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Sulfonamide-derived Four-component Molecular Hybrids as Novel DNA-targeting Membrane Active Potentiators against Clinical *Escherichia coli*

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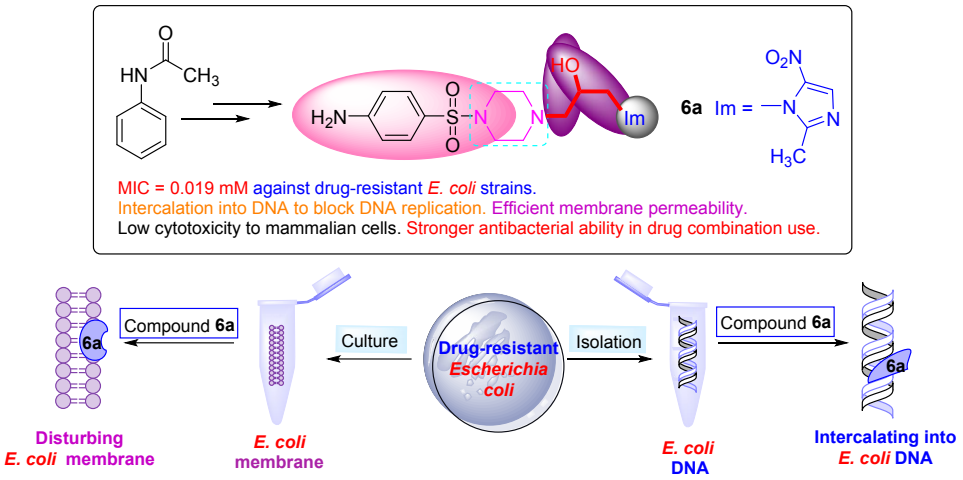
TOC Graphics



ABSTRACT

Novel sulfonamide-based four-component molecular hybrids as potentially DNA-targeting antimicrobial agents were developed from marketed acetanilide through convenient procedures. Biological assays indicated that a few of the target compounds showed significant inhibitory efficiencies toward the tested bacteria and fungi. Noticeably, metronidazole hybrid **6a** exhibited lower minimum inhibitory concentrations (MIC) value of 0.019 mM against clinical drug-resistant *Escherichia coli* (*E. coli*), which showed 84-fold more active than clinical norfloxacin and no obvious toxicity toward human breast cancer MCF-7 cells. Synergistic combinations of compound **6a** with clinical antibacterial or antifungal drugs could improve the antimicrobial efficiency. Further molecular modeling indicated that the active molecule **6a** could bind with THR-199, HIS-64 and GLN-92 residues of human carbonic anhydrase isozyme II through hydrogen bonds and was also able to insert into base-pairs of DNA hexamer duplex by forming hydrogen bonds. The preliminary exploration of antibacterial mechanism suggested that compound **6a** was capable of disturbing *E. coli* membrane effectively, and intercalating into clinical resistant *E. coli* bacterial DNA through non-covalent bonds to form supramolecular complex, thus exerting its powerful antimicrobial activity. This might suggest a great possibility for hybrid **6a** to be a DNA-targeting membrane active potentiator against clinical drug-resistant *E. coli*.

KEYWORDS: sulfonamide, azole, *Escherichia coli*, DNA, antibacterial

INTRODUCTION

Antibiotics have been playing crucial roles in treating deadly microbial infections. In particular, the success of antibacterial sulfonamides immensely promoted the development of antimicrobial agents.[1] However, the frequently clinical use of various types of antimicrobial drugs caused the emergence and spread of resistant strains such as fluoroquinolone-resistant *Escherichia coli* (*E. coli*), vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* (MRSA).[2,3] More seriously, *E. coli* has shown resistance to a variety of clinical antibacterials such as trimethoprim-sulfamethoxazoles, aminoglycosides and carbapenems and so on.[4,5] Consequently, the discovery of antimicrobial agents with new structural hybrids against resistant bacterial strains has been an urgent task worldwide.[6,7]

Sulfonamides with characteristic *p*-aminobenzene sulfonamide skeleton are the first class of artificial synthetic chemotherapeutic agents which were extensively used for the cure and prevention of bacterial infections in early 1935.[8] Antimicrobial mechanism revealed that this type of drugs could competitively bind enzymes with *p*-aminobenzoic acid (PABA) in the biosynthesis of bacterial dihydrofolate and affected the synthesis of nucleic acid precursors, thereby inhibiting bacterial growth and reproduction.[9] Continuous structural modification of the core framework of *p*-aminophenyl sulfonamide has been receiving special interest in the development of novel sulfonamides, and a large number of sulfonamides especially aromatic heterocycle derivatives like pyrimidine, pyridazine, pyridine, isoxazole, and thiazole ones have been marketed and extensively used in clinic, such as sulfadiazine, sulfachlorpyridazine, sulfathiazole and sulfisoxazole. All the sulfonamide drugs provide a great encouragement and guidance for the rational design of broad-spectrum, high-activity, and low-toxicity new sulfonamide derivatives, and put forward idea to develop sulfonamide-based multicomponent

molecular hybrids which might have a large promise as potentially novel DNA-targeting antimicrobial agents.[10-12]

Ethanol as an important component of disinfectant is extensively employed for anti-infective medicinal application. It is generally considered to denature the protein, break bacterial cell wall and destroy enzyme system. Meanwhile, it is prevalent that hydroxyethyl fragment is universally employed to construct new active molecules. Especially, lots of hydroxyethyl derivatives such as antibacterial metronidazole and antifungal fluconazole were clinical drugs.[13,14] Therefore, the exploration of potentially hydroxyethyl-based antimicrobial agents has provided an important step forward for current therapies.

