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Graphical Abstract

Design, synthesis and antimicrobial evaluation of novel benzimidazole-incorporated sulfonamide analogues

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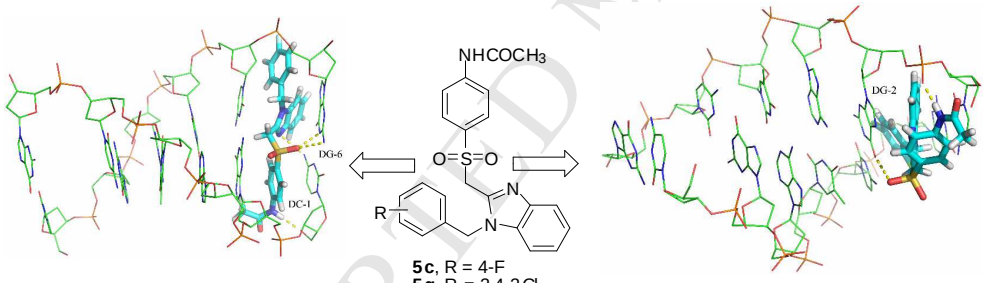
A novel series of benzimidazole-incorporated sulfonamide analogues were developed and screened for their antimicrobial activities. Interaction with calf thymus DNA and molecular docking were investigated. Transportation behavior with HSA was explored and molecular electrostatic potentiality was studied by full geometry optimizations.



5c, R = 4-F
5g, R = 2,4-2Cl

5c-DNA hexamer duplex

5g-DNA hexamer duplex

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Title page

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Abstract:

A novel series of benzimidazole-incorporated sulfonamide analogues were designed and synthesized with an effort to overcome the increasing antibiotic resistance. Compound **5c** gave potent activities against Gram-positive bacteria and fungi, and 2,4-dichlorobenzyl derivative **5g** showed good activities against Gram-negative bacteria. Both of these two active molecules **5c** and **5g** could effectively intercalate into calf thymus DNA to form compound–DNA complex respectively, which might block DNA replication to exert their powerful antimicrobial activity. Molecular docking experiments suggested that compounds **5c** and **5g** could insert into base-pairs of DNA hexamer duplex by the formation of hydrogen bonds with guanine of

DNA. The transportation behavior of these highly active compounds by human serum albumin (HSA) demonstrated that the electrostatic interactions played major roles in the strong association of active compounds with HSA, and which was also confirmed by the full geometry calculation optimizations.

***Keywords*:**

Benzimidazole; Sulfonamide analogues; Antibacterial; Antifungal; Calf thymus DNA; HSA

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1. Introduction

The antibiotic resistance which was accelerated by the use and misuse of antimicrobial drugs has been a major global challenge for public health. During the past decades, a dramatic increase in human-pathogenic bacteria worldwide was seen due to resistance to one or multiple antibiotics. More and more infections caused by resistant microorganisms fail to respond to conventional treatment, and in some cases, even last resort antibiotics have lost their power. Recently, the World Health Organization titled a recent world health day as “Combat drug resistance: no action today means no cure tomorrow”, and triggered an increase in research activity, and several promising strategies have been developed to restore treatment options against infections by resistant bacterial pathogens [1].

Sulfonamides as artificial antifolic agents have been widely used for the prevention and cure of bacterial infections in biological systems and recently have evoked high favor in biology and medicine because of their diverse pharmacological activities [2] including antibacterial [3], antifungal [4], antiviral [5], antitumor [6], anti-inflammatory [7], and carbonic anhydrase inhibitors [8]. As the analogues of aminobenzoic acid, sulfonamides could compete with it to availably prevent the synthesis of nucleic acids and proteins, and then inhibit the growth of various microorganisms. Furthermore, sulfonamide compounds have attracted increasing research in supramolecular chemistry because they could combine the features of different fragments through the coordination of phenylamino and sulfonyl amino groups [9]. Particularly, Ag-sulfadiazine has been significantly employed in burn therapy, which is better than the free ligand or $AgNO_3$. Up to now, numerous sulfonamides bearing aromatic heterocycles such as isoxazole, thiazole, pyridazine and pyrimidine have been successfully developed and used in clinic like sulfadiazine, sulfachlorpyridazine, sulfathiazole and sulfisoxazole with excellent antimicrobial activities. This has stimulated considerable efforts towards the synthesis and development of completely new structural sulfonamide derivatives with excellent activity, broad spectrum and low toxicity.

Our previous efforts have identified that the introduction of aromatic heterocycles such as imidazole [10], triazole [11], tetrazole [12], thiazole [13] and benzene-fused azoles like benzimidazole [14] and benzotriazole [15] into target molecules could highly improve the antimicrobial activities. 1,2,3-Triazole sulfonamide **WXL-1** containing 2,4-difluorobenzyl group was almost 32-fold more potent than precursor sulfonamide against *Pseudomonas aeruginosa* and *Shigella dysenteriae* [16]. The sulfonamide **ZHZ–1** bearing 2-methyl-5-nitroimidazole fragment displayed almost equivalent anti-*P. aeruginosa* efficiency to Chloromycin (MIC = 16 μg/mL) (Fig. 1), and further research found that this compound could effectively intercalate into calf thymus DNA to form compound–DNA complex which might block DNA replication to

exert their powerful antimicrobial activities. Moreover, the transportation behavior of this compound by human serum albumin (HSA) showed that electrostatic interactions played major roles in the strong association of compound **ZHZ-1** and HSA [17].

Fig. 1

In view of such stimulating properties and as an extension of our studies on the development of sulfonamide azoles, the methylene moiety as the bioisostere of amino group was introduced into the sulfonamide fragment with the aim to investigate the effect on antimicrobial activity [18]. Benzimidazole ring as the fused heterocycle of benzene and imidazole was structurally similar to purine, and its derivatives could compete with purine to inhibit the synthesis of nucleic acids and proteins inside the bacterial cell wall, and then kill the bacterial strains or inhibit their growth [19]. Additionally, fluoro- and chloro-substituted benzyl groups were introduced into the target molecules in order to improve the pharmacological properties by enhancing the rate of absorption and the transportation of drugs *in vivo* [20].

The newly synthesized sulfonamide analogues were screened for their antibacterial and antifungal activities *in vitro*. DNA as one of the most important targets has attracted growing interest in investigating the interaction of small molecules with DNA to explore the possible antimicrobial action mechanism [21]. The interactions of most active compounds with calf thymus DNA were evaluated by UV-vis absorption spectroscopy on a molecular level to explore their probable antimicrobial mechanisms. Moreover, molecular docking studies were employed to reconfirm the interaction behaviors between the prepared compounds and DNA hexamer duplex [22]. Additionally, the transportation behavior of the highly active molecules by human serum albumin (HSA) was investigated by fluorescence spectroscopy to preliminarily study their absorption, distribution and metabolism [23].

2. Results and discussion

2.1. Chemistry

The target benzimidazole-incorporated sulfonamide analogues were prepared from commercial acetanilide and chlorosulfonic acid. Their synthetic routes were outlined in Scheme 1. Acetanilide was reacted with chlorosulfonic acid to produce N-protected sulfonyl chloride **2**, and then further treated by sodium sulfite and sodium bicarbonate to give *p*-acetylamino benzenesulfinic acid sodium salt **3**. The latter was subsequently reacted with chloromethyl benzimidazole to afford benzimidazole-incorporated sulfonamide analogue **4**. The N-alkylation of benzimidazole ring of compound **4** with halobenzyl halides in

acetonitrile at 70 ºC using potassium carbonate as base respectively produced the target aralkyl benzimidazole sulfonamide analogues **5a**–**h** in 52.1–73.7% yields which showed that the substituents exhibited effect on the formation of target compounds to some extent. Generally, the chlorobenzyl halides gave higher yields than fluorobenzyl ones. In this series, it was found that the strong electron-withdrawing 3-F group gave the lowest yield (52.1%), while 3-Cl substituted one provided the highest yield of 73.7%. Moreover, compound **4** was also reacted with a series of alkyl bromides to produce compounds **6a**–**i** with a large difference in yields of 24.2–71.2%. Furthermore, the N-alkylation of compound **4** with carbazole alkyl bromide was successfully performed to give carbazole alkylated benzimidazole sulfonamide analogue **7** in yield of 72.0%. Generally, it was thought that the presence of protons on the nitrogen atom of the sulfonamide skeleton was favorable for the bioactivity, and thus compounds **5**–**7** were further transformed into the deprotected sulfonamide analogues **8**–**10** in ethanol in the presence of sodium hydroxide in order to explore their effect on the bioactivities.

Scheme 1

2.2. Analysis of spectra

All the new compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and HRMS spectra. Their spectral analyses were consistent with the assigned structures and listed in the experimental section. The mass spectra for benzimidazole compounds gave a major fragment of $[M+H]^{+}$ according to their molecular formula.

2.2.1. IR spectra

In IR spectra, all the synthesized benzimidazole sulfonamide analogues **4**–**7** gave broad absorption in 3369–3352 cm^{-1} which indicated the presence of NH group of amide moiety, whereas two broad absorption between 3332–3307 and 3209–3185 cm^{-1} were attributed to the NH group in compounds **8**–**10**. The characteristic C=N bands of benzimidazole ring in all benzimidazole derivatives appeared in the region between 1694 and 1639 cm^{-1}. All the other absorption bands were also observed at the expected regions.

2.2.2. ^{1}H NMR spectra

In ^{1}H NMR spectra, compounds **4**–**7** gave singlets at 2.12–2.10 ppm assigned to the CH_3 protons linked to the amide moiety. The singlets at 5.19–5.07 ppm of compounds **5**–**7** were assigned to the CH_2 protons linked to benzimidazole ring, while the N-CH_2 protons of alkyl chain in compound **6** gave lower shift signals at 4.35–4.21 ppm. The substitution of alkyl group by halobenzyl moiety to yield compound **5** led to

downfield shifts of N-CH_2 protons (5.63–5.56 ppm), which were higher than those of CH_2 protons attached C2-benzimidazole ring because of the strong electron-withdrawing ability of halophenyl moieties and nitrogen atom in benzimidazole ring. The corresponding deprotected compounds **8** and **9** gave upfield shifts of SO_2-CH_2 protons down to 4.90–4.87 and 5.03–4.88 ppm due to the absence of acetyl group respectively. Moreover, the peaks for the 3,5-H protons in the sulfonamide analogue ring in the protected compounds **4**–**7** appeared at δ 7.76–7.67 ppm, whereas deprotection led to an upfield shift to 6.61–6.58 ppm. In addition, all the other aromatic and aliphatic protons appeared at the appropriate chemical shifts and integral values.

2.2.3. ^{13}C NMR spectra

The ^{13}C NMR spectral analyses were in accordance with the assigned structures. No large differences were found in the ^{13}C NMR chemical shifts for the carbonyl carbon in compounds **5**–**7** (δ 169.7–169.6 ppm). The signals for the phenyl 3,5-C in the sulfonamide analogue skeletons in derivatives **5**–**7** were observed at 119.0–118.9 ppm, respectively. It was noticeable that the deprotection of these compounds to compounds **8**–**10** resulted in upfield ^{13}C shifts (5.9–5.8 ppm) of these two carbons because of the absence of the strong electron-withdrawing acetyl group. The signals at 56.3–54.5 ppm in compounds **4**–**10** were assigned to the methylene carbon which was connected with sulfonyl group. For compounds **5** and **8**, the methylene carbon linked to the benzimidazole ring was appeared at 50.2–45.1 ppm. All the other carbons gave ^{13}C peaks at the expected regions.

2.3. Biological Activity

The *in vitro* antimicrobial screening for all the synthesized compounds was evaluated against four Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Methicillin-resistant Staphylococcus aureus* N315 (MRSA), *Micrococcus luteus* ATCC 4698 and *Bacillus subtilis* ATCC 21216), four Gram-negative bacteria (*Escherichia coli* ATCC 8099, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus typhi* and *Bacillus proteus* ATCC 13315) and five fungi (*Candida albicans* ATCC 76615, *Candida mycoderma*, *Candida utilis*, *Saccharomyces cerevisia* and *Aspergillus flavus*) using two fold serial dilution technique recommended by National Committee for Clinical Laboratory Standards (NCCLS) with the positive control of clinically antimicrobial drugs Chloromycin, Norfloxacin and Fluconazole [24]. The values of Clog*P*, a partition coefficient as a kind of measurement for hydrophobicity/lipophilicity, were calculated using ChemDraw Ultra 10.0 software integrated with Cambridge Software (Cambridge Soft Corporation). The antibacterial and antifungal data as well as Clog*P* values were depicted in Tables 1 and 2.

2.3.1. Antibacterial activity

The *in vitro* antibacterial screening demonstrated that some of the newly synthesized compounds could effectively inhibit the growth of all the tested microorganisms and exhibit broad antimicrobial spectrum and potent antibacterial activity. Compound **4** exhibited moderate to good activity against the tested bacteria (MIC = 32−128 μg/mL) in comparison with clinical drugs.

Table 1 displayed the significant effects of the substituents on the benzene ring on the biological activity. Noticeably, in this series of halobenzyl benzimidazole sulfonamide analogues, compound **5c** with 4-fluorobenzyl group gave the best activities against the tested Gram-positive bacteria with MIC values of 4–64 μg/mL. Particularly, its anti-*S. aureus* (MIC = 4 μg/mL) activity was 2-fold more potent to clinical Chloromycin and Norfloxacin, and for *B. subtilis* strains, it also showed two times more active than Chloromycin (MIC = 16 μg/mL). Especially, this compound displayed equivalent inhibition activity against MRSA to Chloromycin (MIC = 16 μg/mL). The replacement of 4-fluorobenzyl moiety by 2,4-dichlorobenzyl group, which yielded compound **5g**, resulted in good bioactivities against Gram-negative bacteria with MIC values ranging from 4 to 32 μg/mL. Compound **5g** showed eight times higher activity (MIC = 4 μg/mL) than Chloromycin against *B. typhi*. However, the substitution of 4-fluorobenzyl fragment in compound **5c** by 3,4-dichlorobenzyl group which gave derivative **5h** was not beneficial for the antibacterial activities.

In comparison with halobenzyl compounds **5a**–**h**, most of alkyl derivatives **6a**–**i** exerted relatively lower activities in inhibiting the growth of the tested strains. Compound **6c** with pentyl group gave good anti-*P. aeruginosa* activity with MIC value of 4 μg/mL which was 4-fold more potent than Chloromycin. The replacement of pentyl chain by heptyl group, which yielded compound **6e**, resulted in strong activity towards the tested *S. aureus* strains (MIC = 8 μg/mL). Additionally, when the alkyl substituents were extended to decyl, dodecyl and octadecyl groups, compounds **6f**–**i** gave relatively weaker inhibitory activity. These results suggested that the length of alkyl chain possessed remarkable effects on biological activities. The short alkyl chains with poor lipophilicity or long alkyl chains with poor hydrophilicity in these compounds might make them unfavorable for being delivered to the binding sites.

Some of the deprotected halobenzyl benzimidazole sulfonamide analogues **8a**–**h** exerted relatively better activity than the corresponding protected ones to some extent in inhibiting the growth of the tested bacteria. Specially, compound **8f** with 4-chlorobenzyl moiety was 32-fold or 16-fold more potent than its precursor against *B. subtilis* or *M. luteus* strains (MIC = 8 and 8 μg/mL, respectively). 2,4-Dichlorobenzyl benzimidazole sulfonamide analogue **8g** displayed equivalent bioactivity against *S. aureus* to Chloromycin

and Norfloxacin with MIC value of 8 μg/mL. However, the deprotection of alkyl compounds **6a**–**i**, which yielded compounds **9a**–**i**, led to weak antibacterial activities.

Much research has reported that the introduction of carbazole heterocycle is helpful to improve antimicrobial potency [18]. In our work, it was found that the carbazole incorporated sulfonamide analogue **7** possessed considerable potentiality with MIC value of 16 μg/mL. Its corresponding deprotected compound **10** gave moderate activity against *B. subtilis* and *B. typhi* strains.

Table 1

2.3.2. Antifungal activity

The antifungal evaluation *in vitro* displayed that some prepared benzimidazole sulfonamide analogues exhibited good bioactivities against the tested fungal strains. Compound **4** showed moderate to good antifungal activities with MIC values ranging from 16 to 128 μg/mL.

In the series of sulfonamide analogues **5a**–**h**, compound **5c** bearing 4-fluorobenzyl group exerted the relatively best activities in inhibiting the growth of all the tested fungal strains. The replacement of 4-fluorobenzyl moiety by 2-chlorobenzyl fragment, which yielded compound **5d**, resulted in good activity against Fluconazole-insensitive *A. flavus* (MIC = 8 μg/mL). Moreover, 3,4-dichlorobenzyl substituted compound **5g** gave MIC value of 16 μg/mL against *A. flavus*, which was 16 times more active than reference drug.

