5 μm, 50 mm × 2 mm) using the following method: eluent A: water (0.1% TFA), eluent B: acetonitrile (0.1% TFA), injection volume: 20 μL, flow rate: 1 mL/min, gradient program: 1% B (0.2 min), 100% B (7 min), 100% B (8 min), 1% B (8.1 min), 1% B (9.6 min). The compounds were used as solutions of 200 μM concentration in acetonitrile (2% DMSO).

4.1.1. General synthetic procedures for compounds

The desired benzimidazole derivatives were synthesized according to the previously reported procedures [23–27], and is illustrated as follows.

Step (a): To a solution of *o*-phenylenediamine (1.08 g, 10 mmol, 1.0 equiv) and appropriate benzaldehyde (10.5 mmol, 1.05 equiv) in EtOH (100 ml) was added Na acetate (0.82 g, 10 mmol, 1.0 equiv). The reaction mixture was refluxed for 24−48 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 2-(substituted phenyl)-1*H*-benzimidazole (**3**) in moderate to good yields that was used as such in the next step.

Step (b): To a solution of the appropriate 2-(substituted phenyl)-1*H*-benzimidazole (**3**) (4 mmol, 1.0 equiv) in DMF (10 ml) was added Cs_2CO_3 (1.56 g, 4.8 mmol, 1.2 equiv) and the suspension was stirred at room temperature for 30 min. The appropriate benzyl/alkyl halide (4.4 mmol, 1.1 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 h and the resulting solid was filtered, washed with water, dried and purified by column chromatography (EtOAc/Hexane) to yield the desired products (**4**) in poor to moderate yields.

Step (c): To solution of the appropriate nitro derivative (**4**) (2 mmol, 1 equiv) in EtOAc (50 ml) was added $SnCl_2 \cdot 2H_2O$ (2.26 g, 10 mmol, 5 equiv) and the reaction mixture was refluxed

overnight. Upon completion of the reaction as indicated by TLC, the mixture was cooled, washed with sodium carbonate solution (10%), separated, dried and purified by column chromatography (EtOAc/Hexane) to yield the desired products (**5**) in moderate yields.

Step (d): To a solution of the appropriate amine (**5**) (1 mmol, 1.0 equiv) in DCM (20 ml) was added acetic anhydride (0.1 g, 94 μL, 1.5 mmol, 1.5 equiv) and the reaction mixture was stirred at room temperature overnight. Upon completion of the reaction as indicated by TLC, the mixture was evaporated, purified by column chromatography (DCM/MeOH) to yield the desired final products (**6a**, **6b**).

Step (e): A solution of the appropriate amine (**5**) (1 mmol, 1.0 equiv) in pyridine (20 ml) was cooled at 0 °C, then Methanesulfonyl chloride (0.14 g, 93 μL, 1.2 mmol, 1.2 equiv) and the reaction mixture was stirred at room temperature overnight. Upon completion of the reaction as indicated by TLC, the mixture was evaporated, purified by column chromatography (DCM/MeOH) to yield the desired final products (**6c-s**).

4.1.1.1. 1-Benzyl-2-phenyl-1H-benzimidazole (4a) [19].
$R_f = 0.35$ (EtOAc/Hexane1:6). White solid, yield 45%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.73 (dd, $J = 5.8$, 1.7 Hz, 3H), 7.57−7.50 (m, 3H), 7.46 (dd, $J = 5.8$, 3.0 Hz, 1H), 7.32−7.19 (m, 5H), 7.00 (d, $J = 6.9$ Hz, 2H), 5.58 (s, 2H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.69, 143.12, 137.36, 136.32, 130.59, 130.25, 129.47, 129.21, 127.90, 126.52, 123.12, 122.64, 119.71, 111.53, 47.89. **MS (ESI positive)** m/z [M+H]$^+$: 285.1. **HPLC purity**: 99.3%, **HPLC t_R**: 3.27 min.

4.1.1.2. 1-(4-methylbenzyl)-2-phenyl-1H-benzimidazole (4b) [19].
$R_f = 0.4$ (EtOAc/Hexane 1:4). White solid, yield 30%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.77−7.69 (m, 3H), 7.56−7.50 (m, 3H), 7.45 (dd, $J = 5.3$, 3.4 Hz, 1H), 7.28−7.19 (m, 2H), 7.09 (d, $J = 7.9$ Hz, 2H), 6.89 (d, $J = 7.9$ Hz, 2H), 5.53 (s, 2H), 2.23 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.66, 143.12, 137.10, 136.32, 134.32, 130.61, 130.25, 129.77, 129.46, 129.22, 126.48, 123.08, 122.60, 119.68, 111.56, 47.69, 21.03. **HRMS (ESI positive)** m/z [M+H]$^+$ calcd for $C_{21}H_{19}N_2$, 299.1543; found, 299.1572. **HPLC purity**: 95.5%, **HPLC t_R**: 3.6 min.