Heterocyclic azoles are helpful structural fragments for biological activity and extensively used in drug design and development because they could easily interact with the active sites of organisms through non-covalent interactions like coordination bond, hydrogen bond, π-π stacking, *etc*. to improve the physicochemical and pharmacokinetic properties, thus improving their bioavailability and target selectivity.[15−17] In particular, five-membered azoles such as imidazole,[18−20] triazole[21−23] and tetrazole[24,25] played an important role in the field of medicine, and some azole compounds have been successfully used in clinic like antibacterial secnidazole and ornidazole as well as antifungal fluconazole and voriconazole.[26−28] This further promotes increasingly effort towards fused azoles including benzimidazole,[29−32] benzotriazole and carbazole,[33−35] and some fused azoles like carbazomycins and murrayafoline have been marketed.[36−38]

Piperazine as a significant heterocyclic building block in medicinal chemistry can effectively regulate physicochemical properties such as lipid-water partition coefficient (logP), binding affinity and acid-base equilibrium constant.[39,40] In the meantime, piperazine provides an easy

route to access highly functionalized segments of clinical drugs like quinolones, using straightforward, base-mediated or reductive *N*-alkylation reactions.[41,42]

On the basis of the above considerations, we would like to combine the core antibacterial *p*-aminobenzene sulfonamide skeleton, hydroxyethyl, azole and piperazine four effective structural fragments into one molecule to produce a novel kind of potential four-component antimicrobial hybrids (Figure 1). Meanwhile, this type of new sulfonamide hybrids may be helpful for overcoming drug resistance, broadening antimicrobial spectrum, and opening a new avenue to develop effective multi-targeting antimicrobial agents. All these target molecules would be tested for antimicrobial activities, and bacterial membrane permeabilization, cytotoxicity, resistance of bacteria, bactericidal potential and drug combination of the highly active compound were investigated. To further evaluate the preliminary antibacterial mechanism of this highly active molecule, molecular docking with DNA hexamer duplex and human carbonic anhydrase isozyme II as well as interaction with DNA were also evaluated.

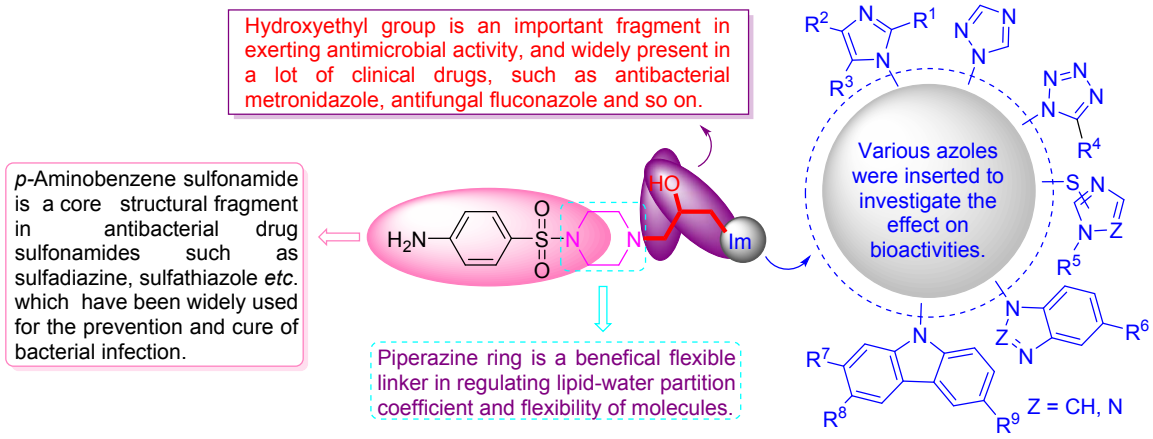


Figure 1. Design of novel four-component sulfonamide hybrids.

MATERIALS AND METHODS

Chemicals and Measurements. Unless specifically stated, all materials were commercially available and did not require further purification for use. Thin layer chromatography (TLC) was

characterized by UV light (254 nm). The masses were measured by a microbalance. Silica gel (500-600 mesh size) was used for column chromatography. Determination of melting point (mp) was employed by a X-6 melting point apparatus. Bruker AVANCE III 600 MHz spectrometer was used to record ^{1}H NMR and ^{13}C NMR (150 MHz) spectra of target compounds. ESI-TOF method was used to analyze high resolution mass spectra (HRMS) by IonSpec FT-ICR mass spectrometer. The following abbreviations were representative for the signal of NMR spectra: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. Hertz unit (Hz) was used to express the coupling constants (*J*). The following abbreviations were used to designate structural fragments: Im = imidazole, Bim = benzimidazole, Ph = phenyl, Cb = carbazole, Pip = piperazine.