The length of aliphatic chain exhibited obvious effects on antifungal activity. The suitable length of alkyl chain to exert the best antifungal efficacy was observed to be $(CH_2)_2$ moiety, and the propyl derivative **6b** generally gave better activity in contrast with other alkyl derivatives with shorter or longer chain length. It displayed comparable inhibitory activity against *S. cerevisiae* to reference (MIC = 16 μg/mL). Compound **6f** containing octyl group was more effective than the reference at 8 μg/mL concentration. Additionally, the deprotected compound **9b** also gave good activity in inhibiting the growth of the tested fungal with MIC values ranging from 4 to 64 μg/mL.

The deprotected compound **8a** bearing 2-fluorobenzyl fragment exhibited good activities against *C. mycoderma*, *C. utilis* and *S. cerevisiae* with MIC values of 8, 4 and 8 μg/mL, respectively. Moreover, its anti-*A. flavus* activity was 8-fold more potent than the reference drug (MIC = 256 μg/mL). Notably, compound **8g** with 2,4-dichlorobenzyl moiety showed much stronger activities against *C. utilis* and *A. flavus* (MIC = 2 and 4 μg/mL) than Fluconazole (MIC = 8 and 256 μg/mL).

Table 2

2.3.3. Effect of ClogP values on antimicrobial activity

Hydrophobic/lipophilic properties possessed an important role in exerting biological activity [25]. The Clog*P* values as one of the most important factors have been extensively employed to predict the bioactivity of target molecules. The calculated liposome/water partition coefficients (Clog*P*) for all newly prepared compounds were shown in Table 1. The results demonstrated that compounds with lower values of Clog*P* showed better antimicrobial activities, and these compounds possessed comparable Clog*P* values to the reference drugs with equivalent potency. As shown in Table 1, the Clog*P* values of compounds **6a**–**i** generally increased with the increasing length of alkyl groups, and the enhancement of the antimicrobial activities was observed in compounds **6a**–**e**, but the bioactivities were decreased in compounds **6f**–**i**. These might be explained by the possibility that higher lipophilic compounds were unfavorable for being delivered to the binding sites in organism, and manifested the significant role of suitable lipophilicity in drug design.

2.4. Interactions with calf thymus DNA

DNA is the informational molecule encoding the genetic instructions and has been widely researched for the advisable design and development of efficient drugs. Calf thymus DNA has always been employed as a model because of its biological importance and commercial available properties. The binding behavior of compound **5c** (exerting good inhibition against Gram-positive bacteria and fungal strains) and **5g** (displaying good inhibition against Gram-negative bacteria strains) with calf thymus DNA was studied to explore the possible antimicrobial mechanism of action on a molecular level *in vitro* with neutral red (NR) dye as a spectral probe using UV-vis spectroscopic methods [26].

*2.4.1. Absorption spectra of DNA in the presence of compounds **5c** and **5g***

The absorption spectroscopy as one of the most important techniques is extensively employed in DNA-binding studies. Generally, it is considered that hypochromism and hyperchromism are vital spectral features to distinguish changes in the DNA double-helical structure. As reported, hyperchromism was generated from the breakage of the DNA duplex secondary structure, while hypochromism was originated from the stabilization of the DNA duplex by either an intercalation binding mode or the electrostatic effects of small molecules [27]. The observed hypochromism intensively recommended a close proximity of the aromatic chromophore to the DNA bases, which might be due to the strong interaction between the electronic states of intercalating chromophore and that of the DNA base.

With a fixed concentration of DNA, the UV-vis absorption spectra were recorded with an increasing amount of compounds **5c** and **5g**. As shown in Fig. 2 and Fig. 3, the UV-vis spectra showed that the maximum absorption of DNA (at 260 nm) displayed a proportional increase with increase in the concentration of compounds **5c** and **5g**. Meanwhile, the absorption value for the measured values of the **5c**–DNA or **5g**–DNA complex was slightly greater than the simply sum of free DNA and free compound **5c** or **5g**, which was observed in the inset of Fig. 2 and Fig. 3. These indicated a weak hyperchromic effect existed between DNA and compound **5c** or **5g**. These demonstrated that a weak hypochromic effect existed between DNA and compound **5c** or **5g**. Moreover, the intercalation of the aromatic chromophore of compound **5c** or **5g** into the helix and the strong overlap of π-π* states in the large π-conjugated system with the electronic states of DNA bases were consistent with the observed spectral changes [28].

Fig. 2

Fig. 3

On the basis of the variations in the absorption spectra of DNA upon binding to **5c** or **5g**, equation 1 can be utilized to calculate the binding constant (K).

$$\frac{A^0}{A-A^0}=\frac{\xi_C}{\xi_{D\text{-}C}-\xi_C}+\frac{\xi_C}{\xi_{D\text{-}C}-\xi_C}\times\frac{1}{K[Q]} \qquad (1)$$

The plot of $A^0/(A\text{-}A^0)$ versus 1/[compound **5c**(or **5g**)] is constructed by using the absorption titration data and linear fitting (Supporting Information: Fig. S1 or Fig. S2), yielding the binding constant, K = 1.26 × 10^4 or 1.52 × 10^4 L/mol, R = 0.999 or 0.999, SD = 0.04 or 0.09 respectively (R is the correlation coefficient. SD is standard deviation).

2.4.2. Absorption spectra of NR interaction with DNA

Neutral Red (NR) as a planar phenazine dye is structurally similar to other planar dyes acridine, thiazine and xanthene. It has been displayed that the binding of NR with DNA is intercalation binding type. Therefore, NR was used as a spectral probe to investigate the binding mode of **5c** or **5g** with DNA in this work.

The absorption spectra of the NR dye upon the addition of DNA were showed in Fig. S3 (Supporting Information). It was apparent that the absorption peak of the NR at around 460 nm gave gradual decrease with the increasing concentration of DNA, and a new band at around 530 nm developed. This was because of the formation of the new DNA–NR complex. An isosbestic point at 504 nm provided evidence of

DNA–NR complex formation.

*2.4.3. Absorption spectra of competitive interaction of compound **5c** or **5g** and NR with DNA*

As showed in Fig. 4 or Fig. 5, the competitive binding between NR and **5c** (or **5g**) with DNA was observed in the absorption spectra. With the increasing concentration of compound **5c** or **5g**, an apparent intensity increase was observed around 275 nm. Compared with the absorption around 275 nm of NR–DNA complex, the absorbance at the same wavelength exhibited the reverse process (inset of Fig. 4 or Fig. 5). These various spectral changes were consistent with the intercalation of compound **5c** or **5g** into DNA by substituting for NR in the DNA–NR complex.

Fig. 4

Fig. 5

As depicted above, although compounds **5c** and **5g** displayed different bioactivities, both of them could intercalate into calf thymus DNA to form compound–DNA complex which might block DNA replication to exert their powerful antimicrobial activities.

*2.5. Molecular docking of compound **5c** or **5g** with DNA hexamer duplex*

Molecular docking study as a useful method is widely employed to investigate the binding modes of small molecules to DNA. Up to now, the full-length 3D structure of calf thymus DNA is not available in Protein Data Bank (PDB). Since calf thymus DNA is B-DNA, the CT-DNA sequence $d(CGATCG)_2$ (PDB code: 3FT6) was chosen as receptor model. In our research, molecular docking study was performed between compound **5c** or **5g** and DNA hexamer duplex to understand the binding model. The docking mode with the lowest binding free energy ($-4.01\ kJ\cdot mol^{-1}$ for **5c** or $-4.26\ kJ\cdot mol^{-1}$ for **5g**) is shown in Fig. 6 and Fig. 7 or Fig. 8 and Fig. 9. The results demonstrated that the hydrogen atom connected to the nitrogen atom and oxygen atom in the sulfonyl group of compounds **5c** and **5g** formed two hydrogen bonds with the guanine of DNA, thus preventing the formation of hydrogen bond between cytosine in DNA. This kind of interaction resulted in decreased stability of DNA, and therefore inhibited its physiological function. All these suggested that the simulation results were in accordance with the above spectral experiment results.

Fig. 6

Fig. 7

Fig. 8

Fig. 9

*2.6. Interactions of compound **5c** or **5g** with HSA*

2.6.1. UV-vis absorption spectral study

UV-vis absorption measurement as operational method is applicable to explore the structural change of protein and to identify the complex formation. In our binding experiment, UV-vis absorption spectroscopic method was employed to evaluate the binding behaviors between compound **5c** or **5g** and HSA. As shown in Fig. S4 or Fig. S5 (Supporting Information), the absorption peak observed at 278 nm was attributed to the aromatic rings in Tryptophan (Trp-214), Tyrosine (Tyr-411) and Phenylalanine (Phe) residues in HSA. With the addition of compound **5c** or **5g**, the peak intensity increased, indicating that compound **5c** or **5g** could interact with HSA and the peptide strands of HSA were extended [29].

2.6.2. Fluorescence quenching mechanism

Fluorescence spectroscopy is a favorable method to investigate the interactions of small molecules with HSA. The fluorescence intensity of Trp-214 may change when HSA interacts with other small molecules, which could be reflected in the fluorescence spectra of HSA in the UV region [30]. The effect of compound **5c** or **5g** on the fluorescence intensity to HSA at 293 K was shown in Fig. 11 or Fig. 12. It was obvious that HSA had a strong fluorescence emission with a peak at 348 nm owing to the single Try-214 residue. The intensity of this characteristic broad emission band regularly decreased with the increased concentrations of compound **5c** or **5g**. In Fig. 10 or Fig. 11, the black line showed the only emission spectrum of compound **5c** or **5g**, which indicated that compound **5c** or **5g** did not possess significant fluorescence features, and therefore the effect of compound **5c** or **5g** on fluorescence of HSA would be negligible at the excitation wavelength (295 nm) [31].

Fig. 10

Fig. 11

The fluorescence quenching data can be analyzed by the well-known Stern-Volmer equation [32]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + K_q \tau_0 [Q] \qquad (2)$$

The Stern-Volmer plots of HSA in the presence of compound **5c** or **5g** at different concentrations and

temperatures could be calculated and were showed in Fig. S6 or Fig. S7 (Supporting Information).

The values of K_{SV} and K_q for the interaction of compound **5c** or **5g** with HSA at different temperatures were showed in Table 3. The K_{SV} values were inversely correlated with the temperature, which indicated that the fluorescence quenching of HSA might be initiated by the formation of compound–HSA complex rather than dynamic collisions. The K_q values obtained at different temperatures were in 10^{12} L/mol s^{-1} (Table 3), which far exceeded the diffusion controlled rate constants of various quenchers with a biopolymer (2.0×10^{10} L/mol s^{-1}), and indicated that the quenching was not initiated by the dynamic diffusion process but occurred in the statically formation of compound–HSA complex [31].

Table 3

2.6.3. Binding constant and site

For a static quenching process, the data could be described by the Modified Stern-Volmer equation [33]:

$$\frac{F_0}{\Delta F} = \frac{1}{f_a K_a}\frac{1}{[Q]} + \frac{1}{f_a} \qquad (3)$$

The modified Stern-Volmer plots were showed in Fig. S8 or Fig. S9 (Supporting Information) and the calculated results were depicted in Table 4.

Table 4

When small molecules bind to a set of equivalent sites on a macromolecule, the equilibrium binding constants and the numbers of binding sites can also be calculated according to the Scatchard equation [34]:

$$r/D_f = nK_b - rK_b \qquad (4)$$

The Scatchard plots were shown in Fig. S10 or Fig. S11 (Supporting Information) and the K_b and n were listed in Table 4.

The modified Stern-Volmer and Scatchard plots for the compound–HSA system at different temperatures were given in Table 4. The decreased trend of K_a and K_b with increased temperatures was in accordance with K_{SV}'s depended on temperatures. The value of the binding site n was approximately 1, which showed one high affinity binding site, was present in the interaction of compound **5c** or **5g** with HSA. The results also showed that the binding constants were moderate and the effects of temperatures were not significant, thus both compounds **5c** and **5g** might be stored and carried by this protein.

2.6.4. Binding mode and thermodynamic parameters

Generally, there are four types of non-covalent interactions including hydrogen bonds, van der Waals forces, electrostatic interactions and hydrophobic bonds, which play substantial roles in small molecules binding to proteins [35]. The thermodynamic parameters enthalpy (ΔH) and entropy (ΔS) change of binding reaction are the main evidence for confirming the interactions between small molecules and protein. If the ΔH does not vary significantly over the studied temperatures range, then its value and ΔS can be evaluated from the van't Hoff equation:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (5)$$

In order to explain the binding model between compound and HSA, the thermodynamic parameters were calculated from the van't Hoff plots. The ΔH was estimated from the slope of the van't Hoff relationship (Fig. 12 or Fig. 13). The free energy change (ΔG) was then calculated from the following equation:

$$\Delta G = \Delta H - T\Delta S \quad (6)$$

Fig. 12

Fig. 13

The values of ΔH, ΔG and ΔS were summarized in Table 5. The negative values of free energy ΔG of the interaction between compound **5c** or **5g** and HSA suggested that the binding process was spontaneous, and the negative values of ΔH indicated that the binding was mainly enthalpy-driven and involved an exothermic reaction, the ΔS was unfavorable for it. A positive ΔS value is frequently taken as a typical evidence for hydrophobic interaction, which was consistent with the above discussion. Therefore, $\Delta H < 0$ and $\Delta S > 0$ obtained in this case indicated that the electrostatic interactions played an important role in the binding of compound **5c** or **5g** to HSA [36].

Table 5

The above experiments displayed that compounds **5c** and **5g** could effectively interact with HSA, thereby causing hypochromic effect of ultraviolet spectroscopy. When the electronic transfer occurred between compound **5c** or **5g** and HSA, it caused energy transfer without radiation, and therefore resulted in the quenching of fluorescence spectrum. Further molecular electrostatic potentiality for the compounds **5c** and **5g** was investigated by full geometry optimizations of the studied systems which was performed by using the B3LYP functional with 6-31G* basis set. Calculation presented in this work was carried out by the GAUSSIAN 09 program package. The results manifested that the nucleophilic effect of carbonyl and sulfonyl groups (Fig. 14, red region) in compound **5c** or **5g** might induce an electrostatic effect with

positive electricity of Lys199 in HSA. Therefore, compound **5c** or **5g** with HSA might interact by electrostatic interactions [37]. This result was also evidenced by the value of enthalpy change (ΔH) and entropy change (ΔS) from the van't Hoff equation, which was accordant with the literature (when $\Delta H < 0$ and $\Delta S > 0$, the main force is electrostatic interaction).

Fig. 14

3. Conclusion

In conclusion, a novel series of benzimidazole-incorporated sulfonamide analogues have been successfully prepared starting from commercially avaliable acetanilide. All the new compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR, MS and HRMS spectra. Among these sulfonamide analogues, compound **5c** bearing 4-fluorobenzyl group gave potent activities against Gram-positive bacteria and fungal strains (MIC = 4–64 μg/mL), and 2,4-dichlorobenzyl derivative **5g** showed good activities against Gram-negative bacteria with MIC values ranging from 4 to 32 μg/mL. These results manifested that compounds **5c** and **5g** should be worthy to be further investigated as potential antimicrobial agents. Further research demonstrated that the two active molecules **5c** and **5g** could effectively intercalate into calf thymus DNA to form the compound–DNA complex, which might block DNA replication to exert their powerful antimicrobial activity. Molecular docking experiments suggested that compounds **5c** and **5g** could intercalate into base-pairs of DNA hexamer duplex by the formation of hydrogen bonds with guanine of DNA. The binding research demonstrated that HSA could effectively store and carry compounds **5c** and **5g** by electrostatic interactions, and which was also confirmed by the full geometry calculation optimizations. All these results opened up a promising starting point to optimize the structures of sulfonamide benzimidazoles as potent antimicrobial agents.

4. Experimental

4.1. General methods

Melting points were recorded on X–6 melting point apparatus and uncorrected. TLC analysis was done using pre-coated silica gel plates. FT-IR spectra were carried out on Bruker RFS100/S spectrophotometer (Bio-Rad, Cambridge, MA, USA) using KBr pellets in the 400–4000 cm^{-1} range. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV 600 spectrometer using TMS as an internal standard. The following abbreviations were used to designate aryl groups: Bim = benzimidazolyl, Ph = phenyl. The chemical shifts were reported in parts per million (ppm), the coupling constants (J) were expressed in hertz (Hz) and

signals were described as singlet (s), doublet (d), triplet (t) as well as multiplet (m). The mass spectra were recorded on LCMS–2010A and HRMS. All chemicals and solvents were commercially available and were used without further purification.

*4.1.1. Synthesis of 4-acetamidobenzene-1-sulfonyl chloride (**2**)*

4-Acetamidobenzene-1-sulfonyl chloride **2** was prepared according to the literature procedure, starting from acetaniline (5.002 g, 0.037 mol) and chlorosulfonic acid (14 mL). Yield: 81.6%; mp: 138–140 ºC. (literature mp: 142–144 ºC) [17].