4.1.1.3. 2-Phenyl-1-(4-(trifluoromethyl)benzyl)-1H-benzimidazole (4c) [29].
$R_f = 0.4$ (EtOAc/Hexane1:6). White solid, yield 53%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.79−7.69 (m, 3H), 7.67 (d, $J = 8.2$ Hz, 2H), 7.56−7.49 (m, 3H), 7.46 (dd, $J = 6.4$, 2.2 Hz, 1H), 7.26 (ddd, $J = 6.1$, 5.2, 3.6 Hz, 2H), 7.21 (d, $J = 7.9$ Hz, 2H), 5.70 (s, 2H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.69, 143.14, 142.17, 136.22, 130.36, 130.33, 129.42, 129.25, 128.73, 128.30, 127.28, 126.23, 126.18, 126.13, 126.08, 123.30, 122.82, 119.82, 111.41, 47.55. **MS (ESI positive)** m/z [M+H]$^+$:

353.1. **HPLC purity**: 98.3%, **HPLC t_R**: 3.76 min.

4.1.1.4. 2-(4-methoxyphenyl)-1-(4-methylbenzyl)-1H-benzimidazole (4d). $R_f = 0.45$ (DCM/MeOH 9.8:0.2). White solid, yield 28%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.73−7.62 (m, 3H), 7.44−7.35 (m, 1H), 7.26−7.16 (m, 2H), 7.09 (t, $J = 7.1$ Hz, 4H), 6.90 (d, $J = 7.8$ Hz, 2H), 5.51 (s, 2H), 3.82 (s, 3H), 2.23 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 160.80, 153.62, 143.13, 137.05, 136.34, 134.40, 130.88, 129.78, 126.40, 122.77, 122.46, 119.42, 114.67, 111.35, 55.74, 47.68, 21.03. **MS (ESI positive)** m/z [M+H]$^+$: 329.2. **HPLC purity**: 99.2%, **HPLC t_R**: 3.76 min.

4.1.1.5. 2-(4-chlorophenyl)-1-(4-methylbenzyl)-1H-benzimidazole (4e) [30]. $R_f = 0.45$ (EtOAc/Hexane 1:4). Off-white solid, yield 16%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.74 (t, $J = 8.3$ Hz, 3H), 7.60 (d, $J = 8.4$ Hz, 2H), 7.50−7.43 (m, 1H), 7.25 (p, $J = 5.7$ Hz, 2H), 7.08 (d, $J = 7.7$ Hz, 2H), 6.88 (d, $J = 7.8$ Hz, 2H), 5.53 (s, 2H), 2.22 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 152.49, 143.04, 137.15, 136.39, 135.13, 134.19, 131.22, 129.78, 129.46, 129.33, 126.46, 123.28, 122.74, 119.75, 111.62, 47.70, 21.03. **MS (ESI positive)** m/z [M+H]$^+$: 333.1 (100%), [(M+2)+H]$^+$: 335.1 (35%). **HPLC purity**: 99%, **HPLC t_R**: 3.84 min.

4.1.1.6. 3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)benzonitrile (4f). $R_f = 0.2$ (EtOAc/Hexane 1:4). White solid, yield 20%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 8.17 (s, 1H), 8.03 (dd, $J = 14.9, 7.8$ Hz, 2H), 7.73 (t, $J = 7.7$ Hz, 2H), 7.59−7.49 (m, 1H), 7.28 (dd, $J = 5.6, 3.0$ Hz, 2H), 7.07 (d, $J = 7.7$ Hz, 2H), 6.87 (d, $J = 7.8$ Hz, 2H), 5.57 (s, 2H), 2.22 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 151.58, 142.96, 137.20, 136.46, 134.14, 134.12, 133.80, 132.90, 131.89, 130.54, 129.76, 126.59, 123.58, 122.90, 119.92, 118.66, 112.44, 111.77, 47.72, 21.02. **MS (ESI positive)** m/z [M+H]$^+$: 324.2. **HPLC purity**: 99.4%, **HPLC t_R**: 3.55 min.

4.1.1.7. 4-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)benzonitrile (4g). $R_f = 0.25$ (EtOAc/Hexane 1:4). Orange crystals, yield 34%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 8.00 (d, $J = 8.2$ Hz, 2H), 7.93 (d, $J = 8.3$ Hz, 2H), 7.76 (dd, $J = 6.2, 2.8$ Hz, 1H), 7.52 (dd, $J = 5.6, 3.2$ Hz, 1H), 7.28 (dd, $J = 6.1, 3.0$ Hz, 2H), 7.08 (d, $J = 7.9$ Hz, 2H), 6.87 (d, $J = 7.9$ Hz, 2H), 5.58 (s, 2H), 2.22 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 151.81, 143.05, 137.22, 136.55, 135.01, 134.05, 133.16, 130.23, 129.81, 126.48, 123.74, 123.00, 120.03, 118.85, 112.70, 111.81, 47.79, 21.03. **MS (ESI positive)** m/z [M+H]$^+$: 324.2. **HPLC purity**: 97.8%, **HPLC t_R**: 3.6 min.