Synthesis. Sulfonamide-based four-component hybrids were conveniently synthesized from commercially available acetanilide **1**. The sulfonylation of acetanilide **1** was carried out with chlorosulfonic acid at 60 °C for 2 h without using any additional solvent to afford acetamido benzenesulfonyl chloride **2** in a high yield of 90.2%, which was further reacted with piperazine in dichloromethane at 0 °C for 1 h to generate intermediate **3** with the yield of 43.4%. The key intermediate **4** with moderate yield of 64.4% was obtained by the reaction of compound **3** with epichlorohydrin in acetonitrile employing potassium carbonate at 80 °C for 18 h. The intermediate **4** was used as scaffold to provide a library of compounds using imidazoles, triazoles and tetrazoles on the basis of the synthetic route outlined in Scheme 1, thioether-bridged azoles in Scheme 2, benzimidazoles, benzotriazole and carbazoles in Scheme 3. Sulfonamide intermediates **5a**−**d**, **7a**−**b**, **9**, **11**, **13a**−**c** and **15a**−**e** with the yields ranging from 18.8% to 35.2% were synthesized in acetonitrile using triethylamine (TEA) as base at 80 °C for 24 h. Finally, all the sulfonamide intermediates were deprotected with hydrochloric acid (40%) in ethanol at 78 °C

for 1 h to afford their deacetylated products **6a**−**d**, **8a**−**b**, **10**, **12**, **14a**−**c** and **16a**−**e** with the yields ranging from 56.4% to 77.3%.

All the sulfonamide hybrids with new structures were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. The spectral data were described in the experimental protocols.

Experimental procedures for the synthesis of intermediate (2). Compound **2** was obtained according to the reported procedures.[12]

Experimental procedures for the synthesis of intermediate (3). Compound **3** was obtained according to the reported procedures.[43]

N-(4-((4-(Oxiran-2-ylmethyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (4). A mixture solution of compound **3** (1.50 g, 5.30 mmol), acetonitrile (25 mL) and potassium carbonate (1.46 g, 10.60 mmol) was stirred at 80 ^{o}C for 1 h, then epichlorohydrin (1.00 mL, 10.60 mmol) was added and allowed to stir another 18 h. After disappearance of the starting material by TLC, potassium carbonate was separated by suction filtration, then the solvent was removed and the crude product was subjected to column chromatography (eluent, petroleum ether/dichloromethane (10/1, V/V)) to afford the desired compound **3** as light yellow liquid. Yield: 64.4%; ^{1}H NMR (DMSO-d_6) δ: 10.43 (s, 1H, N*H*COCH$_3$), 7.84 (d, J = 7.9 Hz, 2H, Ph-2,6-*H*), 7.67 (d, J = 8.7 Hz, 2H, Ph-3,5-*H*), 2.93 (dd, J = 6.2, 3.0 Hz, 1H, epoxypropyl-CH_2), 2.87 (s, 4H, Pip-N-(CH_2)$_2$), 2.65 (dd, J = 8.6, 4.1 Hz, 2H, epoxypropyl-CH_2), 2.56−2.52 (m, 2H, Pip-N-CH_2), 2.49−2.45 (m, 2H, Pip-N-CH_2), 2.42 (dd, J = 4.9, 2.6 Hz, 1H, epoxypropyl-C*H*), 2.16 (dd, J = 13.4, 6.7 Hz, 1H, cyclopropyl-CH_2), 2.10 (s, 3H, COCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 144.06, 129.3, 119.1, 60.0, 52.4, 50.1, 46.3, 44.4, 24.6 ppm.

N-(4-((4-(2-Hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (5a). A mixture solution of 2-methyl-5-nitro-1H-imidazole (0.23

g, 1.80 mmol), acetonitrile (10 mL) and TEA (1.40 mL, 10.00 mmol) was stirred at 80 °C for 1 h, after compound **4** (0.41 g, 1.20 mmol) was added and the system was allowed to stir additional 24 h. After disappearance of the starting material, the solvent was removed and the crude product was subjected to column chromatography (eluent, dichloromethane/methanol (300/1, V/V)) to produce the desired compound **5a** as yellow liquid. Yield: 26.8%; ^{1}H NMR (DMSO-d_6) δ: 10.46 (s, 1H, N*H*COCH$_3$), 8.18 (s, 1H, Im-4-*H*), 7.84 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.67 (d, *J* = 8.6 Hz, 2H, Ph-3,5-*H*), 5.09 (s, 1H, O*H*), 4.02 (d, *J* = 12.0 Hz, 1H, C*H*OH), 3.92−3.84 (m, 2H, Im-N-C*H*$_2$CHOH), 3.08 (dd, *J* = 6.9, 3.6 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.87 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.47 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.37 (s, 1H, Pip-N-C*H*$_2$CHOH), 2.29 (s, 3H, Im-C*H*$_3$), 2.10 (s, 3H, COC*H*$_3$) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.7, 146.1, 145.6, 144.0, 129.2, 128.8, 123.2, 119.2, 67.3, 61.2, 52.7, 51.2, 46.3, 24.6, 13.3 ppm.