*4.1.2. Synthesis of sodium 4-acetamidobenzenesulfinate (**3**)*

A mixture of compound **2** (4.343 g, 18.6 mmol), sodium sulfite (3.486 g, 27.7 mmol) and sodium bicarbonate (2.322 g, 27.6 mmol) was stirred in water at 80 ºC. After the reaction was completed (monitored by TLC, eluent, methanol/ethyl acetate, 1/2, V/V), the system was washed by ethyl acetate, and the water phase was collected and evaporated to afford compound **3** as white solid.

*4.1.3. Synthesis of N-(4-((1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**4**)*

A suspension of compound **3** (3.959 g, 17.9 mmol), chloromethyl benzimidazole (2.992 g, 17.8 mmol) and tetrabutylammonium iodide (0.010 g) was stirred in acetone (30 mL) at 50 ºC. After the reaction was completed (monitored by TLC, eluent, acetone/petroleum ether, 1/1, V/V), the reaction system was filtered, and the residue was collected and washed with water to give compound **4** as yellow solid. Yield: 60%; mp: 155−156 ºC; IR (KBr) ν: 3429 (Bim-NH), 3359 (N−H), 3024 (aromatic C−H), 3024 (CH_2), 1681 (C=N), 1589, 1543 (aromatic frame), 737 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.10 (s, 3H, COCH_3), 4.91 (s, 2H, Bim-CH_2), 7.20−7.19 (m, 2H, Bim-6,7-*H*), 7.53 (d, 2H, *J* = 6.0 Hz, Bim-5,8-*H*), 7.69 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.75 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.39 (s, H, N*H*COCH$_3$), 12.54 (s, H, Bim-1-H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 24.7, 54.6, 115.7, 117.1, 119.9, 123.0, 123.4, 129.0, 130.0, 137.9, 139.0, 145.8, 152.8, 169.8 ppm.

*4.1.4. Synthesis of N-(4-((1-(2-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**5a**)*

A suspension of compound **4** (0.492 g, 1.49 mmol) and potassium carbonate (0.425 g, 3.07 mmol) was stirred in acetonitrile (30 mL) at 50 ºC. After 0.5 h, 1-(chloromethyl)-2-fluorobenzene (0.342 g, 2.37 mmol) was added and the reaction system was stirred at 70 ºC continuously. After the reaction was completed

(monitored by TLC, eluent, acetone/petroleum ether, 1/1, V/V), the solvent was removed and the residue was exacted with chloroform (3 × 20 mL), dried over anhydrous sodium sulfate and purified by silica gel column chromatography (eluent, acetone/petroleum ether, 1/1, V/V) to afford compound **5a** as yellow solid. Yield: 54%; mp: 215−217 ºC; IR (KBr) ν: 3360 (N−H), 3037 (aromatic C−H), 2994 (CH_2), 1687 (C=N), 1584, 1531 (aromatic frame), 739 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.11 (s, 3H, COCH_3), 5.14 (s, 2H, Bim-CH_2), 5.63 (s, 2H, 2-FPh-CH_2), 6.93−6.90 (t, H, *J* = 9.0 Hz, 2-FPh-5-*H*), 7.11−7.09 (t, H, *J* = 6.0 Hz, 2-FPh-4-*H*), 7.24−7.16 (m, 4H, Bim-5,6,7,8-*H*), 7.35−7.32 (m, H, 2-FPh-6-*H*), 7.60−7.59 (m, H, 2-FPh-3-*H*), 7.74 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.77 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 24.6, 49.1, 54.6, 111.4, 116.0, 119.0, 119.90, 122.7, 123.5, 123.7, 125.1, 129.5, 129.9, 130.3, 132.5, 135.6, 142.8, 144.0, 144.8, 160.6, 169.7 ppm; TOF-MS (*m/z*): 460 $[M+Na]^+$; HRMS (TOF) calcd. for $C_{23}H_{20}FN_3O_3SNa$ $[M+Na]^+$, 460.1102; found, 460.1105.

*4.1.5. Synthesis of N-(4-((1-(3-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**5b**)*

Compound **5b** was prepared according to the procedure described for compound **5a**, starting from compound **4** (0.210 g, 0.638), 1-(chloromethyl)-3-fluorobenzene (0.216 g, 1.505 mmol) and potassium carbonate (0.187 g, 1.355 mmol). The pure product **5b** was obtained as yellow solid. Yield: 52.1%; mp: 221−224 ºC; IR (KBr) ν: 3362 (N−H), 3038 (aromatic C−H), 2993 (CH_2), 1689 (C=N), 1582, 1533 (aromatic frame), 736 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.11 (s, 3H, COCH_3), 5.16 (s, 2H, Bim-CH_2), 5.61 (s, 2H, 3-FPh-CH_2), 6.98−6.94 (m, 2H, 3-FPh-2,6-*H*), 7.11−7.08 (m, H, 3-FPh-4-*H*), 7.20−7.18 (m, 2H, Bim-6,7-*H*), 7.30−7.29 (m, H, Bim-8-*H*), 7.37−7.33 (m, H, 3-FPh-5-*H*), 7.61−7.59 (m, H, Bim-5-*H*), 7.76 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.77 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 24.7, 47.1, 54.5, 111.6, 114.3, 114.8, 118.9, 119.9, 122.7, 123.4, 123.5, 130.0, 131.0, 132.6, 135.6, 139.9, 142.9, 144.0, 144.8, 162.8, 169.7 ppm; TOF-MS (*m/z*): 460 $[M+Na]^+$; HRMS (TOF) calcd. for $C_{23}H_{20}FN_3O_3SNa$ $[M+Na]^+$, 460.1102; found, 460.1101.

*4.1.6. Synthesis of N-(4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**5c**)*

Compound **5c** was prepared according to the procedure depicted for compound **5a**, starting from compound **4** (0.201 g, 0.610 mmol), 1-(chloromethyl)-4-fluorobenzene (0.176 g, 1.225 mmol) and potassium carbonate (0.174 g, 1.260 mmol). The pure product **5c** was obtained as yellow solid. Yield: 67.7%; mp: 199−201 ºC; IR (KBr) ν: 3364 (N−H), 3037 (aromatic C−H), 2995 (CH_2), 1687 (C=N), 1585,

1534 (aromatic frame), 740 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ: 2.11 (s, 3H, COCH_3), 5.14 (s, 2H, Bim-CH_2), 5.56 (s, 2H, 4-FPh-CH_2), 7.19−7.12 (m, 6H, 4-FPh-2,3,5,6-*H*, Bim-6,7-*H*), 7.30−7.29 (m, H, Bim-8-*H*), 7.59−7.58 (m, H, Bim-5-*H*), 7.75 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.77 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.40 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 24.7, 47.0, 54.6, 111.7, 115.9, 119.0, 119.9, 122.6, 123.4, 129.5, 130.0, 132.6, 133.1, 135.6, 142.9, 143.9, 144.8, 162.1, 169.7 ppm; TOF-MS (*m/z*): 460 $[M+Na]^+$; HRMS (TOF) calcd. for $C_{23}H_{20}FN_3O_3SNa$ $[M+Na]^+$, 460.1102; found, 460.1104.

*4.1.7. Synthesis of N-(4-((1-(2-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**5d**)*

Compound **5d** was prepared according to the experimental procedure described for compound **5a**, starting from compound **4** (0.271 g, 0.824 mmol), 1-chloro-2-(chloromethyl)benzene (0.200 g, 1.242 mmol) and potassium carbonate (0.180 g, 1.260 mmol). The pure product **5d** was obtained as yellow solid. Yield: 66.7%; mp: 158−160 ºC; IR (KBr) ν: 3367 (N−H), 3039 (aromatic C−H), 2993 (CH_2), 1689 (C=N), 1587, 1535 (aromatic frame), 738 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ: 2.11 (s, 3H, COCH_3), 5.11 (s, 2H, Bim-CH_2), 5.62 (s, 2H, 2-ClPh-CH_2), 6.52 (d, H, *J* = 6.0 Hz, 2-ClPh-6-*H*), 7.22−7.17 (m, 4H, Bim-5,6,7,8-*H*), 7.32−7.30 (t, H, *J* = 6.0 Hz, 2-ClPh-4-*H*), 7.53 (d, H, *J* = 6.0 Hz, 2-ClPh-5-*H*), 7.63 (d, H, *J* = 6.0 Hz, 2-ClPh-3-*H*), 7.73 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.77 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 24.7, 45.5, 54.7, 111.4, 119.0, 120.0, 122.8, 123.7, 127.9, 128.4, 129.8, 129.9, 130.0, 132.1, 132.4, 134.0, 135.6, 142.9, 144.2, 144.9, 169.7 ppm; TOF-MS (*m/z*): 477 $[M+Na]^+$; HRMS (TOF) calcd. for $C_{23}H_{20}ClN_3O_3SNa$ $[M+Na]^+$, 476.0806; found, 476.0805.

*4.1.8. Synthesis of N-(4-((1-(3-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**5e**)*

Compound **5e** was prepared according to the procedure described for compound **5a**, starting from compound **4** (0.239 g, 0.726 mmol), 1-chloro-3-(chloromethyl)benzene (0.176 g, 1.089 mmol) and potassium carbonate (0.156 g, 1.130 mmol). The pure product **5e** was obtained as yellow solid. Yield: 73.7%; mp: 205−207 ºC ; IR (KBr) ν: 3365 (N−H), 3039 (aromatic C−H), 2997 (CH_2), 1686 (C=N), 1589, 1536 (aromatic frame), 739 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ: 2.11 (s, 3H, COCH_3), 5.13 (s, 2H, Bim-CH_2), 5.64 (s, 2H, 3-ClPh-CH_2), 6.48 (d, H, *J* = 6.0 Hz, 3-ClPh-6-*H*), 7.23−7.17 (m, 4H, Bim-5,6,7,8-*H*), 7.39−7.33 (t, H, *J* = 6.0 Hz, 3-ClPh-5-*H*), 7.45 (d, H, *J* = 6.0 Hz, 3-ClPh-4-*H*), 7.57 (d, H, *J* = 6.0 Hz, 3-ClPh-2-*H*), 7.74 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.77 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H,

N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 34.6, 49.9, 54.7, 111.4, 119.0, 120.0, 122.8, 123.3, 123.4, 128.4, 129.7, 129.9, 130.0, 132.2, 132.5, 133.9, 135.6, 142.9, 144.4, 144.9, 169.7 ppm; TOF-MS (*m/z*): 477 $[M+Na]^+$; HRMS (TOF) calcd. for $C_{23}H_{20}ClN_3O_3SNa$ $[M+Na]^+$, 476.0806; found, 476.0803.

*4.1.9. Synthesis of N-(4-((1-(4-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**5f**)*

Compound **5f** was prepared according to the procedure described for compound **5a**, starting from compound **4** (0.287 g, 0.872 mmol), 1-chloro-4-(chloromethyl)benzene (0.254 g, 1.570 mmol) and potassium carbonate (0.182 g, 1.319 mmol). The pure product **5f** was obtained as yellow solid. Yield: 68.1%; mp: 208−210 ºC ; IR (KBr) ν: 3366 (N−H), 3038 (aromatic C−H), 2996 (CH_2), 1687 (C=N), 1588, 1537 (aromatic frame), 738 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.11 (s, 3H, COC*H*$_3$), 5.15 (s, 2H, Bim-C*H*$_2$), 5.59 (s, 2H, 4-ClPh-C*H*$_2$), 7.14 (d, 2H, *J* = 6.0 Hz, 4-ClPh-2,6-*H*), 7.21−7.17 (m, 2H, Bim-6,7-*H*), 7.29−7.27 (m, H, Bim-8-*H*), 7.36 (d, 2H, *J* = 6.0 Hz, 4-ClPh-3,5-*H*), 7.60−7.59 (m, H, Bim-5-*H*), 7.75 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.77 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 24.7, 47.0, 54.6, 111.7, 119.0, 119.8, 122.7, 123.5, 129.0, 129.3, 130.0, 132.5, 132.6, 135.5, 135.9, 142.8, 143.9, 144.8, 169.7 ppm; TOF-MS (*m/z*): 477 $[M+Na]^+$; HRMS (TOF) calcd. for $C_{23}H_{20}ClN_3O_3SNa$ $[M+Na]^+$, 476.0806; found, 476.0809.

*4.1.10. Synthesis of N-(4-((1-(2,4-dichlorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl) acetamide (**5g**)*

Compound **5g** was prepared according to the procedure described for compound **5a**, starting from compound **4** (0.247 g, 0.751 mmol), 2,4-dichloro-1-(chloromethyl)benzene (0.185 g, 0.931 mmol) and potassium carbonate (0.207 g, 1.500 mmol). The pure product **5g** was obtained as white solid. Yield: 59.4%; mp: 166−168 ºC ; IR (KBr) ν: 3363 (N−H), 3039 (aromatic C−H), 2993 (CH_2), 1689 (C=N), 1585, 1531 (aromatic frame), 740 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.12 (s, 3H, COC*H*$_3$), 5.15 (s, 2H, Bim-C*H*$_2$), 5.61 (s, 2H, 2,4-Cl$_2$Ph-C*H*$_2$), 6.49 (d, H, *J* = 6.0 Hz, 2,4-Cl$_2$Ph-6-*H*), 7.28−7.21 (m, 4H, Bim-5,6,7,8-*H*), 7.64 (d, H, *J* = 6.0 Hz, 2,4-Cl$_2$Ph-5-*H*), 7.69 (s, H, 2,4-Cl$_2$Ph-3-*H*), 7.73 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.77 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 24.7, 45.2, 54.7, 111.4, 119.0, 120.1, 122.9, 123.8, 128.1, 129.4, 129.6, 129.9, 132.4, 133.1, 133.3, 133.4, 135.5, 142.9, 144.3, 144.9, 169.7 ppm; ESI-MS (*m/z*): 511 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{23}H_{19}Cl_2N_3O_3SNa$ $[M+Na]^+$, 510.0416; found, 510.0425.

*4.1.11. Synthesis of N-(4-((1-(3,4-dichlorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl) acetamide (**5h**)*

Compound **5h** was prepared according to the procedure described for compound **5a**, starting from compound **4** (0.180 g, 0.547 mmol), 3,4-dichloro-1-(chloromethyl)benzene (0.157 g, 0.803 mmol) and potassium carbonate (0.114 g, 0.826 mmol). The pure product **5h** was obtained as yellow solid. Yield: 61.0%; mp: 167−169 ºC ; IR (KBr) ν: 3365 (N−H), 3038 (aromatic C−H), 2995 (CH_2), 1687 (C=N), 1585, 1535 (aromatic frame), 741 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.11 (s, 3H, COCH_3), 5.19 (s, 2H, Bim-CH_2), 5.61 (s, 2H, 3,4-Cl_2Ph-CH_2), 7.04 (d, H, *J* = 6.0 Hz, 3,4-Cl_2Ph-6-*H*), 7.29−7.19 (m, 4H, Bim-5,6,7,8-*H*), 7.44 (s, H, 3,4-Cl_2Ph-2-*H*), 7.57 (d, H, *J* = 6.0 Hz, 3,4-Cl_2Ph-5-*H*), 7.75 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.77 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 24.7, 46.6, 54.5, 111.6, 118.9, 119.9, 122.8, 123.6, 127.7, 129.5, 129.9, 130.6, 131.2, 131.7, 132.6, 135.4, 138.2, 142.9, 144.0, 144.8, 169.6 ppm; ESI-MS (*m/z*): 511 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{23}H_{19}Cl_2N_3O_3SNa$ $[M+Na]^+$, 510.0416; found, 510.0424.

*4.1.12. Synthesis of N-(4-((1-ethyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**6a**)*

Pure compound **6a** was obtained in process of synthesizing compound **5a** as yellow oil. Yield: 52.8%; mp: >250 ºC; IR (KBr) ν: 3360 (N−H), 3041 (aromatic C−H), 2990 (CH_2), 1691 (C=N), 1587, 1500 (aromatic frame), 738 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.34−1.32 (t, 3H, *J* = 6.0 Hz, CH_2CH_3), 2.10 (s, 3H, COCH_3), 4.35−4.31 (m, 2H, CH_2CH_3), 5.10 (s, 2H, Bim-CH_2), 7.21−7.19 (t, H, *J* = 6.0 Hz, Bim-6-*H*), 7.29−7.26 (t, H, *J* = 9.0 Hz, Bim-7-*H*), 7.59−7.55 (m, 2H, Bim-5,8-*H*), 7.72 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.77 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 15.2, 24.7, 39.2, 54.6, 111.2, 118.9, 119.7, 122.4, 123.2, 129.9, 132.6, 135.2, 142.8, 143.2, 144.8, 169.6 ppm; ESI-MS (*m/z*): 358 $[M+H]^+$; HRMS (ESI) calcd. for $C_{18}H_{20}N_3O_3S$ $[M+H]^+$, 358.1220; found, 358.1223.