4.1.1.8. 1-(4-methylbenzyl)-2-(3-(trifluoromethyl)phenyl)-1H-benzimidazole (4h). $R_f = 0.35$ (EtOAc/Hexane 1:6). Light yellow crystals, yield 33%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 8.02 (s, 2H), 7.90 (d, $J = 7.9$ Hz, 1H), 7.77 (t, $J = 7.0$ Hz, 2H), 7.54 (dd, $J = 5.9, 2.8$ Hz, 1H), 7.33−7.25 (m, 2H), 7.09 (d, $J = 7.8$ Hz, 2H), 6.89 (d, $J = 7.6$ Hz, 2H), 5.57 (s, 2H), 2.23 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 151.99, 142.96, 137.20, 136.59, 134.21, 133.25, 131.64, 130.50, 130.19, 129.80, 129.34, 126.90, 126.85, 126.80, 126.74, 126.45, 126.04, 125.99, 123.58, 122.90, 119.91, 111.68, 47.82, 21.02. **HRMS (ESI positive)** m/z [M+H]$^+$ calcd for $C_{22}H_{18}F_3N_2$, 367.1417; found, 367.0932. **HPLC purity**: 99.5%, **HPLC t_R**: 3.97 min.

4.1.1.9. 1-(4-methylbenzyl)-2-(4-(trifluoromethyl)phenyl)-1H-benzimidazole (4i). $R_f = 0.3$ (EtOAc/Hexane 1:6). White crystals, yield 35%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.97 (d, $J = 8.3$ Hz, 2H), 7.90 (d, $J = 8.3$ Hz, 2H), 7.76 (dd, $J = 6.2, 2.8$ Hz, 1H), 7.50 (dd, $J = 6.3, 2.7$ Hz, 1H), 7.27 (dd, $J = 5.8, 3.4$ Hz, 2H), 7.08 (d, $J = 7.9$ Hz, 2H), 6.89 (d, $J = 7.9$ Hz, 2H), 5.58 (s, 2H), 2.22 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 152.08, 143.05, 137.18, 136.47, 134.60, 134.58, 134.11, 130.47, 130.29, 130.05, 129.79, 126.46, 126.21, 126.16, 126.11, 126.06, 123.58, 122.91, 119.95, 111.77, 47.76, 21.01. **MS (ESI positive)** m/z

[M+H]$^+$: 367.1. **HPLC purity**: 98.7%, **HPLC t_R**: 4.04 min.

4.1.1.10. 1-(4-methylbenzyl)-2-(4-(trifluoromethoxy)phenyl)-1H-benzimidazole (4j). $R_f = 0.5$ (EtOAc/Hexane 1:1). White crystals, yield 40%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.90 (d, $J = 8.7$ Hz, 2H), 7.81−7.72 (m, 1H), 7.56 (d, $J = 8.2$ Hz, 1H), 7.53−7.47 (m, 1H), 7.33−7.24 (m, 2H), 7.12 (d, $J = 7.9$ Hz, 2H), 6.91 (d, $J = 7.9$ Hz, 2H), 5.58 (s, 2H), 2.26 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 152.27, 149.67, 149.64, 143.02, 137.16, 136.39, 134.15, 131.57, 129.85, 129.78, 126.48, 123.34, 122.77, 121.70, 119.79, 111.66, 47.72, 21.02. **HRMS (ESI positive)** m/z [M+H]$^+$ calcd for $C_{22}H_{18}F_3N_2O$, 383.1366; found, 383.1439. **HPLC purity**: 99%, **HPLC t_R**: 4.1 min.

4.1.1.11. 1-(4-methylbenzyl)-2-(3-nitrophenyl)-1H-benzimidazole (4k). $R_f = 0.35$ (EtOAc/Hexane 1:4). Brown crystals, yield 35%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 8.52 (s, 1H), 8.37 (dd, $J = 7.9, 1.8$ Hz, 1H), 8.20 (d, $J = 7.8$ Hz, 1H), 7.84 (d, $J = 8.0$ Hz, 1H), 7.81−7.74 (m, 1H), 7.55 (dd, $J = 5.8, 3.4$ Hz, 1H), 7.33−7.24 (m, 2H), 7.10 (d, $J = 7.9$ Hz, 2H), 6.92 (d, $J = 8.0$ Hz, 2H), 5.61 (s, 2H), 2.23 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 151.28, 148.36, 142.94, 137.22, 136.62, 135.60, 134.10, 132.01, 130.97, 129.82, 126.54, 124.83, 124.07, 123.72, 123.00, 119.99, 111.79, 47.85, 21.03. **MS (ESI positive)** m/z [M+H]$^+$: 344.1. **HPLC purity**: 99%, **HPLC t_R**: 3.7 min.

4.1.1.12. 3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)aniline (5a). $R_f = 0.25$ (DCM/MeOH 9.8:0.2). Light yellow solid, yield 56%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.68 (dd, $J = 6.4, 2.0$ Hz, 1H), 7.38 (dd, $J = 6.3, 2.0$ Hz, 1H), 7.20 (ddd, $J = 6.7, 5.7, 3.6$ Hz, 2H), 7.15 (d, $J = 7.7$ Hz, 1H), 7.12−7.05 (m, 2H), 7.02 (s, 1H), 6.90 (d, $J = 8.0$ Hz, 2H), 6.77 (d, $J = 7.6$ Hz, 1H), 6.71 (dd, $J = 7.7, 1.8$ Hz, 1H), 5.51 (s, 2H), 5.33 (s, 2H), 2.22 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 154.53, 149.50, 143.14, 137.03, 136.11, 134.38, 131.10, 129.70, 129.55, 126.57, 122.76, 122.40, 119.50, 116.34, 115.65, 115.04, 111.50, 47.65, 21.03. **HRMS (ESI positive)** m/z [M+H]$^+$ calcd for $C_{21}H_{20}N_3$, 314.1652; found, 314.1667. **HPLC purity**: 97%, **HPLC t_R**: 3.17 min.