N-(4-((4-(2-Hydroxy-3-(4-nitro-1H-imidazol-1-yl)propyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (5b). Sulfonamide intermediate **5b** was generated by the same experimental procedure for the synthesis of molecule **5a**. Compound **4** (0.41 g, 1.20 mmol), 4-nitro-1H-imidazole (0.20 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used as starting materials. The pure product **5b** was obtained as white solid. Yield: 33.1%; mp: 180−182 °C; ^{1}H NMR (DMSO-d_6) δ: 10.38 (s, 1H, N*H*COCH$_3$), 8.26 (s, 1H, Im-2-*H*), 7.83 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.72 (s, 1H, Im-5-*H*), 7.68 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 5.12 (d, *J* = 4.0 Hz, 1H, O*H*), 4.11 (dd, *J* = 17.2, 6.6 Hz, 1H, C*H*OH), 3.91−3.87 (m, 2H, Im-N-C*H*$_2$CHOH), 2.89 (d, *J* = 8.3 Hz, 4H, Pip-N-(C*H*$_2$)$_2$), 2.52 (s, 2H, Pip-N-C*H*$_2$), 2.46 (d, *J* = 4.6 Hz, 2H, Pip-N-C*H*$_2$), 2.28 (dd, *J* = 12.7, 5.0 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.22 (dd, *J* = 12.7, 5.9 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.10 (s, 3H, COC*H*$_3$) ppm; ^{13}C NMR (DMSO-d_6) δ: 147.1, 144.0, 138.3, 129.3, 128.9, 122.7, 67.1, 61.0, 52.7, 52.2, 46.3, 24.6 ppm.

N-(4-((4-(2-Hydroxy-3-(2-phenyl-1H-imidazol-1-yl)propyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (5c). Sulfonamide intermediate **5c** was generated by the similar experimental procedure for the synthesis of molecule **5a**. Compound **4** (0.41 g, 1.20 mmol), 2-phenyl-1H-imidazole (0.26 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used as starting materials. The pure product **5c** was obtained as white solid. Yield: 35.2%; mp: > 250 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 10.52 (s, 1H, N*H*COCH$_3$), 7.91 (d, *J* = 8.8 Hz, 2H, Im-2-Ph-2,6-*H*), 7.68 (d, *J* = 8.8 Hz, 2H, Ph-2,6-*H*), 7.54−7.51 (m, 2H, Ph-3,5-*H*), 7.28 (d, *J* = 1.0 Hz, 1H, Im-5-*H*), 7.25 (t, *J* = 7.4 Hz, 1H, Im-2-Ph-4-*H*), 7.16 (t, *J* = 7.6 Hz, 2H, Im-2-Ph-3,5-*H*), 6.94 (d, *J* = 1.0 Hz, 1H, Im-4-*H*), 5.13 (s, 1H, O*H*), 4.12 (q, *J* = 6.7 Hz, 1H, C*H*OH), 3.77 (d, *J* = 9.7 Hz, 2H, Im-N-CH_2CHOH), 2.72 (s, 4H, Pip-N-(CH_2)$_2$), 2.39 (d, *J* = 21.0 Hz, 4H, Pip-N-(CH_2)$_2$), 2.22−2.19 (m, 2H, Pip-N-CH_2CHOH), 2.14 (s, 3H, COCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 147.2, 144.1, 131.6, 129.3, 128.5, 127.9, 122.7, 119.2, 68.3, 61.4, 52.7, 50.8, 46.6, 24.6 ppm.