*4.1.13. Synthesis of N-(4-((1-propyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**6b**)*

Pure compound **6b** was obtained in process of synthesizing compound **5a** as yellow solid. Yield: 51.2%; mp: 116−118 ºC; IR (KBr) ν: 3362 (N−H), 3037 (aromatic C−H), 2991 (CH_2), 1687 (C=N), 1589, 1508 (aromatic frame), 739 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.88−0.86 (t, 3H, *J* = 6.0 Hz, $CH_2CH_2CH_3$), 1.77−1.71 (m, 2H, $CH_2CH_2CH_3$), 2.10 (s, 3H, COCH_3), 4.24−4.21 (t, 2H, *J* = 9.0 Hz, $CH_2CH_2CH_3$), 5.13 (s, 2H, Bim-CH_2), 7.24−7.22 (t, H, *J* = 6.0 Hz, Bim-6-*H*), 7.30−7.28 (t, H, *J* = 6.0 Hz, Bim-7-*H*), 7.58 (d, H, *J*

= 6.0 Hz, Bim-8-*H*), 7.63 (d, H, *J* = 6.0 Hz, Bim-5-*H*), 7.71 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.77 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.44 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 15.2, 22.1, 24.6, 39.2, 54.5, 111.2, 119.0, 119.7, 122.4, 123.2, 129.9, 132.6, 135.2, 142.8, 143.2, 144.7, 169.6 ppm; ESI-MS (*m/z*): 372 [M+H]$^+$; HRMS (ESI) calcd. for $C_{19}H_{22}N_3O_3S$ [M+H]$^+$, 372.1376; found, 372.1385.

*4.1.14. Synthesis of N-(4-((1-pentyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**6c**)*

Pure compound **6c** was obtained in process of synthesizing compound **5a** as yellow solid. Yield: 28.1%; mp: 94−96 ºC; IR (KBr) ν: 3359 (N−H), 3030 (aromatic C−H), 2994 (CH$_2$), 1680 (C=N), 1589, 1491 (aromatic frame), 741 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.84 (t, 3H, *J* = 6.0 Hz, (CH$_2$)$_4$C*H*$_3$), 1.31−1.26 (m, 4H, (CH$_2$)$_2$(C*H*$_2$)$_2$CH$_3$), 1.72−1.67 (m, 2H, CH$_2$C*H*$_2$(CH$_2$)$_2$CH$_3$), 2.10 (s, 3H, COC*H*$_3$), 4.24−4.21 (t, 2H, *J* = 9.0 Hz, C*H*$_2$(CH$_2$)$_3$CH$_3$), 5.08 (s, 2H, Bim-C*H*$_2$), 7.20−7.18 (t, H, *J* = 6.0 Hz, Bim-6-*H*), 7.28−7.25 (t, H, *J* = 9.0 Hz, Bim-7-*H*), 7.57−7.55 (t, 2H, *J* = 6.0 Hz, Bim-5,8-*H*), 7.71 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.76 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.40 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.3, 22.3, 24.6, 29.2, 29.4, 44.2, 54.7, 111.3, 118.9, 119.7, 122.4, 123.2, 129.9, 132.6, 135.5, 142.6, 143.5, 144.8, 169.7 ppm; ESI-MS (*m/z*): 401 [M+H]$^+$; HRMS (ESI) calcd. for $C_{21}H_{26}N_3O_3S$ [M+H]$^+$, 400.1685; found, 400.1693.

*4.1.15. Synthesis of N-(4-((1-hexyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**6d**)*

Pure compound **6d** was obtained in process of synthesizing compound **5a** as yellow oil. Yield: 47.5%; mp: 108−110 ºC; IR (KBr) ν: 3352 (N−H), 3037 (aromatic C−H), 2999 (CH$_2$), 1687 (C=N), 1589, 1493 (aromatic frame), 738 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.84 (t, 3H, *J* = 6.0 Hz, (CH$_2$)$_5$C*H*$_3$), 1.30−1.25 (m, 6H, (CH$_2$)$_2$(C*H*$_2$)$_3$CH$_3$), 1.70−1.65 (m, 2H, CH$_2$C*H*$_2$(CH$_2$)$_3$CH$_3$), 2.10 (s, 3H, COC*H*$_3$), 4.24−4.21 (t, 2H, *J* = 9.0 Hz, C*H*$_2$(CH$_2$)$_4$CH$_3$), 5.08 (s, 2H, Bim-C*H*$_2$), 7.21−7.18 (t, H, *J* = 9.0 Hz, Bim-6-*H*), 7.28−7.25 (t, H, *J* = 9.0 Hz, Bim-7-*H*), 7.57−7.55 (t, 2H, *J* = 6.0 Hz, Bim-5,8-*H*), 7.71 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.76 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.3, 22.5, 24.6, 26.3, 29.6, 31.3, 44.2, 54.6, 111.3, 118.9, 119.6, 122.4, 123.2, 129.9, 132.6, 135.5, 142.6, 143.4, 144.8, 169.6 ppm; ESI-MS (*m/z*): 415 [M+H]$^+$; HRMS (ESI) calcd. for $C_{22}H_{28}N_3O_3S$ [M+H]$^+$, 414.1846; found, 414.1855.

*4.1.16. Synthesis of N-(4-((1-heptyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**6e**)*

Compound **6e** was obtained as yellow solid. Yield: 24.2%; mp: 102−103 ºC; IR (KBr) ν: 3354 (N−H), 3032 (aromatic C−H), 2994 (CH$_2$), 1685 (C=N), 1592, 1498 (aromatic frame), 738 cm^{-1}; ^{1}H NMR (600

MHz, DMSO-d_6) δ: 0.86−0.83 (t, 3H, *J* = 9.0 Hz, $(CH_2)_6CH_3$), 1.27−1.23 (m, 8H, $(CH_2)_2(CH_2)_4CH_3$), 1.70−1.66 (m, 2H, $CH_2CH_2(CH_2)_4CH_3$), 2.10 (s, 3H, $COCH_3$), 4.23−4.21 (t, 2H, *J* = 6.0 Hz, $CH_2(CH_2)_5CH_3$), 5.08 (s, 2H, Bim-CH_2), 7.21−7.18 (t, H, *J* = 9.0 Hz, Bim-6-*H*), 7.27−7.25 (t, H, *J* = 6.0 Hz, Bim-7-*H*), 7.57−7.55 (t, 2H, *J* = 6.0 Hz, Bim-5,8-*H*), 7.70 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.76 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.40 (s, H, $NHCOCH_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.4, 22.5, 24.6, 26.6, 28.8, 29.7, 31.6, 44.1, 54.6, 111.3, 118.9, 119.7, 122.4, 123.2, 129.9, 132.6, 135.5, 142.7, 143.4, 144.8, 169.6 ppm; ESI-MS (*m/z*): 429 $[M+H]^+$; HRMS (ESI) calcd. for $C_{23}H_{30}N_3O_3S$ $[M+H]^+$, 428.2002; found, 428.2010.

*4.1.17. Synthesis of N-(4-((1-octyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**6f**)*

Pure compound **6f** was obtained in process of synthesizing compound **5a** as yellow solid. Yield: 71.2%; mp: 97–99 ºC; IR (KBr) ν: 3357 (N−H), 3038 (aromatic C−H), 2996 (CH_2), 1685 (C=N), 1590, 1498 (aromatic frame), 739 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.83 (t, 3H, *J* = 9.0 Hz, $(CH_2)_7CH_3$), 1.27−1.23 (m, 10H, $(CH_2)_2(CH_2)_5CH_3$), 1.69−1.66 (m, 2H, $CH_2CH_2(CH_2)_5CH_3$), 2.10 (s, 3H, $COCH_3$), 4.23−4.21 (t, 2H, *J* = 6.0 Hz, $CH_2(CH_2)_6CH_3$), 5.08 (s, 2H, Bim-CH_2), 7.21−7.18 (t, H, *J* = 9.0 Hz, Bim-6-*H*), 7.28−7.25 (t, H, *J* = 9.0 Hz, Bim-7-*H*), 7.57−7.55 (t, 2H, *J* = 6.0 Hz, Bim-5,8-*H*), 7.70 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.76 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, $NHCOCH_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.4, 22.5, 24.6, 26.6, 29.0, 29.1, 29.7, 31.7, 44.1, 54.6, 111.3, 118.9, 119.7, 122.4, 123.2, 129.9, 132.6, 135.5, 142.7, 143.4, 144.8, 169.6 ppm; ESI-MS (*m/z*): 443 $[M+H]^+$; HRMS (ESI) calcd. for $C_{24}H_{32}N_3O_3S$ $[M+H]^+$, 442.2159; found, 442.2168.

*4.1.18. Synthesis of N-(4-((1-decyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**6g**)*

Compound **6g** was synthesized according to the experimental procedure reported for compound **5a**, starting from compound **4** (0.407 g, 1.237 mmol), 1-bromononane (0.410 g, 1.856 mmol) and potassium carbonate (0.256 g, 1.856 mmol). The crude product **6g** was obtained as yellow solid. Yield: 47.7%; mp: 78–80 ºC; IR (KBr) ν: 3352 (N−H), 3034 (aromatic C−H), 2996 (CH_2), 1687 (C=N), 1590, 1505 (aromatic frame), 741 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.84 (t, 3H, *J* = 6.0 Hz, $(CH_2)_9CH_3$), 1.26−1.22 (m, 14H, $(CH_2)_2(CH_2)_7CH_3$), 1.70−1.66 (m, 2H, $CH_2CH_2(CH_2)_7CH_3$), 2.10 (s, 3H, $COCH_3$), 4.23−4.21 (t, 2H, *J* = 6.0 Hz, $CH_2(CH_2)_8CH_3$), 5.08 (s, 2H, Bim-CH_2), 7.21−7.18 (t, H, *J* = 9.0 Hz, Bim-6-*H*), 7.27−7.25 (t, H, *J* = 6.0 Hz, Bim-7-*H*), 7.57−7.55 (t, 2H, *J* = 6.0 Hz, Bim-5,8-*H*), 7.70 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.76 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, $NHCOCH_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.4, 22.5, 24.6, 26.6, 29.1, 29.4, 29.4, 29.6, 31.7, 44.1, 54.7, 111.3, 118.9, 119.7, 122.4, 123.2, 129.9, 132.7,

135.6, 142.7, 143.4, 144.8, 169.6 ppm; ESI-MS (*m/z*): 471 $[M+H]^+$; HRMS (ESI) calcd. for $C_{26}H_{36}N_3O_3S$ $[M+H]^+$, 470.2472; found, 470.2476.

*4.1.19. Synthesis of N-(4-((1-dodecyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**6h**)*

Prepared according to the general procedure described for compound **5a**, starting from compound **4** (0.363 g, 1.103 mmol), 1-bromododecane (0.412 g, 1.655 mmol) and potassium carbonate (0.228 g, 1.655 mmol), the pure compound **6h** (0.243 g) was obtained as yellow solid. Yield: 44.3%; mp: 135–137 ºC; IR (KBr) ν: 3357 (N−H), 3038 (aromatic C−H), 2991 (CH_2), 1687 (C=N), 1590, 1508 (aromatic frame), 736 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.83 (t, 3H, *J* = 9.0 Hz, $(CH_2)_{11}CH_3$), 1.26−1.22 (m, 18H, $(CH_2)_2(CH_2)_9CH_3$), 1.69−1.67 (m, 2H, $CH_2CH_2(CH_2)_9CH_3$), 2.10 (s, 3H, $COCH_3$), 4.24−4.21 (t, 2H, *J* = 9.0 Hz, $CH_2(CH_2)_{10}CH_3$), 5.08 (s, 2H, Bim-CH_2), 7.21−7.18 (t, H, *J* = 9.0 Hz, Bim-6-*H*), 7.27−7.25 (t, H, *J* = 6.0 Hz, Bim-7-*H*), 7.56 (d, 2H, *J* = 6.0 Hz, Bim-5,8-*H*), 7.71 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.76 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, N*H*$COCH_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.4, 22.5, 24.6, 26.6, 29.1, 29.2, 29.4, 29.4, 29.5, 29.5, 29.7, 31.8, 44.2, 54.7, 111.3, 118.9, 119.7, 122.4, 123.2, 129.9, 132.7, 135.6, 142.7, 143.4, 144.8, 169.6 ppm; ESI-MS (*m/z*): 499 $[M+H]^+$; HRMS (ESI) calcd. for $C_{28}H_{40}N_3O_3S$ $[M+H]^+$, 498.2785; found, 498.2793.

*4.1.20. Synthesis of N-(4-((1-octadecyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)phenyl)acetamide (**6i**)*

Compound **6i** was synthesized according to the experimental procedure reported for compound **5a**, starting from compound **4** (0.350 g, 1.064 mmol), 1-bromooctadecane (0.495 g, 1.486 mmol) and potassium carbonate (0.220 g, 1.596 mmol). The crude product was obtained and purified *via* silica gel column chromatography (eluent, acetone/petroleum ether, 1/1, V/V) to give pure compound **6i** (0.328 g) as yellow solid. Yield: 53.0%; mp: 143–145 ºC; IR (KBr) ν: 3356 (N−H), 3034 (aromatic C−H), 2997 (CH_2), 1689 (C=N), 1593, 1501 (aromatic frame), 738 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.85−0.83 (t, 3H, *J* = 6.0 Hz, $(CH_2)_{17}CH_3$), 1.26−1.22 (m, 30H, $(CH_2)_2(CH_2)_{15}CH_3$), 1.69−1.66 (m, 2H, $CH_2CH_2(CH_2)_{15}CH_3$), 2.10 (s, 3H, $COCH_3$), 4.23−4.21 (t, 2H, *J* = 6.0 Hz, $CH_2(CH_2)_{16}CH_3$), 5.07 (s, 2H, Bim-CH_2), 7.20−7.17 (t, H, *J* = 9.0 Hz, Bim-6-*H*), 7.26−7.24 (t, H, *J* = 6.0 Hz, Bim-7-*H*), 7.56 (d, 2H, *J* = 6.0 Hz, Bim-5,8-*H*), 7.70 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.76 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 10.41 (s, H, N*H*$COCH_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.3, 22.5, 24.6, 26.6, 29.1, 29.2, 29.4, 29.4, 29.5, 29.5, 29.6, 31.8, 44.1, 54.7, 111.2, 118.9, 119.7, 122.3, 123.1, 129.9, 132.7, 135.6, 142.8, 143.4, 144.8, 169.6 ppm; ESI-MS (*m/z*): 583 $[M+H]^+$; HRMS (ESI) calcd. for $C_{34}H_{52}N_3O_3S$ $[M+H]^+$, 582.3724; found, 582.3727.

*4.1.21. Synthesis of N-(4-((1-(5-(9H-carbazol-9-yl)pentyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl) phenyl)acetamide (**7**)*

Prepared the same way as the general procedure described for compound **5a**, starting from compound **4** (0.176 g, 0.535 mmol), 9-(5-bromopentyl)-9H-carbazole (0.342 g, 1.082 mmol) and potassium carbonate (0.152 g, 1.101 mmol), pure compound **7** (0.216 g) was synthesized as white solid. Yield: 72.0%; mp: 118–120 ºC; IR (KBr) ν: 3369 (N−H), 3042 (aromatic C−H), 2984 (CH_2), 1694 (C=N), 1591, 1525 (aromatic frame), 741 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.46−1.32 (m, 2H, carbazole-$(CH_2)_2CH_2$), 1.75−1.71 (m, 2H, carbazole-CH_2CH_2), 1.82−1.78 (m, 2H, carbazole-$(CH_2)_3CH_2$), 2.10 (s, 3H, $COCH_3$), 4.20−4.18 (t, H, *J* = 6.0 Hz, carbazole-CH_2), 4.39−4.36 (t, H, *J* = 9.0 Hz, carbazole-$(CH_2)_4CH_2$), 5.00 (s, 2H, Bim-CH_2), 7.24−7.18 (m, 4H, Bim-5,6,7,8-*H*), 7.48−7.42 (m, 3H, carbazole-4,5,10-*H*), 7.57−7.53 (m, 3H, carbazole-3,11,12-*H*), 7.67 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.76 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 8.15−8.13 (d, 2H, carbazole-6,9-*H*), 10.39 (s, H, N*H*$COCH_3$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 24.5, 24.7, 28.7, 29.5, 42.7, 44.1, 54.6, 109.7, 111.3, 119.0, 119.1, 119.7, 120.7, 122.3, 122.6, 123.2, 126.1, 129.9, 132.6, 135.6, 140.5, 142.7, 143.4, 144.8, 169.6 ppm; ESI-MS (*m/z*): 566 $[M+H]^+$; HRMS (ESI) calcd. for $C_{33}H_{33}N_4O_3S$ $[M+H]^+$, 565.2268; found, 565.2271.