4.1.1.13. 3-(1-(4-(trifluoromethyl)benzyl)-1H-benzimidazol-2-yl)aniline (5i). $R_f = 0.3$ (DCM/MeOH 9.8:0.2). Yellow solid, yield 47%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.75−7.63 (m, 3H), 7.39 (d, $J = 7.1$ Hz, 1H), 7.23 (dd, $J = 13.8, 7.4$ Hz, 4H), 7.13 (t, $J = 7.8$ Hz, 1H), 6.99 (s, 1H), 6.72 (t, $J = 8.7$ Hz, 2H), 5.68 (s, 2H), 5.34 (s, 2H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 154.54, 149.55, 143.14, 142.26, 142.24, 136.04, 130.82, 129.63, 128.67, 128.25, 127.31, 126.21, 126.16, 126.11, 126.06, 123.02, 122.64, 119.64, 116.25, 115.75, 114.89, 111.33, 47.53. **HRMS (ESI positive)** m/z [M+H]$^+$ calcd for $C_{21}H_{17}F_3N_3$, 368.1369; found, 368.1405. **HPLC purity**: 97.9%, **HPLC t_R**: 3.43 min.

4.1.1.14. 3-(1-(3,4-dichlorobenzyl)-1H-benzimidazol-2-yl)aniline (5l). $R_f = 0.3$ (DCM/MeOH 9.8:0.2). Buff solid, yield 29%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.70 (dd, $J = 5.6, 2.5$ Hz, 1H), 7.54 (d, $J = 8.4$ Hz, 1H), 7.43 (dd, $J = 5.8, 2.6$ Hz, 1H), 7.31 (s, 1H), 7.23 (p, $J = 7.4$ Hz, 2H), 7.15 (t, $J = 7.7$ Hz, 1H), 6.96 (s, 1H), 6.88 (d, $J = 8.4$ Hz, 1H), 6.73 (t, $J = 8.7$ Hz, 2H), 5.58 (s, 2H), 5.35 (s, 2H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 154.45, 149.56, 143.09, 138.61, 135.94, 131.76, 131.43, 130.77, 130.50, 129.69, 128.84, 126.81, 123.08, 122.69, 119.66, 116.31, 115.76, 114.80, 111.33, 46.79. **HRMS (ESI positive)** m/z [M+H]$^+$ calcd for $C_{20}H_{16}Cl_2N_3$, 368.0716; found, 368.0724, [(M+2)+H]$^+$ calcd 370.0687; found, 370.0693 (66.8%). **HPLC purity**: 99.2%, **HPLC t_R**: 3.53 min.

4.1.1.15. N-(3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl) acetamide (6a). $R_f = 0.2$ (DCM/MeOH 9.5:0.5). White solid, yield 35%. **^{1}H NMR (400 MHz, DMSO-d_6)** δ 10.15 (s, 1H), 8.12 (s, 1H), 7.71 (d, $J = 5.6$ Hz, 2H), 7.43 (s, 2H), 7.34 (s, 1H), 7.23 (s, 2H), 7.07 (d, $J = 5.9$ Hz, 2H), 6.88 (d, $J = 5.9$ Hz, 2H), 5.55 (s, 2H), 2.21 (s, 3H), 2.07

(s, 3H).

4.1.1.16. N-(3-(1-(4-chlorobenzyl)-1H-benzimidazol-2-yl)phenyl) acetamide (6b). $R_f = 0.25$ (DCM/MeOH 9.5:0.5). White solid, yield 27%. **^{1}H NMR (400 MHz, DMSO-d_6)** δ 10.14 (s, 1H), 8.09 (s, 1H), 7.71 (dd, $J = 14.6$, 8.1 Hz, 2H), 7.44 (dd, $J = 14.4$, 7.0 Hz, 2H), 7.33 (t, $J = 8.1$ Hz, 3H), 7.29−7.22 (m, 2H), 7.00 (d, $J = 8.2$ Hz, 2H), 5.60 (s, 2H), 2.06 (s, 3H).

4.1.1.17. N-(3-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6c). $R_f = 0.3$ (DCM/MeOH 9.8:0.2). White solid, yield 37%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.97 (s, 1H), 7.76−7.70 (m, 1H), 7.63 (s, 1H), 7.51−7.40 (m, 3H), 7.35 (d, $J = 8.0$ Hz, 1H), 7.29−7.20 (m, 2H), 7.08 (d, $J = 7.9$ Hz, 2H), 6.87 (d, $J = 7.9$ Hz, 2H), 5.54 (s, 2H), 2.98 (s, 3H), 2.22 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.09, 143.01, 139.31, 137.12, 136.33, 134.20, 131.62, 130.21, 129.72, 126.56, 124.46, 123.20, 122.68, 121.34, 120.67, 119.73, 111.62, 47.68, 39.81, 21.03. **HRMS (ESI positive)** m/z [M+H]$^+$ calcd for $C_{22}H_{22}N_3O_2S$, 392.1427; found, 392.1435. **HPLC purity**: 95.8%, **HPLC t_R**: 3.47 min.