N-(4-((4-(2-Hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (5d). Sulfonamide intermediate **5d** was generated by the similar experimental procedure for the synthesis of molecule **5a**. Compound **4** (0.41 g, 1.20 mmol), triazole (0.12 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used as starting materials. The pure product **5d** was obtained as yellow liquid. Yield: 30.6%; ^{1}H NMR (DMSO-d_6) δ: 10.37 (s, 1H, N*H*COCH$_3$), 8.36 (s, 1H, triazole 5-*H*), 7.89 (s, 1H, triazole 3-*H*), 7.83 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.67 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 4.95 (d, *J* = 5.0 Hz, 1H, O*H*), 4.17 (dd, *J* = 13.8, 3.6 Hz, 1H, triazole N-CH_2CHOH), 4.03 (dd, *J* = 13.8, 7.5 Hz, 1H, triazole N-CH_2CHOH), 3.92−3.87 (m, 1H, C*H*OH), 2.85 (s, 4H, Pip-N-(CH_2)$_2$), 2.49 (s, 4H, Pip-N-(CH_2)$_2$), 2.28 (dq, *J* = 13.0, 6.5 Hz, 2H, Pip-N-CH_2CHOH), 2.10 (s, 3H, COCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 151.5, 145.1, 144.0, 129.3, 128.8, 119.2, 66.9, 61.3, 53.6, 52.8, 46.4, 24.6 ppm.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (6a). A mixture solution of compound **5a** (0.10 g, 0.20 mmol), ethanol (10 mL) and hydrochloric acid (5 mL, 40%) was stirred at 78 °C for 1 h. After disappearance of the starting material, the reaction solvent was evaporated and neutralized with saturated sodium bicarbonate solution to produce the desired compound **6a** as yellow liquid. Yield: 61.5%; mp: > 250 °C; ^{1}H NMR (DMSO-d_6) δ: 8.19 (s, 1H, Im-4-*H*), 7.35 (d, *J* = 8.6 Hz, 2H, Ph-2,6-*H*), 6.66 (d, *J* = 8.6 Hz, 2H, Ph-3,5-*H*), 6.08 (s, 2H, Ph-NH_2), 5.09 (d, *J* = 5.0 Hz, 1H, O*H*), 4.03 (dd, *J* = 14.0, 2.3 Hz, 1H, Im-N-CH_2CHOH), 3.87 (d, *J* = 5.4 Hz, 1H, C*H*OH), 3.79 (dd, *J* = 14.0, 8.4 Hz, 1H, Im-N-CH_2CHOH), 2.81 (s, 4H, Pip-N-(CH_2)$_2$), 2.52 (s, 2H, Pip-N-CH_2), 2.47 (s, 2H, Pip-N-CH_2), 2.30 (s, 5H, Pip-N-CH_2CHOH, COCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ: 153.7, 146.1, 145.6, 130.1, 123.2, 119.9, 113.2, 67.4, 61.4, 52.8, 51.3, 46.3, 13.3 ppm; HR-ESIMS m/z calcd for $C_{17}H_{24}N_6O_5S$ $[M + H]^+$, 425.1607; found, 425.1606.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(4-nitro-1H-imidazol-1-yl)propan-2-ol (6b). Sulfonamide derivative **6b** was produced by the similar experimental procedure for the synthesis of molecule **6a**. Compound **5b** (0.10 g, 0.22 mmol) and hydrochloric acid (5 mL, 40%) were used as starting materials. The pure product **6b** was acquired as yellow solid. Yield: 68.9%; mp: 120−122 °C; ^{1}H NMR (DMSO-d_6) δ: 8.27 (d, *J* = 0.7 Hz, 1H, Im-2-*H*), 7.73 (d, *J* = 0.9 Hz, 1H, Im-5-*H*), 7.35 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 6.66 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 6.08 (s, 2H, Ph-NH_2), 5.13 (d, *J* = 4.3 Hz, 1H, O*H*), 4.12 (q, *J* = 6.5 Hz, 1H, C*H*OH), 3.88 (q, *J* = 7.6 Hz, 2H, Im-N-CH_2CHOH), 2.81 (s, 4H, Pip-N-(CH_2)$_2$), 2.51 (s, 2H, Pip-N-CH_2), 2.45 (s, 2H, Pip-N-CH_2), 2.27 (dd, *J* = 12.7, 5.0 Hz, 1H, Pip-N-CH_2CHOH), 2.22 (dd, *J* = 12.7, 6.1 Hz, 1H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 153.7, 147.1, 138.4, 130.1, 122.7, 119.9, 113.2, 67.0,

61.2, 52.8, 52.2, 46.3 ppm; HR-ESIMS m/z calcd for $C_{16}H_{22}N_6O_5S$ $[M + H]^+$, 411.1451; found, 411.1450.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(2-phenyl-1H-imidazol-1-yl)propan-2-ol (6c). Sulfonamide derivative **6c** was produced by the same experimental procedure for the synthesis of molecule **6a**. Compound **5c** (0.10 g, 0.21 mmol), and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **6c** was acquired as yellow solid. Yield: 66.6%; mp: 76−78 ^oC; ^1H NMR (DMSO-d_6) δ: 7.56 (d, J = 7.3 Hz, 2H, Ph-2,6-*H*), 7.38−7.34 (m, 4H, Im-2-Ph-2,3,5,6-*H*), 7.20 (t, J = 7.7 Hz, 2H, Ph-3,5-*H*), 7.03 (s, 1H, Im-2-Ph-4-*H*), 6.73 (d, J = 8.7 Hz, 2H, Im-4,5-*H*), 6.19 (s, 2H, Ph-NH_2), 5.17 (s, 1H, O*H*), 4.16 (q, J = 6.5 Hz, 1H, C*H*OH), 3.78 (d, J = 9.3 Hz, 2H, Im-N-CH_2CHOH), 2.69 (s, 4H, Pip-N-(CH_2)$_2$), 2.41 (d, J = 28.6 Hz, 4H, Pip-N-(CH_2)$_2$), 2.24 (d, J = 4.4 Hz, 2H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 153.8, 147.0, 130.7, 130.2, 129.4, 129.0, 128.7, 127.1, 123.0, 119.5, 113.2, 68.2, 61.5, 52.8, 51.0, 46.4 ppm; HR-ESIMS m/z calcd for $C_{22}H_{27}N_5O_3S$ $[M + H]^+$, 442.1913; found, 442.1915.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (6d). Sulfonamide derivative **6d** was produced by the similar process depicted for compound **6a**. Compound **5d** (0.10 g, 0.24 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **6d** was acquired as yellow liquid. Yield: 69.2%; ^1H NMR (DMSO-d_6) δ: 8.37 (s, 1H, triazole 5-*H*), 7.90 (s, 1H, triazole 3-*H*), 7.35 (d, J = 8.7 Hz, 2H, Ph-2,6-*H*), 6.66 (d, J = 8.7 Hz, 2H, Ph-3,5-*H*), 6.08 (s, 2H, Ph-NH_2), 4.98 (s, 1H, O*H*), 4.18 (dd, J = 13.8, 3.6 Hz, 1H, triazole N-CH_2CHOH), 4.03 (dd, J = 13.8, 7.5 Hz, 1H, triazole N-CH_2CHOH), 3.91−3.88 (m, 1H, C*H*OH), 2.79 (s, 4H, Pip-N-(CH_2)$_2$), 2.48 (s, 4H, Pip-N-(CH_2)$_2$), 2.31−2.25 (m, 2H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 153.7, 151.5, 145.1, 130.1, 113.2,