*4.1.22. Synthesis of 4-((1-(2-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**8a**)*

To a solution of compound **5a** (0.300 g, 0.685 mmol) in ethanol 15 mL was added 0.4 mL 2 mol/L sodium hydroxide solution. The mixture was refluxed for 10 h (monitored by TLC, eluent, acetone/petroleum ether, 1/1, V/V). After cooling to the room temperature, the solvent was removed in vacuo to give the deprotected compound **8a** as yellow solid. Yield: 97.4%; mp: 205–207 ºC; IR (KBr) ν: 3318, 3207 (N−H), 3058 (aromatic C−H), 2988 (CH_2), 1647 (C=N), 1587, 1506 (aromatic frame), 739 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.92 (s, 2H, Bim-CH_2), 5.57 (s, 2H, 2-FPh-CH_2), 6.21 (s, 2H, NH_2), 6.60 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 6.91−6.89 (t, H, *J* = 6.0 Hz, 2-FPh-5-*H*), 7.10−7.08 (t, H, *J* = 6.0 Hz, 2-FPh-4-*H*), 7.24−7.19 (m, 4H, Bim-5,6,7,8-*H*), 7.34−7.31 (m, H, 2-FPh-6-*H*), 7.37 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.62−7.61 (m, H, 2-FPh-3-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 49.1, 55.2, 111.3, 113.1, 116.0, 119.9, 122.6, 123.4, 123.6, 123.8, 125.1, 129.5, 130.3, 130.5, 135.6, 142.9, 144.5, 154.6, 160.4 ppm; ESI-MS (*m/z*): 418 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{21}H_{18}FN_3O_2SNa$ $[M+Na]^+$, 418.0996; found, 418.1005.

*4.1.23. Synthesis of 4-((1-(3-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**8b**)*

Compound **8b** was prepared according to the experimental procedure described for compound **8a**,

starting from compound **5b** (0.095 g, 0.217 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution. The pure product **8b** was obtained as yellow solid. Yield: 95.3%; mp: 212–214 ºC; IR (KBr) ν: 3314, 3209 (N−H), 3061 (aromatic C−H), 2993 (CH_2), 1649 (C=N), 1588, 1501 (aromatic frame), 738 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.95 (s, 2H, Bim-CH_2), 5.57 (s, 2H, 3-FPh-CH_2), 6.21 (s, 2H, NH_2), 6.61 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 6.97−6.92 (m, 2H, 3-FPh-2,6-*H*), 7.11−7.08 (m, H, 3-FPh-4-*H*), 7.20−7.18 (m, 2H, Bim-6,7-*H*), 7.31−7.30 (m, H, Bim-8-*H*), 7.37−7.33 (m, H, 3-FPh-5-*H*), 7.39 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.63−7.61 (m, H, Bim-5-*H*), 10.41 (s, H, N*H*COCH$_3$) ppm; ^{13}C NMR (600 MHz, DMSO-d_6) δ: 47.1, 55.2, 111.5, 113.1, 114.3, 114.9, 119.8, 122.6, 123.4, 123.8, 130.5, 131.1, 135.6, 139.9, 139.9, 142.9, 144.5, 154.6, 162.8 ppm; ESI-MS (*m/z*): 418 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{21}H_{18}FN_3O_2SNa$ $[M+Na]^+$, 418.0996; found, 418.1004.

*4.1.24. Synthesis of 4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**8c**)*

Compound **8c** was prepared according to the procedure depicted for compound **8a**, starting from compound **5c** (0.140 g, 0.320 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution. The pure product **5h** was obtained as yellow solid. Yield: 95.2%; mp: 224–226 ºC; IR (KBr) ν: 3307, 3200 (N−H), 3069 (aromatic C−H), 2997 (CH_2), 1654 (C=N), 1594, 1507 (aromatic frame), 740 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.93 (s, 2H, Bim-CH_2), 5.52 (s, 2H, 4-FPh-CH_2), 6.21 (s, 2H, NH_2), 6.60 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.19−7.12 (m, 6H, 4-FPh-2,3,5,6-*H*, Bim-6,7-*H*), 7.31−7.30 (m, H, Bim-8-*H*), 7.39 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.61−7.60 (m, H, Bim-5-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 46.9, 55.3, 111.6, 113.1, 115.9, 119.8, 122.6, 123.3, 123.8, 129.5, 130.6, 133.2, 135.6, 143.0, 144.4, 154.6, 162.0 ppm; ESI-MS (*m/z*): 418 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{21}H_{18}FN_3O_2SNa$ $[M+Na]^+$, 418.0996; found, 418.1000.

*4.1.25. Synthesis of 4-((1-(2-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**8d**)*

Compound **8d** was prepared according to the experimental procedure reported for compound **8a**, starting from compound **5d** (0.148 g, 0.325 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution. The pure product **5i** was obtained as yellow solid. Yield: 97.5%; mp: 184–186 ºC; IR (KBr) ν: 3311, 3207 (N−H), 3062 (aromatic C−H), 2989 (CH_2), 1659 (C=N), 1588, 1501 (aromatic frame), 741 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.88 (s, 2H, Bim-CH_2), 5.56 (s, 2H, 2-ClPh-CH_2), 6.22 (s, 2H, NH_2), 6.50 (d, H, *J* = 6.0 Hz, 2-ClPh-6-*H*), 6.60 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.24−7.17 (m, 4H, Bim-5,6,7,8-*H*), 7.32−7.29 (t, H, *J* = 6.0 Hz, 2-ClPh-4-*H*), 7.35 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.53 (d, H, *J* = 6.0 Hz, 2-ClPh-5-*H*), 7.65 (d, H, *J* = 6.0 Hz, 2-ClPh-3-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 45.3, 56.3, 111.3, 113.1, 120.0,

122.7, 123.5, 123.6, 128.0, 128.4, 129.8, 130.0, 130.5, 132.1, 134.1, 135.7, 142.9, 144.7, 154.6 ppm; ESI-MS (*m/z*): 435 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{21}H_{18}ClN_3O_2SNa$ $[M+Na]^+$, 434.0700; found, 434.0712.

*4.1.26. Synthesis of 4-((1-(3-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**8e**)*

Pure compound **8e** was obtained in process of synthesizing compound **8a** as yellow solid. Yield: 93.7%; mp: 237–239 ºC; IR (KBr) ν: 3314, 3202 (N−H), 3067 (aromatic C−H), 2993 (CH_2), 1657 (C=N), 1585, 1503 (aromatic frame), 740 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ: 4.92 (s, 2H, Bim-CH_2), 5.59 (s, 2H, 3-ClPh-CH_2), 6.21 (s, 2H, NH_2), 6.61 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.07 (d, H, *J* = 6.0 Hz, 3-ClPh-6-*H*), 7.24−7.18 (m, 4H, Bim-5,6,7,8-*H*), 7.29−7.26 (t, H, *J* = 9.0 Hz, 3-ClPh-5-*H*), 7.35 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.51 (d, H, *J* = 6.0 Hz, 3-ClPh-4-*H*), 7.52 (d, H, *J* = 6.0 Hz, 3-ClPh-2-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 50.2, 56.3, 111.2, 113.3, 119.5, 122.8, 123.7, 123.8, 127.8, 128.3, 129.7, 130.1, 130.4, 132.0, 134.2, 135.8, 142.9, 144.8, 154.7 ppm; ESI-MS (*m/z*): 435 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{21}H_{18}ClN_3O_2SNa$ $[M+Na]^+$, 434.0700; found, 434.0712.

*4.1.27. Synthesis of 4-((1-(4-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**8f**)*

Compound **8f** was prepared according to the experimental procedure described for compound **8a**, starting from compound **5f** (0.150 g, 0.329 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution. The pure product **8f** was obtained as yellow solid. Yield: 90.4%; mp: 223–225 ºC; IR (KBr) ν: 3319, 3206 (N−H), 3064 (aromatic C−H), 2989 (CH_2), 1654 (C=N), 1587, 1505 (aromatic frame), 739 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ: 4.93 (s, 2H, Bim-CH_2), 5.54 (s, 2H, 4-ClPh-CH_2), 6.21 (s, 2H, NH_2), 6.61 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.13 (d, 2H, *J* = 6.0 Hz, 4-ClPh-2,6-*H*), 7.20−7.16 (m, 2H, Bim-6,7-*H*), 7.30−7.28 (m, H, Bim-8-*H*), 7.36 (d, 2H, *J* = 6.0 Hz, 4-ClPh-3,5-*H*), 7.38 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.62−7.61 (m, H, Bim-5-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 47.0, 55.2, 111.6, 113.1, 119.8, 122.6, 123.4, 123.7, 129.0, 129.3, 130.6, 132.7, 135.6, 136.0, 143.0, 144.5, 154.6 ppm; ESI-MS (*m/z*): 413 $[M+H]^+$; HRMS (ESI) calcd. for $C_{21}H_{19}ClN_3O_2S$ $[M+H]^+$, 412.0881; found, 412.0885.

*4.1.28. Synthesis of 4-((1-(2,4-dichlorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**8g**)*

Compound **8g** was prepared according to the experimental procedure depicted for compound **8a**, starting from compound **5g** (0.198 g, 0.405 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution. The pure product **8g** was obtained as yellow solid. Yield: 93.4%; mp: 216–218 ºC; IR (KBr) ν: 3329, 3201 (N−H), 3066 (aromatic C−H), 2993 (CH_2), 1639 (C=N), 1593, 1500 (aromatic frame), 736 cm^{-1}; ^1H NMR (600

MHz, DMSO-d_6) δ: 4.91 (s, 2H, Bim-CH_2), 5.55 (s, 2H, 2,4-Cl_2Ph-CH_2), 6.21 (s, 2H, NH_2), 6.47 (d, H, *J* = 6.0 Hz, 2,4-Cl_2Ph-6-*H*), 6.60 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.29−7.18 (m, 4H, Bim-5,6,7,8-*H*), 7.34 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.65 (d, H, *J* = 6.0 Hz, 2,4-Cl_2Ph-5-*H*), 7.70 (s, H, 2,4-Cl_2Ph-3-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 45.1, 55.2, 111.3, 113.1, 120.0, 122.8, 123.5, 123.6, 128.1, 129.4, 129.6, 130.5, 133.0, 133.3, 133.4, 135.5, 142.9, 144.8, 154.6 ppm; ESI-MS (*m/z*): 447 $[M+H]^+$; HRMS (ESI) calcd. for $C_{21}H_{18}Cl_2N_3O_2S$ $[M+H]^+$, 446.0491; found, 446.0500.

*4.1.29. Synthesis of 4-((1-(3,4-dichlorobenzyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**8h**)*

Compound **8h** was prepared according to the experimental procedure reported for compound **8a**, starting from compound **5h** (0.140 g, 0.287 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution. The pure product **8h** was obtained as yellow solid. Yield: 94.7%; mp: 235–237 ºC; IR (KBr) ν: 3324, 3205 (N−H), 3061 (aromatic C−H), 2988 (CH_2), 1642 (C=N), 1594, 1503 (aromatic frame), 740 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.03 (s, 2H, Bim-CH_2), 5.62 (s, 2H, 3,4-Cl_2Ph-CH_2), 6.25 (s, 2H, NH_2), 6.66 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.09 (d, H, *J* = 6.0 Hz, 3,4-Cl_2Ph-6-*H*), 7.26−7.23 (m, 2H, Bim-6,7-*H*), 7.34−7.33 (m, H, Bim-8-*H*), 7.44 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.49 (s, H, 3,4-Cl_2Ph-2-*H*), 7.62 (d, H, *J* = 6.0 Hz, 3,4-Cl_2Ph-5-*H*), 7.68−7.66 (m, H, Bim-5-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 46.6, 56.1, 111.5, 113.1, 119.9, 122.7, 123.5, 123.8, 127.7, 129.5, 130.5, 130.6, 131.2, 131.7, 135.5, 138.2, 143.0, 144.6, 154.5 ppm; ESI-MS (*m/z*): 469 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{21}H_{17}Cl_2N_3O_2SNa$ $[M+Na]^+$, 468.0311; found, 468.0314.

*4.1.30. Synthesis of 4-((1-ethyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**9a**)*

Prepared according to the general procedure described for **8a**, starting from compound **6a** (0.135 g, 0.378 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution, pure compound **9a** was obtained as yellow solid. Yield: 90.8%; mp: 232–234 ºC; IR (KBr) ν: 3332, 3208 (N−H), 3061 (aromatic C−H), 2988 (CH_2), 1675 (C=N), 1589, 1493 (aromatic frame), 740 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 1.32−1.30 (t, 3H, *J* = 6.0 Hz, CH_2CH_3), 4.30−4.27 (m, 2H, CH_2CH_3), 4.90 (s, 2H, Bim-CH_2), 6.19 (s, 2H, NH_2), 6.60 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.21−7.19 (t, H, *J* = 6.0 Hz, Bim-6-*H*), 7.27−7.25 (t, H, *J* = 6.0 Hz, Bim-7-*H*), 7.35 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.58−7.56 (t, 2H, *J* = 6.0 Hz, Bim-5,8-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 15.2, 39.1, 55.2, 111.1, 113.1, 119.7, 122.3, 123.1, 123.9, 130.5, 135.2, 143.0, 143.8, 154.5 ppm; ESI-MS (*m/z*): 316 $[M+H]^+$; HRMS (ESI) calcd. for $C_{16}H_{18}N_3O_2S$ $[M+H]^+$, 316.1114; found, 316.1121.

*4.1.31. Synthesis of 4-((1-propyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**9b**)*

Prepared according to the general procedure described for **8a**, starting from compound **6b** (0.122 g, 0.329 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution, pure compound **9b** was obtained as yellow solid. Yield: 97.2%; mp: 237–239 ºC; IR (KBr) ν: 3327, 3205 (N−H), 3051 (aromatic C−H), 2993 (CH_2), 1684 (C=N), 1595, 1487 (aromatic frame), 737 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.87−0.84 (t, 3H, *J* = 9.0 Hz, $CH_2CH_2CH_3$), 1.75−1.69 (m, 2H, $CH_2CH_2CH_3$), 4.17−4.15 (t, 2H, *J* = 6.0 Hz, $CH_2CH_2CH_3$), 4.89 (s, 2H, Bim-CH_2), 6.19 (s, 2H, NH_2), 6.58 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.20−7.18 (t, H, *J* = 6.0 Hz, Bim-6-*H*), 7.26−7.24 (t, H, *J* = 6.0 Hz, Bim-7-*H*), 7.33 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.58 (d, 2H, *J* = 6.0 Hz, Bim-5,8-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 11.4, 22.9, 45.5, 55.3, 111.3, 113.1, 119.7, 122.2, 123.0, 123.9, 130.5, 135.6, 142.8, 144.1, 154.5 ppm; ESI-MS (*m/z*): 330 $[M+H]^+$; HRMS (ESI) calcd. for $C_{17}H_{20}N_3O_2S$ $[M+H]^+$, 330.1271; found, 330.1277.

*4.1.32. Synthesis of 4-((1-pentyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**9c**)*

Compound **9c** was prepared according to the procedure depicted for compound **8a**, starting from compound **6c** (0.042 g, 0.105 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution. The product **9c** (0.033 g) was obtained as yellow solid. Yield: 89.1%; mp: 233–235 ºC; IR (KBr) ν: 3316, 3200 (N−H), 3046 (aromatic C−H), 2987 (CH_2), 1680 (C=N), 1587, 1494 (aromatic frame), 741 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.84 (t, 3H, *J* = 6.0 Hz, $(CH_2)_4CH_3$), 1.31−1.24 (m, 4H, $(CH_2)_2(CH_2)_2CH_3$), 1.72−1.67 (m, 2H, $CH_2CH_2(CH_2)_2CH_3$), 4.19−4.16 (t, 2H, *J* = 9.0 Hz, $CH_2(CH_2)_3CH_3$), 4.88 (s, 2H, Bim-CH_2), 6.18 (s, 2H, NH_2), 6.59 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.20−7.18 (t, H, *J* = 6.0 Hz, Bim-6-*H*), 7.27−7.24 (t, H, *J* = 9.0 Hz, Bim-7-*H*), 7.33 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.58−7.54 (m, 2H, Bim-5,8-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.3, 22.3, 28.8, 29.3, 44.0, 55.3, 111.2, 113.1, 119.7, 122.3, 123.0, 123.8, 130.5, 135.6, 142.8, 144.0, 154.5 ppm; ESI-MS (*m/z*): 358 $[M+H]^+$; HRMS (ESI) calcd. for $C_{19}H_{24}N_3O_2S$ $[M+H]^+$, 358.1584; found, 358.1587.

*4.1.33. Synthesis of 4-((1-hexyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**9d**)*

Compound **9d** was prepared according to the procedure depicted for compound **8a**, starting from compound **6d** (0.134 g, 0.324 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution. The product **9d** (0.108 g) was obtained as yellow solid. Yield: 94.2%; mp: 238–240 ºC; IR (KBr) ν: 3319, 3201 (N−H), 3042 (aromatic C−H), 2986 (CH_2), 1672 (C=N), 1583, 1494 (aromatic frame), 740 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.84 (t, 3H, *J* = 6.0 Hz, $(CH_2)_5CH_3$), 1.26−1.25 (m, 6H, $(CH_2)_2(CH_2)_3CH_3$), 1.70−1.66 (m, 2H, $CH_2CH_2(CH_2)_3CH_3$), 4.19−4.16 (t, 2H, *J* = 9.0 Hz, $CH_2(CH_2)_4CH_3$), 4.88 (s, 2H, Bim-CH_2), 6.20 (s, 2H, NH_2), 6.59 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.20−7.18 (t, H, *J* = 6.0 Hz, Bim-6-*H*),

7.27−7.24 (t, H, *J* = 9.0 Hz, Bim-7-*H*), 7.33 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.58−7.54 (m, 2H, Bim-5,8-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.3, 22.5, 26.3, 29.6, 31.3, 44.1, 55.3, 111.2, 113.1, 119.7, 122.2, 123.0, 123.9, 130.5, 135.6, 142.9, 144.0, 154.5 ppm; ESI-MS (*m/z*): 373 $[M+H]^+$; HRMS (ESI) calcd. for $C_{20}H_{26}N_3O_2S$ $[M+H]^+$, 372.1740; found, 372.1744.