4.1.1.18. N-(3-(1-benzyl-1H-benzimidazol-2-yl)phenyl)meth- anesulfonamide (6d). $R_f = 0.3$ (DCM/MeOH 9.8:0.2). Buff solid, yield 21%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.97 (s, 1H), 7.77−7.70 (m, 1H), 7.63 (s, 1H), 7.52−7.44 (m, 2H), 7.41 (d, $J = 7.9$ Hz, 1H), 7.35 (d, $J = 7.8$ Hz, 1H), 7.25 (dq, $J = 8.6$, 4.9, 3.7 Hz, 5H), 6.98 (d, $J = 7.2$ Hz, 2H), 5.60 (s, 2H), 2.98 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.13, 143.02, 139.34, 137.25, 136.36, 131.59, 130.22, 129.19, 127.92, 126.59, 124.46, 123.25, 122.73, 121.35, 120.64, 119.76, 111.60, 47.89, 39.84. **MS (ESI positive)** m/z [M+H]$^+$: 378.1. **HPLC purity**: 94.3%, **HPLC t_R**: 3.25 min.

4.1.1.19. N-(3-(1-(3-methylbenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6e). $R_f = 0.4$ (DCM/MeOH 9.8:0.2). White solid, yield 37.5%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.98 (s, 1H), 7.78−7.71 (m, 1H), 7.63 (s, 1H), 7.53−7.40 (m, 3H), 7.36 (d, $J = 8.2$ Hz, 1H), 7.31−7.21 (m, 2H), 7.16 (t, $J = 7.4$ Hz, 1H), 7.05 (d, $J = 7.8$ Hz, 1H), 6.84 (s, 1H), 6.76 (d, $J = 7.1$ Hz, 1H), 5.55 (s, 2H), 2.99 (s, 3H), 2.20 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.14, 143.00, 139.33, 138.36, 137.17, 136.34, 131.62, 130.21, 129.09, 128.60, 127.18, 124.47, 123.66, 123.24, 122.71, 121.36, 120.65, 119.74, 111.60, 47.86, 39.81, 21.42. **HRMS (ESI positive)** m/z [M+H]$^+$ calcd for $C_{22}H_{22}N_3O_2S$, 392.1427; found, 392.1441. **HPLC purity**: 95.3%, **HPLC t_R**: 3.44 min.

4.1.1.20. N-(3-(1-(3-fluorobenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6f). $R_f = 0.4$ (DCM/MeOH 9.8:0.2). Off-white solid, yield 35%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.97 (s, 1H), 7.79−7.71 (m, 1H), 7.60 (s, 1H), 7.53−7.45 (m, 2H), 7.41 (d, $J = 7.6$ Hz, 1H), 7.38−7.30 (m, 2H), 7.26 (dd, $J = 9.5$, 5.1 Hz, 2H), 7.07 (td, $J = 8.7$, 2.3 Hz, 1H), 6.83 (d, $J = 9.7$ Hz, 1H), 6.77 (d, $J = 7.8$ Hz, 1H), 5.61 (s, 2H), 2.98 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 164.28, 153.09, 142.98, 140.21, 140.11, 139.34, 136.27, 131.42, 131.36, 131.25, 130.28, 124.49, 123.39, 122.85, 122.56, 122.52, 121.39, 120.53, 119.82, 114.95, 114.67, 113.75, 113.45, 111.50, 109.99, 47.39, 39.81. **MS (ESI positive)** m/z [M+H]$^+$: 396.1. **HPLC purity**: 94.2%, **HPLC t_R**: 3.26 min.

4.1.1.21. N-(3-(1-(4-fluorobenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6g). $R_f = 0.35$ (DCM/MeOH 9.8:0.2). Buff solid, yield 38%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.97 (s, 1H), 7.78−7.68 (m, 1H), 7.60 (s, 1H), 7.53−7.45 (m, 2H), 7.42 (d, $J = 7.6$ Hz, 1H), 7.36 (d, $J = 8.1$ Hz, 1H), 7.30−7.21 (m, 2H), 7.11 (t, $J = 8.8$ Hz, 2H), 7.02 (dd, $J = 8.2$, 5.7 Hz, 2H), 5.58 (s, 2H), 2.99 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 163.42, 160.20, 153.05, 143.02, 139.30, 136.25, 133.43, 133.39, 131.52, 130.26, 128.75, 128.64, 124.53, 123.31, 122.77, 121.35, 120.55, 119.79, 116.15, 115.87, 111.55, 47.20, 39.83. **MS (ESI positive)** m/z [M+H]$^+$: 396.1. **HPLC purity**: 94.9%, **HPLC t_R**: 3.27 min.