66.9, 61.4, 53.7, 52.8, 46.3 ppm; HR-ESIMS m/z calcd for $C_{15}H_{22}N_6O_3S$ $[M + H]^+$, 367.1552; found, 367.1551.

N-(4-((4-(2-Hydroxy-3-(1H-tetrazol-1-yl)propyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (7a). Sulfonamide intermediate **7a** was generated by the similar process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), tetrazole (0.13 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **7a** was acquired as yellow liquid. Yield: 30.5%; ^{1}H NMR (DMSO-d_6) δ: 10.46 (s, 1H, N*H*COCH$_3$), 9.24 (s, 1H, tetrazole 5-*H*), 7.84 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.67 (d, *J* = 8.8 Hz, 2H, Ph-3,5-*H*), 4.48 (dd, *J* = 14.0, 3.3 Hz, 1H, tetrazole N-C*H*$_2$CHOH), 4.31 (dd, *J* = 14.0, 7.8 Hz, 1H, tetrazole N-C*H*$_2$CHOH), 3.97−3.92 (m, 1H, C*H*OH), 2.86 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.50 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.30 (d, *J* = 6.2 Hz, 2H, Pip-N-C*H*$_2$CHOH), 2.10 (s, 3H, COC*H*$_3$) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 145.0, 144.8, 144.0, 129.3, 119.2, 66.7, 61.0, 52.7, 52.3, 46.4, 24.6 ppm.

N-(4-((4-(2-Hydroxy-3-(5-methyl-1H-tetrazol-1-yl)propyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (7b). Sulfonamide intermediate **7b** was generated by the same process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), 5-methyl-1H-tetrazole (0.15 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **7b** was acquired as white solid. Yield: 29.5%; mp: 95−97 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 10.38 (s, 1H, N*H*COCH$_3$), 7.83 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.67 (d, *J* = 8.8 Hz, 2H, Ph-3,5-*H*), 5.10 (s, 1H, O*H*), 4.37 (dd, *J* = 14.3, 3.4 Hz, 1H, tetrazole N-C*H*$_2$CHOH), 4.16 (dd, *J* = 14.3, 7.9 Hz, 1H, tetrazole N-C*H*$_2$CHOH), 3.92 (d, *J* = 3.6 Hz, 1H, C*H*OH), 2.86 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.51 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.45 (s, 3H, tetrazole C*H*$_3$), 2.37 (dd, *J* = 12.9, 6.6 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.32 (dd, *J* = 12.8, 6.0 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.09 (s, 3H, COC*H*$_3$) ppm; ^{13}C