*4.1.34. Synthesis of 4-((1-heptyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**9e**)*

Compound **9e** was synthesized according to the experimental procedure reported for compound **8a**, starting from compound **6e** (0.079 g, 0.185 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution. The desired compound **9e** (0.067 g) was obtained as yellow solid. Yield: 94.4%; mp: 219−219 ºC; IR (KBr) ν: 3316, 3196 (N−H), 3044 (aromatic C−H), 2981 (CH_2), 1675 (C=N), 1579, 1482 (aromatic frame), 738 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.84 (t, 3H, *J* = 6.0 Hz, $(CH_2)_6CH_3$), 1.27−1.23 (m, 8H, $(CH_2)_2(CH_2)_4CH_3$), 1.69−1.67 (m, 2H, $CH_2CH_2(CH_2)_4CH_3$), 4.19−4.16 (t, 2H, *J* = 9.0 Hz, $CH_2(CH_2)_5CH_3$), 4.88 (s, 2H, Bim-CH_2), 6.19 (s, 2H, NH_2), 6.58 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.20−7.18 (t, H, *J* = 6.0 Hz, Bim-6-*H*), 7.27−7.24 (t, H, *J* = 9.0 Hz, Bim-7-*H*), 7.33 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.58−7.54 (m, 2H, Bim-5,8-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.4, 22.5, 26.6, 28.8, 29.6, 31.6, 44.1, 55.3, 111.2, 113.1, 119.7, 122.2, 123.0, 123.9, 130.4, 135.6, 142.8, 144.0, 154.5 ppm; ESI-MS (*m/z*): 387 $[M+H]^+$; HRMS (ESI) calcd. for $C_{21}H_{28}N_3O_2S$ $[M+H]^+$, 386.1897; found, 386.1898.

*4.1.35. Synthesis of 4-((1-octyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**9f**)*

Compound **9f** was synthesized according to the experimental procedure reported for compound **8a**, starting from compound **6f** (0.288 g, 0.652 mmol) and 0.4 mL 2 mol/L sodium hydroxide solution. The desired compound **9f** (0.256 g) was obtained as yellow solid. Yield: 98.5%; mp: 213−215 ºC; IR (KBr) ν: 3319, 3203 (N−H), 3040 (aromatic C−H), 2985 (CH_2), 1670 (C=N), 1584, 1478 (aromatic frame), 736 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.83 (t, 3H, *J* = 9.0 Hz, $(CH_2)_7CH_3$), 1.26−1.23 (m, 10H, $(CH_2)_2(CH_2)_5CH_3$), 1.69−1.67 (m, 2H, $CH_2CH_2(CH_2)_5CH_3$), 4.19−4.16 (t, 2H, *J* = 9.0 Hz, $CH_2(CH_2)_6CH_3$), 4.88 (s, 2H, Bim-CH_2), 6.19 (s, 2H, NH_2), 6.58 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.20−7.18 (t, H, *J* = 6.0 Hz, Bim-6-*H*), 7.27−7.24 (t, H, *J* = 9.0 Hz, Bim-7-*H*), 7.33 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.58−7.54 (m, 2H, Bim-5,8-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.4, 22.5, 26.6, 29.0, 29.1, 29.6, 31.6, 44.1, 55.3, 111.2, 113.1, 119.7, 122.2, 123.0, 123.9, 130.4, 135.6, 142.8, 144.0, 154.5 ppm; ESI-MS (*m/z*): 401 $[M+H]^+$; HRMS (ESI) calcd. for $C_{22}H_{30}N_3O_2S$ $[M+H]^+$, 400.2053; found, 400.2055.

*4.1.36. Synthesis of 4-((1-decyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**9g**)*

Compound **9g** was prepared according to the experimental procedure reported for compound **8a**, starting from compound **6g** (0.235 g, 0.500 mmol) and 0.4 mL 2 mol/L sodium hydroxide solution. The desired compound **9g** (0.179 g) was obtained as yellow solid. Yield: 85.1%; mp: 214−216 ºC; IR (KBr) ν: 3309, 3192 (N−H), 3038 (aromatic C−H), 2977 (CH_2), 1672 (C=N), 1589, 1485 (aromatic frame), 739 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.84 (t, 3H, *J* = 6.0 Hz, $(CH_2)_9CH_3$), 1.26−1.23 (m, 14H, $(CH_2)_2(CH_2)_7CH_3$), 1.69−1.66 (m, 2H, $CH_2CH_2(CH_2)_7CH_3$), 4.19−4.16 (t, 2H, *J* = 9.0 Hz, $CH_2(CH_2)_8CH_3$), 4.87 (s, 2H, Bim-CH_2), 6.19 (s, 2H, NH_2), 6.59 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.20−7.18 (t, H, *J* = 6.0 Hz, Bim-6-*H*), 7.26−7.24 (t, H, *J* = 6.0 Hz, Bim-7-*H*), 7.33 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.58−7.54 (m, 2H, Bim-5,8-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.4, 22.5, 26.6, 29.1, 29.4, 29.4, 29.6, 31.7, 44.1, 55.3, 111.2, 113.1, 119.7, 122.2, 123.0, 123.9, 130.4, 135.6, 142.8, 144.0, 154.5 ppm; ESI-MS (*m/z*): 429 $[M+H]^+$; HRMS (ESI) calcd. for $C_{24}H_{34}N_3O_2S$ $[M+H]^+$, 428.2366; found, 428.2373.

*4.1.37. Synthesis of 4-((1-dodecyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**9h**)*

Compound **9h** was obtained as yellow solid. Yield: 95.1%; mp: 208–210 ºC; IR (KBr) ν: 3313, 3192 (N−H), 3031 (aromatic C−H), 2977 (CH_2), 1670 (C=N), 1589, 1482 (aromatic frame), 736 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.84 (t, 3H, *J* = 6.0 Hz, $(CH_2)_{11}CH_3$), 1.26−1.23 (m, 18H, $(CH_2)_2(CH_2)_9CH_3$), 1.69−1.67 (m, 2H, $CH_2CH_2(CH_2)_9CH_3$), 4.19−4.16 (t, 2H, *J* = 9.0 Hz, $CH_2(CH_2)_{10}CH_3$), 4.87 (s, 2H, Bim-CH_2), 6.18 (s, 2H, NH_2), 6.59 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.20−7.18 (t, H, *J* = 6.0 Hz, Bim-6-*H*), 7.26−7.24 (t, H, *J* = 6.0 Hz, Bim-7-*H*), 7.33 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.58−7.54 (m, 2H, Bim-5,8-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.4, 22.5, 26.6, 29.1, 29.2, 29.4, 29.4, 29.4, 29.5, 29.6, 31.8, 44.1, 55.3, 111.2, 113.1, 1197, 122.2, 123.0, 123.9, 130.4, 135.6, 142.8, 144.0, 154.5 ppm; ESI-MS (*m/z*): 457 $[M+H]^+$; HRMS (ESI) calcd. for $C_{26}H_{38}N_3O_2S$ $[M+H]^+$, 456.2679; found, 456.2683.

*4.1.38. Synthesis of 4-((1-octadecyl-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**9i**)*

Compound **9i** was obtained as yellow solid. Yield: 76.4%; mp: 186–188 ºC; IR (KBr) ν: 3310, 3185 (N−H), 3025 (aromatic C−H), 2981 (CH_2), 1675 (C=N), 1589, 1476 (aromatic frame), 738 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ: 0.86−0.83 (t, 3H, *J* = 9.0 Hz, $(CH_2)_{17}CH_3$), 1.26−1.22 (m, 30H, $(CH_2)_2(CH_2)_{15}CH_3$), 1.69−1.67 (m, 2H, $CH_2CH_2(CH_2)_{15}CH_3$), 4.19−4.16 (t, 2H, *J* = 9.0 Hz, $CH_2(CH_2)_{16}CH_3$), 4.87 (s, 2H, Bim-CH_2), 6.19 (s, 2H, NH_2), 6.58 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.20−7.17 (t, H, *J* = 9.0 Hz, Bim-6-*H*), 7.26−7.23 (t, H, *J* = 9.0 Hz, Bim-7-*H*), 7.32 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.58−7.53 (m, 2H, Bim-5,8-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.4, 22.5, 26.6, 29.1, 29.2, 29.4, 29.4, 29.5, 29.5, 29.6, 31.8, 44.1, 55.3, 111.1, 113.1, 119.7, 122.2, 123.0, 123.9, 130.4, 136.6, 142.8, 144.0,

154.5 ppm; ESI-MS (*m/z*): 541 $[M+H]^+$; HRMS (ESI) calcd. for $C_{32}H_{50}N_3O_2S$ $[M+H]^+$, 540.3618; found, 540.3627.

*4.1.39. Synthesis of 4-((1-(5-(9H-carbazol-9-yl)pentyl)-1H-benzo[d]imidazol-2-yl)methylsulfonyl)aniline (**10**)*

Compound **10** was prepared according to the experimental procedure reported for compound **8a**, starting from compound **7** (0.184 g, 0.327 mmol) and 0.2 mL 2 mol/L sodium hydroxide solution. The desired compound **10** (0.156 g) was obtained as yellow solid. Yield: 91.8%; mp: 188−190 ºC; IR (KBr) ν: 3329, 3209 (N−H), 3055 (aromatic C−H), 2995 (CH_2), 1681 (C=N), 1597, 1485 (aromatic frame), 744 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ: 1.36−1.31 (m, 2H, carbazole-$(CH_2)_2CH_2$), 1.73−1.70 (m, 2H, carbazole-CH_2CH_2), 1.81−1.78 (m, 2H, carbazole-$(CH_2)_3CH_2$), 4.15−4.12 (t, H, *J* = 9.0 Hz, carbazole-CH_2), 4.38−4.36 (t, H, *J* = 6.0 Hz, carbazole-$(CH_2)_4CH_2$), 4.80 (s, 2H, Bim-CH_2), 6.18 (s, 2H, NH_2), 6.58 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.23−7.17 (m, 4H, Bim-5,6,7,8-*H*), 7.32 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 7.47−7.42 (m, 3H, carbazole-4,5,10-*H*), 7.57−7.55 (m, 3H, carbazole-3,11,12-*H*), 8.15−8.13 (d, 2H, carbazole-6,9-*H*) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 24.6, 28.7, 29.4, 42.7, 44.0, 55.3, 109.7, 111.2, 1131, 119.1, 119.6, 120.7, 122.2, 122.5, 123.0, 123.8, 126.1, 130.4, 135.6, 140.5, 142.8, 143.9, 154.5 ppm; ESI-MS (*m/z*): 524 $[M+H]^+$; HRMS (ESI) calcd. for $C_{31}H_{31}N_4O_2S$ $[M+H]^+$, 523.2162; found, 523.2170.

4.2. Antibacterial and antifungal assays

Minimal inhibitory concentration (MIC, μg/mL) is defined as the lowest concentration of target compounds that completely inhibit the growth of bacteria, by means of standard two-fold serial dilution method in 96-well microtest plates according to the National Committee for Clinical Laboratory Standards (NCCLS). The tested microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University. Chloromycin, Norfloxacin and Fluconazole, were used as control drugs. DMSO with inoculation bacterial not medicine was used as positive control to ensure that the solvent had no effect on bacteria growth. All the bacteria and fungi growth was monitored visually and spectrophotometrically, and the experiments were performed in triplicate. The MIC values in μg/mL were summarized in Tables 1 and 2.

4.2.1. Antibacterial assays

The prepared compounds **4**–**10** were evaluated for their antibacterial activities against Gram-positive bacteria (*S. aureus* ATCC 6538, *Methicillin-resistant Staphylococcus aureus* N315 (MRSA), *M. luteus* and

B. subtilis ATCC 21216), Gram-negative bacteria (*E. coli* ATCC 8099, *P. aeruginosa* ATCC 27853, *B. typhi* and *B. proteus* ATCC 13315). The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^5 CFU. Initially the compounds were dissolved in DMSO to prepare the stock solutions, then the tested compounds and reference drugs were prepared in Mueller–Hinton broth (Guangdong huaikai microbial sci. & tech co., Ltd, Guangzhou, Guangdong, China) to obtain the required concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 μg/mL. These dilutions were inoculated and incubated at 37 ºC for 24 h.

4.2.2. Antifungal assays

The newly synthesized compounds **4**–**10** were evaluated for their antifungal activities against *C. albicans* ATCC 76615, *A. fumigatus* ATCC 96918, *C. utilis*, *S. cerevisia* and *A. flavus*. A spore suspension in sterile distilled water was prepared from one day old culture of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was $1-5 \times 10^3$ spore mL^{-1}. From the stock solutions of the tested compounds and reference antifungal drug Fluconazole, dilutions in sterile RPMI 1640 medium (Neuronbc Laboraton Technology CO., Ltd, Beijing, China) were made resulting in eleven wanted concentrations (0.5 to 512 μg/mL) of each tested compound. These dilutions were inoculated and incubated at 35 ºC for 24 hours.

4.3. Molecular docking

All the docking studies were carried out using Surflex-Docking 2.0 on a window 7 workstation. The crystal structure of DNA (PDB entry 3FT6) was downloaded from the protein data bank and used for docking studies. Water molecules were removed from protein PDB files, and hydrogen atoms were added. The 3D structures of compounds **5c** and **5g** were first built using Surflex-Docking 2.0 sketch followed by energy minimization. At last we used the Surflex-docking program to automatically dock the drugs into the binding pockets of DNA. In the docking process, 20 conformations were obtained, and among them the lowest free energy solution (or the highest total score) was chosen for our compounds modeling. To confirm our docking experiment reliable, we redocked the native ligand into the 3FT6 (Supporting Information: Fig. S12), the RMSD value is 0.68 (< 2), suggesting the methodology is reliable.

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Lists of table and scheme captions

Table 1 ClogP values and antibacterial data as MIC (μg/mL) for compounds **4**–**10**.

Table 2 Antifungal data as MIC (μg/mL) for compounds **4**–**10**.

Table 3 Stern-Volmer quenching constants for the interaction of compounds **5c** and **5g** with HSA at various temperatures.

Table 4 Binding constants and sites of **5c**–HSA and **5g**–HSA systems at pH = 7.4.

Table 5 Thermodynamic parameters of **5g**–HSA and **5g**–HSA systems at different temperatures.

Figure 1 Structures of some antimicrobial sulfonamides.

Figure 2 UV absorption spectra of DNA with different concentrations of compound **5c** (pH = 7.4, T = 293 K). Inset: comparison of absorption at 260 nm between the **5c**–DNA complex and the sum values of free DNA and free compound **5c**. *c*(DNA) = 1.28 × 10^{-5} mol/L, and *c*(compound **5c**) = 0–1.6 × 10^{-5} mol/L for curves *a*–*g* respectively at increment 0.2 × 10^{-5}.

Figure 3 UV absorption spectra of DNA with different concentrations of compound **5g** (pH = 7.4, T = 293 K). Inset: comparison of absorption at 260 nm between the **5g**–DNA complex and the sum values of free DNA and free compound **5g**. *c*(DNA) = 1.28 × 10^{-5} mol/L, and *c*(compound **5g**) = 0–1.6 × 10^{-5} mol/L for curves *a*–*g* respectively at increment 0.2 × 10^{-5}.

Figure 4 UV Absorption spectra of the competitive reaction between **5c** and neutral red with DNA. *c*(DNA) = 1.28 × 10^{-5} mol/L, *c*(NR) = 2 × 10^{-5} mol/L, and *c*(compound **5c**) = 0–4.8 × 10^{-5} mol/L for curves *a*–*i* respectively at increment 0.6 × 10^{-5}. (Inset) Absorption spectra of the system with the increasing concentration of **5c** in the wavelength range of 260–285 nm absorption spectra of competitive reaction between compound **5c** and NR with DNA.

Figure 5 UV Absorption spectra of the competitive reaction between **5g** and neutral red with DNA. *c*(DNA) = 1.28 × 10^{-5} mol/L, *c*(NR) = 2 × 10^{-5} mol/L, and *c*(compound **5g**) = 0–4.8 × 10^{-5} mol/L for curves *a*–*i* respectively at increment 0.6 × 10^{-5}. (Inset) Absorption spectra of the system with the increasing concentration of **5g** in the wavelength range of 260–285 nm absorption spectra of competitive reaction between compound **5g** and NR with DNA.

Figure 6 Molecular modeling of compound **5c** and DNA hexamer duplex (PDB: 3FT6). The dashed lines represent the hydrogen bonding interactions between compound **5c** and DNA hexamer duplex (Total binding score is 5.04).