4.1.1.22. N-(3-(1-(3-chlorobenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6h). $R_f = 0.3$ (DCM/MeOH 9.8:0.2). Buff solid, yield 47%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.98 (s, 1H), 7.74 (d, $J = 6.0$ Hz, 1H), 7.60 (s, 1H), 7.49 (t, $J = 7.3$ Hz, 2H), 7.41 (d, $J = 7.6$ Hz, 1H), 7.36 (d, $J = 8.0$ Hz, 1H), 7.30 (d, $J = 4.9$ Hz, 4H), 7.07 (s, 1H), 6.88 (s, 1H), 5.61 (s, 2H), 2.99 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.08, 142.99, 139.79, 139.34, 136.26, 133.79, 131.41, 131.14, 130.31, 127.96, 126.59, 125.19, 124.51, 123.43, 122.89, 121.40, 120.49, 119.85, 111.50, 47.30, 39.81. **MS (ESI positive)** m/z [M+H]$^+$: 412.1 (100%), [(M+2)+H]$^+$: 414.1 (38%). **HPLC purity**: 93.6%, **HPLC t_R**: 3.5 min.

4.1.1.23. N-(3-(1-(4-chlorobenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6i). $R_f = 0.3$ (DCM/MeOH 9.8:0.2). White solid, yield 34%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.97 (s, 1H), 7.74 (dd, $J = 4.9$, 3.8 Hz, 1H), 7.59 (s, 1H), 7.48 (dd, $J = 9.1$, 6.1 Hz, 2H), 7.40 (d, $J = 7.8$ Hz, 1H), 7.35 (d, $J = 8.4$ Hz, 3H), 7.30−7.22 (m, 2H), 7.00 (d, $J = 8.4$ Hz, 2H), 5.59 (s, 2H), 2.99 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.06, 143.01, 139.31, 136.27, 136.26, 132.48, 131.43, 130.27, 129.17, 128.47, 124.50, 123.36, 122.82, 121.38, 120.54, 119.81, 111.51, 47.28, 39.81. **MS (ESI positive)** m/z [M+H]$^+$: 412.1 (100%), [(M+2)+H]$^+$: 414.1 (38.5%). **HPLC purity**: 97%, **HPLC t_R**: 3.52 min.

4.1.1.24. N-(3-(1-(4-bromobenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6j). $R_f = 0.4$ (DCM/MeOH 9.8:0.2). White solid, yield 46%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.97 (s, 1H), 7.77−7.70 (m, 1H), 7.59 (s, 1H), 7.52−7.44 (m, 4H), 7.40 (d, $J = 7.7$ Hz, 1H), 7.35 (d, $J = 7.9$ Hz, 1H), 7.26 (p, $J = 6.5$ Hz, 2H), 6.93 (d, $J = 8.3$ Hz, 2H), 5.57 (s, 2H), 2.99 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.07, 143.00, 139.32, 136.70, 136.25, 132.09, 131.42, 130.27, 128.80, 124.47, 123.36, 122.83, 121.39, 120.99, 120.54, 119.81, 111.51, 47.39, 39.83. **HRMS (ESI positive)** m/z [M+H]$^+$ calcd for $C_{21}H_{19}BrN_3O_2S$, 456.0376; found, 456.0389, [(M+2)+H]$^+$ calcd 458.0356; found, 458.0372 (1:1). **HPLC purity**: 94%, **HPLC t_R**: 3.6 min.

4.1.1.25. N-(3-(1-(4-(trifluoromethyl)benzyl)-1H-benzimidazol-2-yl) phenyl)methanesulfonamide (6k). $R_f = 0.45$ (DCM/MeOH 9.8:0.2). Buff solid, yield 25%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.97 (s, 1H), 7.67 (dd, $J = 26.4$, 20.7 Hz, 4H), 7.42 (dd, $J = 24.9$, 10.8 Hz, 4H), 7.24 (d, $J = 18.3$ Hz, 4H), 5.70 (s, 2H), 2.96 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.10, 143.02, 142.05, 142.03, 139.32, 136.26, 131.33, 130.29, 128.70, 128.28, 127.31, 126.22, 126.16, 126.11, 126.06, 124.45, 123.44, 122.91, 121.39, 120.45, 119.86, 111.46, 47.56, 39.81. **MS (ESI positive)** m/z [M+H]$^+$: 446.1. **HPLC purity**: 98.3%, **HPLC t_R**: 3.65 min.

4.1.1.26. N-(3-(1-(4-(trifluoromethoxy)benzyl)-1H-benzimidazol-2-yl)phenyl)methanesulfonamide (6l). $R_f = 0.15$ (DCM/MeOH 9.9:0.1). Buff solid, yield 30%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.98 (s, 1H), 7.78−7.71 (m, 1H), 7.60 (s, 1H), 7.53−7.45 (m, 2H), 7.41 (d, $J = 7.6$ Hz, 1H), 7.35 (d, $J = 8.0$ Hz, 1H), 7.28 (t, $J = 7.4$ Hz, 4H), 7.10 (d, $J = 8.5$ Hz, 2H), 5.64 (s, 2H), 2.98 (s, 3H). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 153.06, 147.99, 147.97, 147.95, 147.93, 143.02, 139.31, 136.67, 136.23, 131.41, 130.28, 128.52, 124.49, 123.38, 122.84, 122.11, 121.79, 121.36, 120.46, 119.83, 111.51, 47.22, 39.81. **MS (ESI positive)** m/z [M+H]$^+$: 462.1. **HPLC purity**: 98.6%, **HPLC t_R**: 3.74 min.