NMR (DMSO-d_6) δ: 169.6, 153.4, 144.0, 129.3, 128.8, 119.2, 67.3, 61.1, 52.8, 51.2, 46.4, 24.6, 9.1 ppm.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(1H-tetrazol-1-yl)propan-2-ol (8a). Sulfonamide derivative **8a** was produced by the similar process depicted for compound **6a**. Compound **7a** (0.10 g, 0.24 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **8a** was acquired as white solid. Yield: 67.6%; mp: 94−96 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 9.24 (s, 1H, tetrazole 5-*H*), 7.35 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 6.66 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 6.08 (s, 2H, Ph-NH_2), 5.15 (d, *J* = 5.1 Hz, 1H, O*H*), 4.49 (dd, *J* = 14.0, 3.2 Hz, 1H, tetrazole N-CH_2CHOH), 4.31 (dd, *J* = 14.0, 7.8 Hz, 1H, tetrazole N-CH_2CHOH), 3.95 (dd, *J* = 9.4, 6.5 Hz, 1H, C*H*OH), 2.80 (s, 4H, Pip-N-(CH_2)$_2$), 2.50 (s, 4H, Pip-N-(CH_2)$_2$), 2.30 (d, *J* = 6.2 Hz, 2H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 153.7, 144.9, 130.1, 119.9, 113.2, 66.7, 61.1, 52.8, 52.3, 46.3 ppm; HR-ESIMS m/z calcd for $C_{14}H_{21}N_7O_3S$ $[M + H]^+$, 368.1505; found, 368.1505.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(5-methyl-1H-tetrazol-1-yl)propan-2-ol (8b). Sulfonamide derivative **8b** was produced by the similar process depicted for compound **6a**. Compound **7b** (0.10 g, 0.24 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **8b** was acquired as white solid. Yield: 70.2%; mp: 100−102 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 7.35 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 6.66 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 6.08 (s, 2H, Ph-NH_2), 5.12 (s, 1H, O*H*), 4.38 (dd, *J* = 14.3, 3.2 Hz, 1H, tetrazole N-CH_2CHOH), 4.17 (dd, *J* = 14.2, 7.8 Hz, 1H, tetrazole N-CH_2CHOH), 3.94 (s, 1H, C*H*OH), 2.80 (s, 4H, Pip-N-(CH_2)$_2$), 2.51 (s, 4H, Pip-N-(CH_2)$_2$), 2.46 (s, 3H, tetrazole CH_3), 2.37 (d, *J* = 15.9 Hz, 2H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 153.7, 153.4, 130.1, 119.8, 113.2,

67.3, 61.2, 52.8, 51.2, 46.3, 9.1 ppm; HR-ESIMS m/z calcd for $C_{15}H_{23}N_7O_3S$ $[M + H]^+$, 382.1661; found, 382.1659.

N-(4-((4-(3-((1H-1,2,4-Triazol-3-yl)thio)-2-hydroxypropyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (9). Sulfonamide intermediate **9** was generated by the similar process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), 1H-1,2,4-triazole-3-thiol (0.18 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **9** was acquired as yellow liquid. Yield: 28.4%; ^{1}H NMR (DMSO-d_6) δ: 10.38 (s, 1H, N*H*COCH$_3$), 8.31 (s, 1H, triazole 5-*H*), 7.83 (d, *J* = 8.6 Hz, 2H, Ph-2,6-*H*), 7.66 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 3.80 (dt, *J* = 11.5, 5.9 Hz, 1H, C*H*OH), 3.23 (dd, *J* = 13.2, 4.5 Hz, 1H, triazole N-CH_2CHOH), 2.99 (dd, *J* = 13.2, 7.0 Hz, 1H, triazole N-CH_2CHOH), 2.84 (s, 4H, Pip-N-(CH_2)$_2$), 2.46 (s, 4H, Pip-N-(CH_2)$_2$), 2.38 (dd, *J* = 12.8, 5.5 Hz, 1H, Pip-N-CH_2CHOH), 2.33 (dd, *J* = 12.7, 6.4 Hz, 1H, Pip-N-CH_2CHOH), 2.10 (s, 3H, COCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 147.0, 144.0, 129.3, 128.9, 119.2, 67.6, 62.8, 52.7, 46.4, 37.5, 24.6 ppm.

1-((1H-1,2,4-Triazol-3-yl)thio)-3-(4-((4-aminophenyl)sulfonyl)piperazin-1-yl)propan-2-ol (10). Sulfonamide derivative **10** was produced by the similar process depicted for compound **6a**. Compound **9** (0.10 g, 0.23 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **10** was acquired as white solid. Yield: 69.9%; mp: 96−98 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 8.31 (s, 1H, triazole 5-*H*), 7.34 (d, *J* = 8.5 Hz, 2H, Ph-2,6-*H*), 6.65 (d, *J* = 8.6 Hz, 2H, Ph-3,5-*H*), 6.06 (s, 2H, Ph-NH_2), 4.94 (s, 1H, O*H*), 3.82−3.78 (m, 1H, C*H*OH), 3.23 (dd, *J* = 13.2, 4.5 Hz, 1H, triazole N-CH_2CHOH), 3.00 (dd, *J* = 13.2, 7.0 Hz, 1H, triazole N-CH_2CHOH), 2.78 (s, 4H, Pip-N-(CH_2)$_2$), 2.46 (d, *J* = 15.3 Hz, 4H, Pip-N-(CH_2)$_2$), 2.39−2.31 (m, 2H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 153.7, 147.0, 130.1, 120.0, 113.2, 67.6,

62.9, 52.8, 46.3, 37.6 ppm; HR-ESIMS m/z calcd for $C_{15}H_{22}N_6O_3S_2$ $[M + H]^+$, 399.1273; found, 399.1271.