Figure 7 Stereoview of the conformation of compound **5c** intercalated to DNA hexamer duplex to form compound **5c**–DNA complex.

Figure 8 Molecular modeling of compound **5g** and DNA hexamer duplex (PDB: 3FT6). The dashed lines represent the hydrogen bonding interactions between compound **5g** and DNA hexamer duplex (Total binding score is 5.59).

Figure 9 Stereoview of the conformation of compound **5g** intercalated to DNA hexamer duplex to form compound **5g**–DNA complex.

Figure 10 Emission spectra of HSA in the presence of various concentrations of compound **5c**. *c*(HSA) = 1.0×10^{-5} mol/L; *c*(compound **5c**)/(10^{-5} mol/L), *a*–*m*: from 0.0 to 1.2 at increments of 0.2; black line shows the emission spectrum of compound **5c** only; T = 293 K, λ_{ex} = 295 nm.

Figure 11 Emission spectra of HSA in the presence of various concentrations of compound **5g**. *c*(HSA) = 1.0×10^{-5} mol/L; *c*(compound **5g**)/(10^{-5} mol/L), *a*–*m*: from 0.0 to 1.2 at increments of 0.2; black line shows the emission spectrum of compound **5g** only; T = 293 K, λ_{ex} = 295 nm.

Figure 12 Van't Hoff plots of the **5c**–HSA system.

Figure 13 Van't Hoff plots of the **5g**–HSA system.

Figure 14 Electrostatic potential of compounds **5c** and **5g**.

Scheme 1 Synthesis of benzimidazole sulfonamide analogues **4**–**10**

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Table 1 ClogP values and antibacterial data as MIC (μg/mL) for compounds **4**–**10**[a,b,c]

Compds	ClogP	Gram-Positive bacteria				Gram-Negative bacteria			
		S. aureus	MRSA	*B. subtilis*	*M. luteus*	*B. proteus*	*E. coli*	*P. aeruginosa*	*B. typhi*
4	1.16	32	128	32	128	64	64	32	128
5a	3.08	512	128	64	32	128	256	128	64
5b	3.08	512	256	512	512	256	128	256	128
5c	3.08	4	16	16	64	32	32	256	16
5d	3.65	32	32	32	64	512	32	16	16
5e	3.65	32	128	128	64	128	64	256	128
5f	3.65	512	512	256	128	256	128	512	64
5g	4.37	64	128	16	128	32	8	16	4
5h	4.25	64	512	128	256	128	256	512	512
6a	1.70	128	128	32	128	64	256	32	64
6b	2.23	128	64	16	256	64	128	128	32
6c	3.29	32	128	64	32	256	256	4	32
6d	3.82	512	512	128	256	256	512	512	512
6e	4.35	8	512	256	512	256	64	512	512
6f	4.87	64	512	128	256	256	512	256	512
6g	5.93	512	512	512	512	512	512	512	512
6h	6.99	512	512	512	512	512	256	512	512
6i	10.16	128	512	512	512	512	512	512	512
7	6.05	256	512	512	128	512	512	16	64
8a	2.89	64	64	16	32	256	4	512	16
8b	2.89	512	128	512	512	256	128	512	512
8c	2.89	16	512	512	512	128	256	16	16
8d	3.46	16	32	16	64	256	128	16	16
8e	3.46	32	512	512	256	256	32	256	512
8f	3.46	256	256	8	8	256	64	256	512
8g	4.18	8	128	32	64	128	16	32	128
8h	4.06	512	128	512	256	512	512	512	64
9a	1.51	64	128	64	128	64	64	64	128
9b	2.04	256	128	32	256	256	64	256	32
9c	3.10	512	512	512	256	512	512	512	512
9d	3.63	128	512	512	512	256	512	128	512
9e	4.16	512	512	512	128	512	512	512	512
9f	4.68	512	512	512	512	512	512	512	512
9g	5.74	512	512	512	512	512	512	512	512
9h	6.80	512	512	512	256	512	512	512	512
9i	9.97	512	512	512	256	512	512	512	512
10	5.86	512	128	32	512	512	64	512	64
Chloromycin	–1.09	8	16	32	8	32	16	16	32
Norfloxacin	0.58	8	1	2	1	4	1	1	1

[a] Minimal inhibitory concentrations were determined by micro broth dilution method for microdilution plates.

[b] *S. aureus*, Staphylococcus aureus (ATCC25923); MRSA, Methicillin-Resistant Staphylococcus aureus (N315); *B. subtilis*, Bacillus subtilis; *M. luteus*, Micrococcus luteus (ATCC4698); *B. proteus*, Bacillus proteus (ATCC13315); *E. coli*, Escherichia coli (JM109); *P. aeruginosa*, Pseudomonas aeruginosa; *B. typhi*, Bacillus typhi; *C. albicans*, Candida albicans (ATCC76615); *C. mycoderma*, Candida mycoderma; *C. utilis*, Candida utilis; *S. cerevisia*, Saccharomyces cerevisia; *A. flavus*, Aspergillus flavus.

[c] ClogP values were calculated by ChemDraw Ultra 10.0.

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Table 2 Antifungal data as MIC (μg/mL) for compounds **4**–**10**[a,b,c]

Compds	*C. albicans*	*C. mycoderma*	*C. utilis*	*S. cerevisiae*	*A. flavus*
4	32	64	16	32	128
5a	256	512	32	512	64
5b	256	512	512	32	256
5c	64	64	16	64	64
5d	32	512	512	512	8
5e	512	256	512	128	256
5f	128	32	512	64	512
5g	512	512	128	128	32
5h	64	512	512	32	16
6a	64	256	64	512	64
6b	64	16	64	16	128
6c	256	16	32	64	32
6d	512	512	512	512	512
6e	512	512	128	512	256
6f	512	512	512	8	512
6g	512	512	16	512	512
6h	512	512	512	512	512
6i	512	512	512	512	512
7	512	32	512	32	128
8a	128	8	4	8	32
8b	128	512	512	8	256
8c	32	512	128	32	256
8d	16	512	512	512	32
8e	512	512	512	256	16
8f	256	16	32	64	128
8g	16	512	2	256	4
8h	64	512	256	256	512
9a	256	512	64	512	32
9b	64	4	64	32	8
9c	512	512	512	128	256
9d	512	512	512	512	512
9e	512	512	512	64	512
9f	64	256	512	512	512
9g	512	512	8	512	512
9h	512	512	512	256	512
9i	512	64	512	64	512
10	512	16	512	64	128
Fluconazole	1	4	8	16	256

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Table 3 Stern-Volmer quenching constants for the interaction of compounds **5c** and **5g** with HSA at various temperatures

pH	T (K)	*K*sv (L mol^{-1})		*K*q (L mol^{-1} S^{-1})		*R*[a]		S.D.[b]	
		5c	**5g**	**5c**	**5g**	**5c**	**5g**	**5c**	**5g**
7.4	273	3.26×10^4	5.25×10^4	5.09×10^{12}	8.20×10^{12}	0.975	0.996	0.049	0.030
	293	2.20×10^4	4.08×10^4	3.44×10^{12}	6.38×10^{12}	0.997	0.999	0.011	0.007
	313	1.83×10^4	3.02×10^4	2.86×10^{12}	4.72×10^{12}	0.996	0.998	0.011	0.011

R[a] is the correlation coefficient. S.D.[b] is standard deviation

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Table 4 Binding constants and sites of **5c**–HSA and **5g**–HSA systems at pH = 7.4.

T (K)	Modified Stern-Volmer method						Scatchard method							
	10^{-4} K_a (L/mol)		R		S.D.		10^{-4} K_b (L/mol)		R		S.D.		n	
	5c	**5g**	**5c**	**5g**	**5c**	**5g**	**5c**	**5g**	**5c**	**5g**	**5c**	**5g**	**5c**	**5g**
273	5.43	1.11	0.997	0.197	0.211	0.197	7.86	6.56	0.998	0.998	0.114	0.029	0.97	1.07
293	3.69	1.08	0.999	0.552	0.217	0.552	5.84	5.93	0.999	0.999	0.108	0.020	0.95	1.06
313	3.58	1.06	0.998	0.258	0.355	0.258	4.16	4.21	0.999	0.999	0.013	0.015	1.14	1.18

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Table 5 Thermodynamic parameters of **5c**–HSA and **5g**–HSA systems at different temperatures.

T(K)	ΔH (kJ mol^{-1})		ΔG (kJ mol^{-1})		ΔS (J mol^{-1} K^{-1})	
	5c	**5g**	**5c**	**5g**	**5c**	**5g**
273			-24.310	-21.139		
293	-1.757	-0.820	-25.542	-22.042	82.610	74.429
313			-26.774	-24.116		

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Fig. 1. Structures of some antimicrobial sulfonamides.

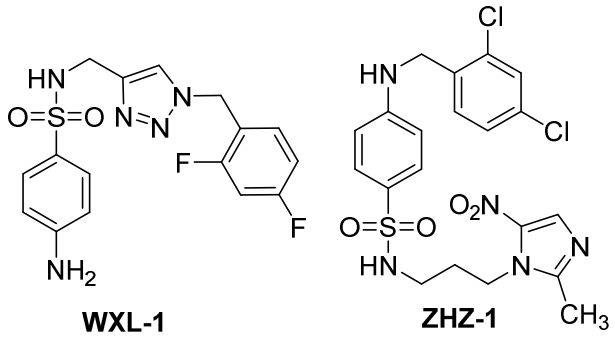

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Fig. 2. UV absorption spectra of DNA with different concentrations of compound **5c** (pH = 7.4, T = 293 K). Inset: comparison of absorption at 260 nm between the **5c**–DNA complex and the sum values of free DNA and free compound **5c**. *c*(DNA) = 1.28 × 10^{-5} mol/L, and *c*(compound **5c**) = 0–1.6 × 10^{-5} mol/L for curves *a*–*g* respectively at increment 0.2 × 10^{-5}.

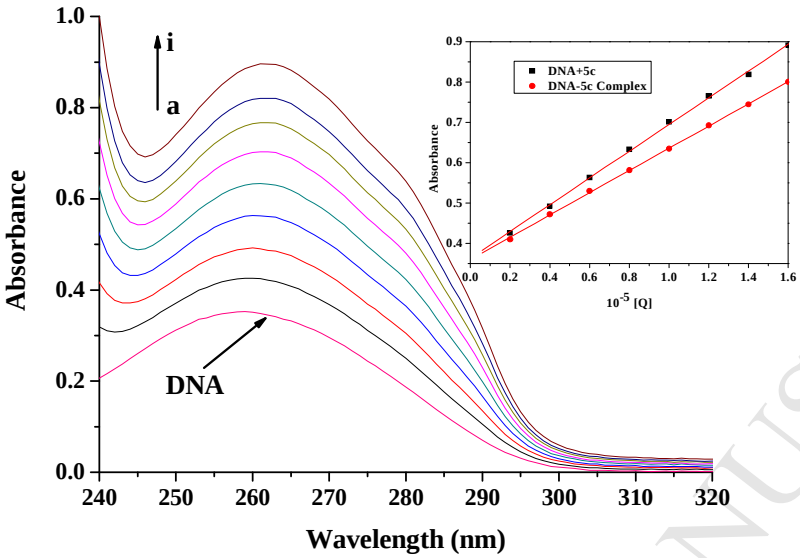

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Fig. 3. UV absorption spectra of DNA with different concentrations of compound **5g** (pH = 7.4, T = 293 K). Inset: comparison of absorption at 260 nm between the **5g**–DNA complex and the sum values of free DNA and free compound **5g**. *c*(DNA) = 1.28 × 10^{-5} mol/L, and *c*(compound **5g**) = 0–1.6 × 10^{-5} mol/L for curves *a*–*g* respectively at increment 0.2 × 10^{-5}.

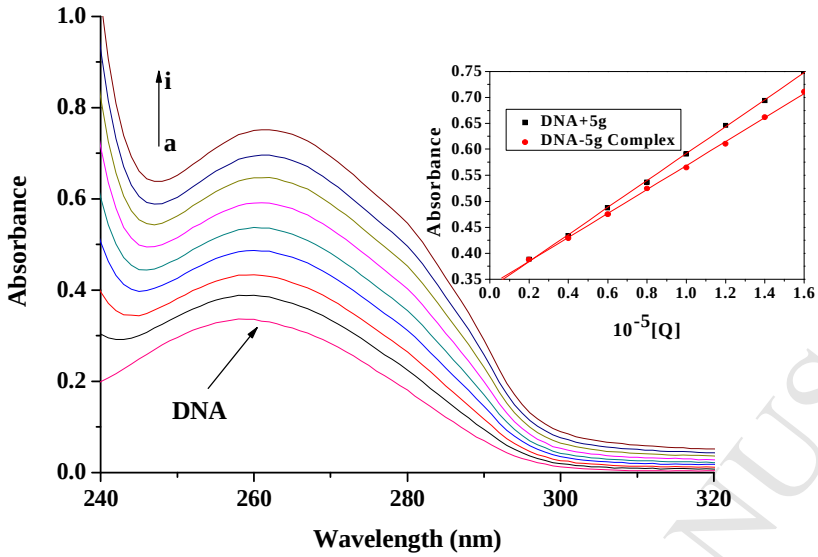

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Fig. 4. UV Absorption spectra of the competitive reaction between **5c** and neutral red with DNA. *c*(DNA) = 1.28 × 10^{-5} mol/L, *c*(NR) = 2 × 10^{-5} mol/L, and *c*(compound **5c**) = 0–4.8 × 10^{-5} mol/L for curves *a*–*i* respectively at increment 0.6 × 10^{-5}. (Inset) Absorption spectra of the system with the increasing concentration of **5c** in the wavelength range of 260–285 nm absorption spectra of competitive reaction between compound **5c** and NR with DNA.

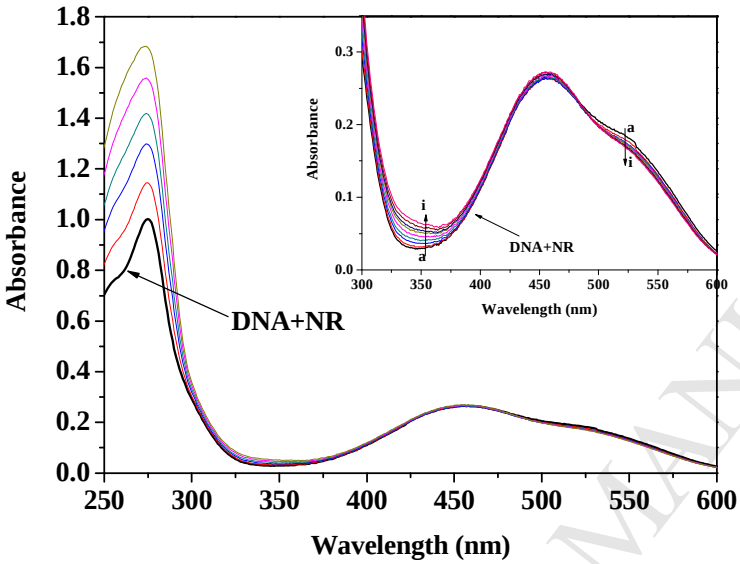

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Fig. 5. UV Absorption spectra of the competitive reaction between **5g** and neutral red with DNA. *c*(DNA) = 1.28 × 10^{-5} mol/L, *c*(NR) = 2 × 10^{-5} mol/L, and *c*(compound **5g**) = 0–4.8 × 10^{-5} mol/L for curves *a*–*i* respectively at increment 0.6 × 10^{-5}. (Inset) Absorption spectra of the system with the increasing concentration of **5g** in the wavelength range of 260–285 nm absorption spectra of competitive reaction between compound **5g** and NR with DNA.

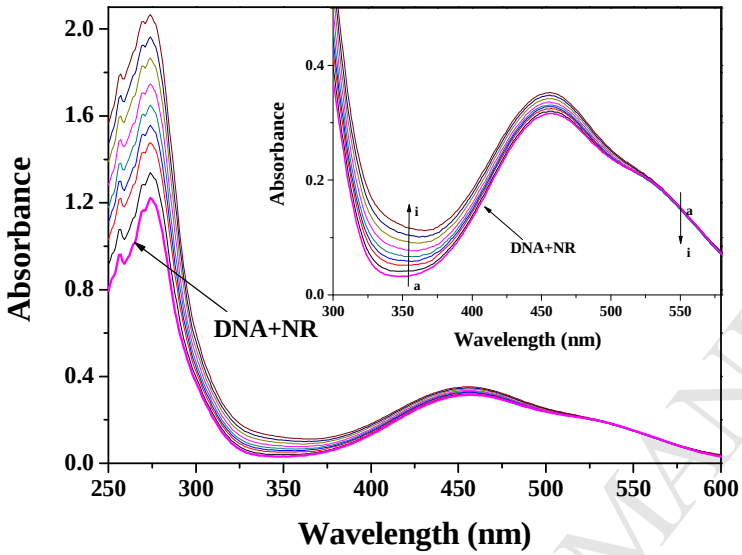

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Fig. 6. Molecular modeling of compound **5c** and DNA hexamer duplex (PDB: 3FT6). The dashed lines represent the hydrogen bonding interactions between compound **5c** and DNA hexamer duplex (Total binding score is 5.04).