4.1.1.27. N-(3-(1-(2,4-dichlorobenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6m). $R_f = 0.45$ (DCM/MeOH 9.5:0.5). White solid, yield 34%. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 9.97 (s, 1H), 7.77 (d, $J = 6.4$ Hz, 1H), 7.69 (s, 1H), 7.51 (s, 1H), 7.49−7.38 (m, 2H), 7.37−7.23

(m, 5H), 6.62 (dd, $J = 8.7$, 3.1 Hz, 1H), 5.57 (s, 2H), 2.96 (s, 3H). **13C NMR (75 MHz, DMSO-d_6)** δ 153.26, 142.99, 139.38, 136.23, 133.57, 133.38, 132.84, 131.24, 130.29, 129.64, 129.03, 128.32, 124.27, 123.55, 123.00, 121.39, 120.15, 119.94, 111.39, 46.05, 39.78. **MS (ESI positive)** m/z [M+Na]$^+$: 468 (100%), [(M+2)+Na]$^+$: 470 (68%). **HPLC purity**: 97.4%, **HPLC t_R**: 3.72 min.

4.1.1.28. N-(3-(1-(3,4-dichlorobenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6n). $R_f = 0.45$ (DCM/MeOH 9.5:0.5). White solid, yield 42%. **1H NMR (300 MHz, DMSO-d_6)** δ 9.96 (s, 1H), 7.75 (dd, $J = 4.2$, 2.3 Hz, 1H), 7.56 (s, 1H), 7.55–7.44 (m, 3H), 7.41 (d, $J = 7.6$ Hz, 1H), 7.36 (d, $J = 7.7$ Hz, 1H), 7.32–7.24 (m, 3H), 6.88 (d, $J = 8.2$ Hz, 1H), 5.60 (s, 2H), 2.99 (s, 3H). **13C NMR (75 MHz, DMSO-d_6)** δ 153.06, 142.99, 139.36, 138.43, 136.18, 131.77, 131.45, 131.30, 130.54, 130.35, 128.84, 126.81, 124.56, 123.50, 122.95, 121.41, 120.38, 119.88, 111.45, 46.88, 39.81. **HRMS (ESI positive)** m/z [M+H]$^+$ calcd for $C_{21}H_{18}Cl_2N_3O_2S$, 446.0491; found, 446.0506, [(M+2)+H]$^+$ calcd 448.0463; found, 448.0472 (72.7%). **HPLC purity**: 95.9%, **HPLC t_R**: 3.76 min.

4.1.1.29. N-(3-(1-(3-cyanobenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6o). $R_f = 0.4$ (DCM/MeOH 9.5:0.5). White solid, yield 48%. **1H NMR (300 MHz, DMSO-d_6)** δ 9.97 (s, 1H), 7.74 (t, $J = 6.0$ Hz, 2H), 7.56 (s, 1H), 7.48 (d, $J = 9.1$ Hz, 4H), 7.40 (d, $J = 7.1$ Hz, 1H), 7.35 (d, $J = 8.3$ Hz, 1H), 7.26 (dd, $J = 10.4$, 6.7 Hz, 3H), 5.65 (s, 2H), 2.98 (s, 3H). **13C NMR (75 MHz, DMSO-d_6)** δ 153.15, 143.02, 143.01, 139.39, 138.90, 136.18, 131.82, 131.37, 130.55, 130.41, 130.31, 124.54, 123.46, 122.93, 121.37, 120.40, 119.88, 118.93, 112.09, 111.46, 47.27, 39.83. **MS (ESI positive)** m/z [M+H]$^+$: 403.1. **HPLC purity**: 95.6%, **HPLC t_R**: 3.04 min.

4.1.1.30. N-(3-(1-(4-cyanobenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6p). $R_f = 0.4$ (DCM/MeOH 9.5:0.5). White solid, yield 12%. **1H NMR (300 MHz, DMSO-d_6)** δ 9.96 (s, 1H), 7.77 (d, $J = 8.2$ Hz, 3H), 7.56 (s, 1H), 7.47 (t, $J = 7.7$ Hz, 2H), 7.38 (d, $J = 7.9$ Hz, 1H), 7.34 (d, $J = 8.8$ Hz, 1H), 7.26 (dt, $J = 11.3$, 3.9 Hz, 2H), 7.16 (d, $J = 8.2$ Hz, 2H), 5.70 (s, 2H), 2.98 (s, 3H). **13C NMR (75 MHz, DMSO-d_6)** δ 153.09, 142.99, 142.98, 139.30, 136.25, 133.17, 131.27, 130.30, 127.49, 124.49, 123.47, 122.94, 121.39, 120.38, 119.88, 118.99, 111.42, 110.73, 47.65, 39.83. **MS (ESI positive)** m/z [M+H]$^+$: 403.1. **HPLC purity**: 96.9%, **HPLC t_R**: 2.98 min.

4.1.1.31. N-(3-(1-(naphthalen-1-ylmethyl)-1H-benzimidazol-2-yl) phenyl)methanesulfonamide (6q). $R_f = 0.4$ (DCM/MeOH 9.8:0.2). White solid, yield 38%. **1H NMR (300 MHz, DMSO-d_6)** δ 9.93 (s, 1H), 8.16–8.08 (m, 1H), 8.03–7.95 (m, 1H), 7.85 (d, $J = 8.2$ Hz, 1H), 7.80 (d, $J = 7.9$ Hz, 1H), 7.66 (s, 1H), 7.61 (dd, $J = 5.7$, 3.7 Hz, 2H), 7.34 (t, $J = 7.8$ Hz, 4H), 7.27 (d, $J = 6.9$ Hz, 2H), 7.24–7.17 (m, 1H), 6.60 (d, $J = 7.1$ Hz, 1H), 6.07 (s, 2H), 2.82 (s, 3H). **13C NMR (75 MHz, DMSO-d_6)** δ 153.37, 143.07, 139.34, 136.63, 133.71, 132.58, 131.43, 130.25, 130.17, 129.10, 128.28, 126.99, 126.70, 125.97, 123.99, 123.40, 123.35, 122.86, 122.76, 121.45, 120.38, 119.88, 111.52, 46.43, 39.65. **MS (ESI positive)** m/z [M+H]$^+$: 428.1. **HPLC purity**: 92.7%, **HPLC t_R**: 3.68 min.

4.1.1.32. N-(3-(1-(cyclohexylmethyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6r). $R_f = 0.35$ (DCM/MeOH 9.8:0.2). White solid, yield 34%. **1H NMR (300 MHz, DMSO-d_6)** δ 9.97 (s, 1H), 7.67 (t, $J = 6.7$ Hz, 2H), 7.61 (s, 1H), 7.57–7.49 (m, 2H), 7.41–7.34 (m, 1H), 7.32–7.20 (m, 2H), 4.22 (d, $J = 7.3$ Hz, 2H), 3.04 (s, 3H), 1.64 (ddd, $J = 9.8$, 6.4, 3.3 Hz, 1H), 1.49 (d, $J = 8.5$ Hz, 3H), 1.30 (d, $J = 12.0$ Hz, 2H), 1.03 (dt, $J = 34.8$, 8.2 Hz, 3H), 0.78 (t, $J = 11.1$ Hz, 2H). **13C NMR (75 MHz, DMSO-d_6)** δ 153.18, 142.81, 139.11, 136.46, 132.33, 130.24, 125.06, 122.87, 122.34, 121.22, 120.72, 119.59, 111.76, 50.19, 39.71, 38.00, 30.33, 26.05, 25.37. **MS (ESI positive)** m/z [M+H]$^+$: 384.2.

HPLC purity: 98%, **HPLC t_R**: 3.58 min.

4.1.1.33. N-(4-(1-(4-methylbenzyl)-1H-benzimidazol-2-yl)phenyl) methanesulfonamide (6s). $R_f = 0.3$ (DCM/MeOH 9.8:0.2). White solid, yield 15%. **1H NMR (300 MHz, DMSO-d_6)** δ 10.12 (s, 1H), 7.70 (d, $J = 8.6$ Hz, 3H), 7.43 (dd, $J = 5.8$, 2.9 Hz, 1H), 7.32 (d, $J = 8.6$ Hz, 2H), 7.22 (p, $J = 7.6$ Hz, 2H), 7.09 (d, $J = 7.8$ Hz, 2H), 6.89 (d, $J = 7.9$ Hz, 2H), 5.53 (s, 2H), 3.08 (s, 3H), 2.23 (s, 3H). **13C NMR (75 MHz, DMSO-d_6)** δ 153.24, 143.10, 140.35, 137.07, 136.39, 134.36, 130.56, 129.76, 126.47, 125.36, 122.94, 122.54, 119.52, 119.11, 111.44, 47.72, 40.09, 21.03. **MS (ESI positive)** m/z [M+H]$^+$: 392.1. **HPLC purity**: 99.4%, **HPLC t_R**: 3.51 min.

4.2. Biology

4.2.1. Determination of minimum inhibitory concentration (MIC) against clinically-important gram-negative bacteria

The minimum inhibitory concentrations (MICs) of the tested compounds and control drugs were determined using the broth microdilution method, according to guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI) [33,34]. Bacterial strains were grown aerobically overnight on tryptone soy agar plates at 37 °C. Afterwards, a bacterial solution equivalent to 0.5 McFarland standard was prepared and diluted in cation-adjusted Mueller-Hinton broth (CAMHB), to achieve a bacterial concentration of about 5×10^5 CFU/ml. Compounds and control drugs were added in the first row of the 96-well plates, and serially diluted with media containing bacteria. Plates were then, incubated aerobically at 37 °C for 18–20 h. MICs reported in Tables 1–2 are the minimum concentration of the compounds and control drugs that could completely inhibit the visual growth of bacteria.

4.2.2. Cytotoxicity of benzimidazole derivative 6c

Compound **6c** was assayed for its potential cytotoxicity against human colorectal adenocarcinoma (Caco-2) and monkey kidney fibroblast (Vero) cells as described previously [35,36]. Caco-2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), non-essential amino acids (1X), penicillin-streptomycin at 37 °C with 5% CO_2. Vero cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, and penicillin-streptomycin at 37 °C with 5% CO_2. Compound **6c** was incubated with Caco-2 or Vero cells for 2 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 4 h before measuring absorbance values (OD_{490}).

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111850.

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