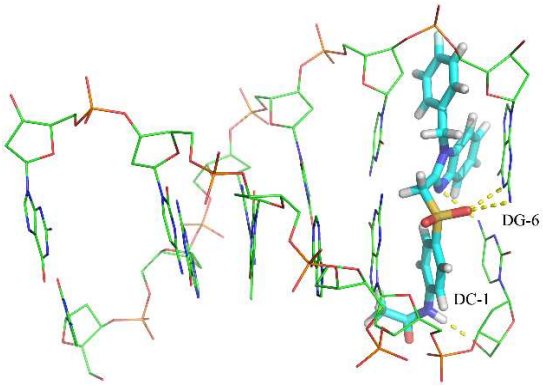

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Fig. 7. Stereoview of the conformation of compound **5c** intercalated to DNA hexamer duplex to form compound **5c**–DNA complex.

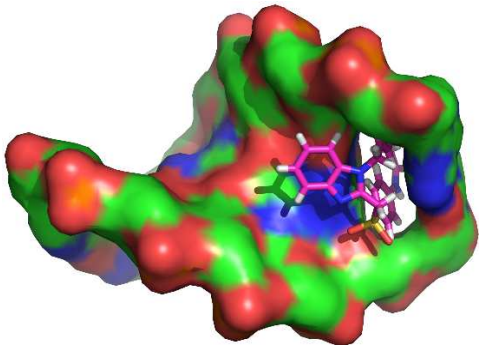

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Fig. 8. Molecular modeling of compound **5g** and DNA hexamer duplex (PDB: 3FT6). The dashed lines represent the hydrogen bonding interactions between compound **5g** and DNA hexamer duplex (Total binding score is 5.59).

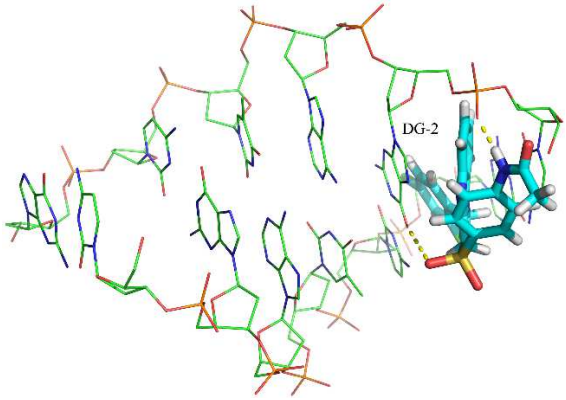

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Fig. 9. Stereoview of the conformation of compound **5g** intercalated to DNA hexamer duplex to form compound **5g**–DNA complex.

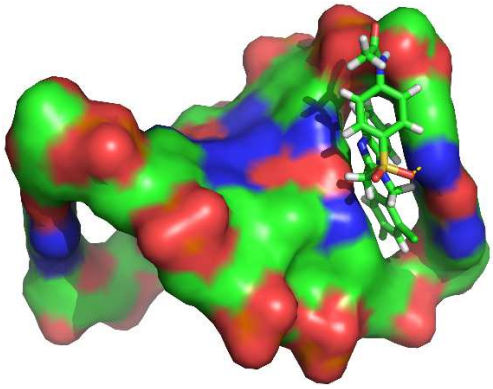

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Fig. 10. Emission spectra of HSA in the presence of various concentrations of compound **5c**. *c*(HSA) = 1.0 × 10^{-5} mol/L; *c*(compound **5c**)/(10^{-5} mol/L), *a*–*m*: from 0.0 to 1.2 at increments of 0.2; black line shows the emission spectrum of compound **5c** only; T = 293 K, λ_{ex} = 295 nm.

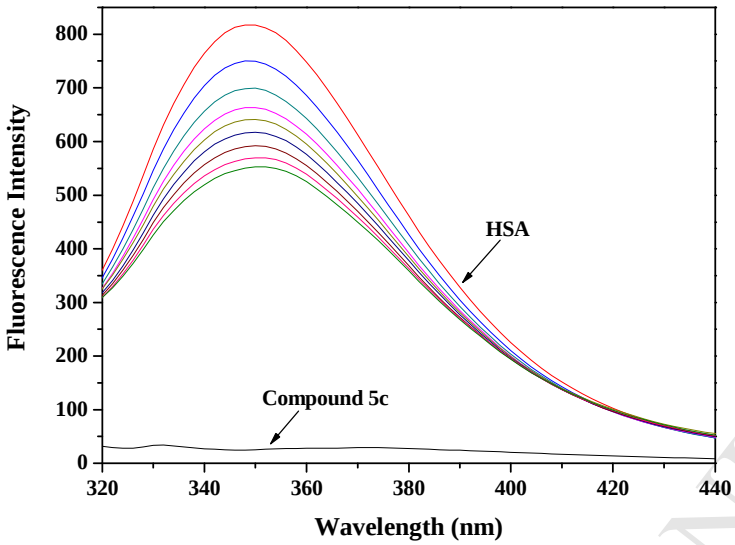

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Fig. 11. Emission spectra of HSA in the presence of various concentrations of compound **5g**. *c*(HSA) = 1.0 × 10^{-5} mol/L; *c*(compound **5g**)/(10^{-5} mol/L), *a*–*m*: from 0.0 to 1.2 at increments of 0.2; black line shows the emission spectrum of compound **5g** only; T = 293 K, λ_{ex} = 295 nm.

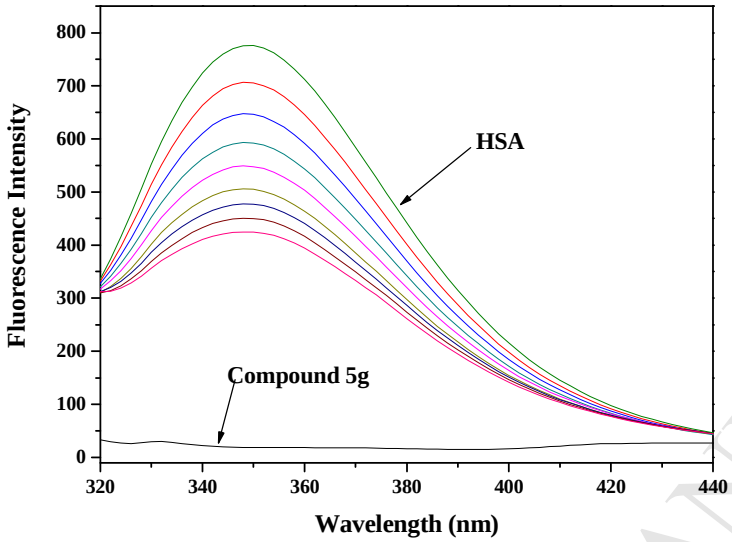

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Fig. 12. Van't Hoff plots of the **5c**–HSA system.

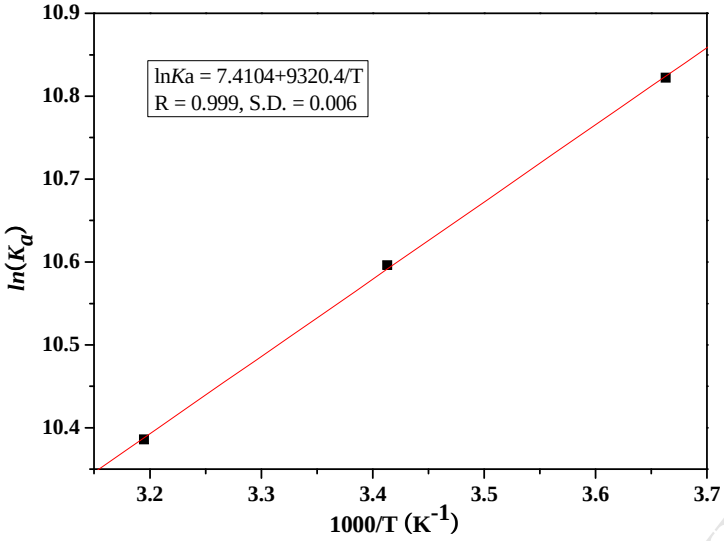

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Fig. 13. Van't Hoff plots of the **5g**–HSA system.

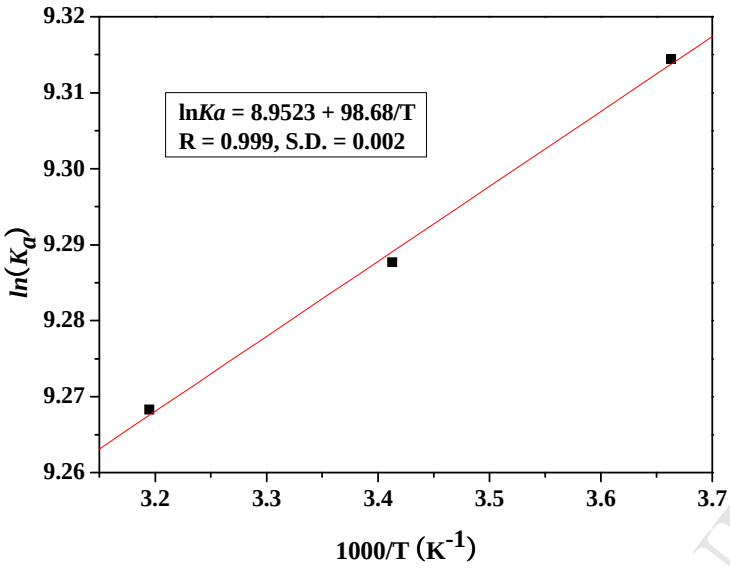

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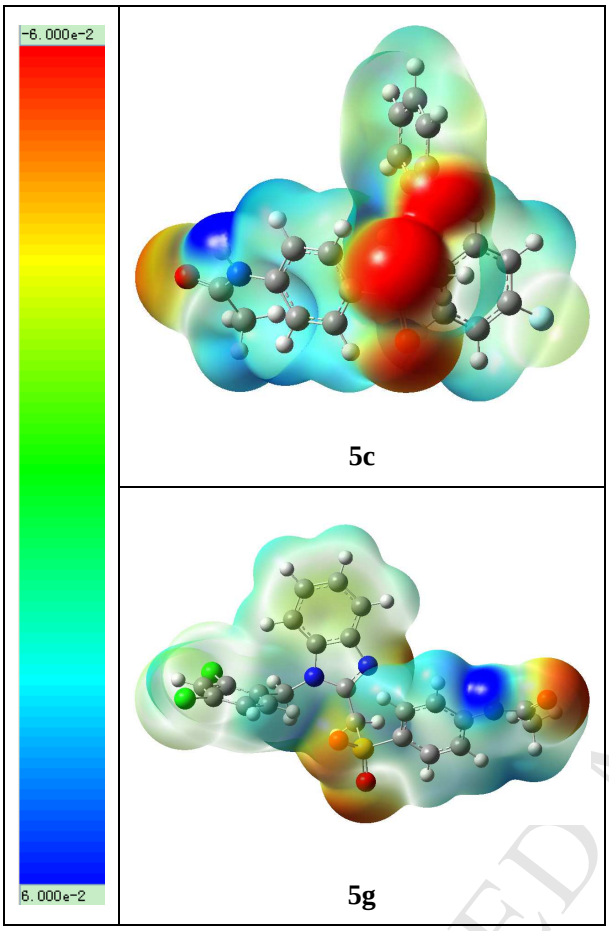
Fig. 14. Electrostatic potential of compounds **5c** and **5g**.



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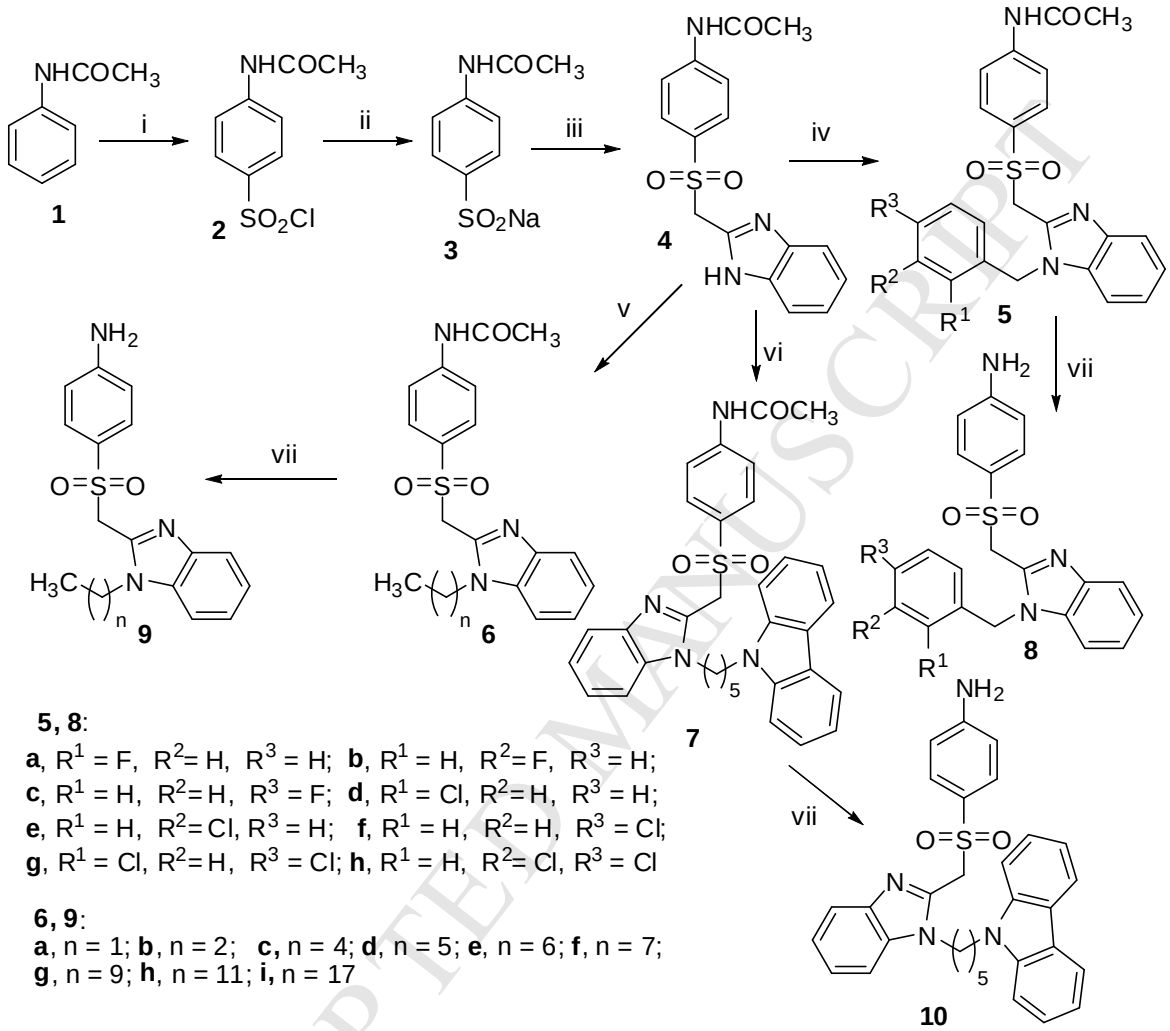
Scheme 1 Synthesis of benzimidazole sulfonamide analogues **4**–**10**



5, 8:
a, R^1 = F, R^2 = H, R^3 = H; **b**, R^1 = H, R^2 = F, R^3 = H;
c, R^1 = H, R^2 = H, R^3 = F; **d**, R^1 = Cl, R^2 = H, R^3 = H;
e, R^1 = H, R^2 = Cl, R^3 = H; **f**, R^1 = H, R^2 = H, R^3 = Cl;
g, R^1 = Cl, R^2 = H, R^3 = Cl; **h**, R^1 = H, R^2 = Cl, R^3 = Cl

6, 9:
a, n = 1; **b**, n = 2; **c,** n = 4; **d**, n = 5; **e**, n = 6; **f**, n = 7;
g, n = 9; **h**, n = 11; **i,** n = 17

Reagents and conditions: (i) chlorosulfonic acid, 0 ºC; (ii) sodium sulfite, sodium bicarbonate, 80 ºC; (iii) chloromethyl benzimidazole, acetone, 50 ºC; (iv) substituted halobenzyl halide, potassium carbonate, acetonitrile, 70 ºC; (v) alkyl bromide, potassium carbonate, acetonitrile, 70 ºC; (vi) 9-(5-bromopentyl)-9*H*-carbazole, potassium carbonate, acetonitrile, 70 ºC; (vii) 2 mol/L NaOH, ethanol, reflux.

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- ► Novel series of potentially antimicrobial sulfonamide benzimidazoles were developed
- ► Intercalation mechanism into DNA was suggested by experiments and molecular docking study
- ► Effective transportation by HSA towards highly active compounds were revealed
- ► Molecular electrostatic potentiality was studied by full geometry optimizations

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