N-(4-((4-(2-Hydroxy-3-((1-methyl-1H-imidazol-2-yl)thio)propyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (11). Sulfonamide intermediate **11** was prepared according to the same process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), 1-methyl-1H-imidazole-2-thiol (0.21 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **11** was acquired as light yellow liquid. Yield: 27.6%; ^{1}H NMR (DMSO-d_6) δ: 10.42 (s, 1H, N*H*COCH$_3$), 7.84 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.66 (d, *J* = 8.8 Hz, 2H, Ph-3,5-*H*), 7.17 (s, 1H, Im-5-*H*), 6.86 (d, *J* = 1.0 Hz, 1H, Im-4-*H*), 3.78−3.74 (m, 1H, C*H*OH), 3.52 (s, 3H, Im-C*H*$_3$), 3.10 (dd, *J* = 13.3, 4.5 Hz, 1H, Im-N-C*H*$_2$CHOH), 2.92 (dd, *J* = 13.3, 6.7 Hz, 1H, Im-N-C*H*$_2$CHOH), 2.82 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.47 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.37 (dd, *J* = 12.8, 5.8 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.32 (dd, *J* = 12.7, 6.4 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.10 (s, 3H, COC*H*$_3$) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 144.0, 141.5, 129.3, 128.6, 123.4, 119.2, 67.9, 62.5, 52.7, 46.3, 39.4, 33.3, 24.6 ppm.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-((1-methyl-1H-imidazol-2-yl)thio)propan-2-ol (12). Sulfonamide derivative **12** was produced by the similar process depicted for compound **6a**. Compound **11** (0.10 g, 0.22 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **12** was acquired as white solid. Yield: 58.7%; mp: 178−180 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 7.34 (d, *J* = 8.6 Hz, 2H, Ph-2,6-*H*), 7.17 (s, 1H, Im-5-*H*), 6.87 (s, 1H, Im-4-*H*), 6.66 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 6.09 (s, 2H, Ph-N*H*$_2$), 3.80−3.75 (m, 1H, C*H*OH), 3.53 (s, 3H, Im-C*H*$_3$), 3.11 (dd, *J* = 13.3, 4.5 Hz, 1H, Im-N-C*H*$_2$CHOH), 2.94−2.91 (m, 1H, Im-N-C*H*$_2$CHOH), 2.77 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.48 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.40 (dd, *J* = 12.8, 5.6 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.35 (dd, *J* = 12.7, 6.5 Hz, 1H, Pip-N-C*H*$_2$CHOH) ppm;

^{13}C NMR (DMSO-d_6) δ: 153.7, 141.5, 130.1, 128.5, 123.4, 119.8, 113.2, 67.8, 62.5, 52.7, 46.2, 39.4, 33.3 ppm; HR-ESIMS m/z calcd for $C_{17}H_{25}N_5O_3S_2$ $[M + H]^+$, 412.1477; found, 412.1478.

N-(4-((4-(3-(1H-Benzo[d]imidazol-1-yl)-2-hydroxypropyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (13a). Sulfonamide intermediate **13a** was prepared according to the similar process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), benzimidazole (0.21 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **13a** was acquired as yellow liquid. Yield: 27.3%; ^{1}H NMR (DMSO-d_6) δ: 10.42 (s, 1H, N*H*COCH$_3$), 8.07 (s, 1H, Bim-2-*H*), 7.85 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.67 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 7.56 (d, *J* = 7.7 Hz, 1H, Bim-7-*H*), 7.49 (d, *J* = 7.6 Hz, 1H, Bim-4-*H*), 7.17−7.12 (m, 2H, Bim-5,6-*H*), 5.02 (d, *J* = 4.6 Hz, 1H, O*H*), 4.24 (dd, *J* = 14.3, 3.7 Hz, 1H, Bim-N-C*H*$_2$CHOH), 4.09 (dd, *J* = 14.3, 7.0 Hz, 1H, Bim-N-C*H*$_2$CHOH), 3.93 (d, *J* = 5.1 Hz, 1H, C*H*OH), 2.83 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.45 (dd, *J* = 30.3, 3.2 Hz, 4H, Pip-N-(C*H*$_2$)$_2$), 2.27 (d, *J* = 6.2 Hz, 2H, Pip-N-C*H*$_2$CHOH), 2.10 (s, 3H, COC*H*$_3$) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 145.1, 144.0, 143.7, 134.9, 129.3, 122.3, 121.6, 119.6, 119.2, 111.1, 66.9, 61.4, 55.38, 52.8, 46.3, 24.6 ppm.

N-(4-((4-(2-Hydroxy-3-(5-methyl-1H-benzo[d]imidazol-1-yl)propyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (13b). Sulfonamide intermediate **13b** was prepared according to the same process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), 5-methyl-1H-benzoimidazole (0.24 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **13b** was acquired as yellow liquid. Yield: 26.5%; ^{1}H NMR (DMSO-d_6) δ: 10.44 (s, 1H, N*H*COCH$_3$), 8.00 (d, *J* = 12.0 Hz, 1H, Bim-2-*H*), 7.86 (dd, *J* = 8.7, 1.6 Hz, 2H, Ph-2,6-*H*), 7.68 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 7.44−7.25 (m, 2H, Bim-6,7-*H*), 6.97 (dd, *J* = 15.3, 8.4 Hz, 1H, Bim-4-*H*), 5.03 (d, *J* = 9.4 Hz, 1H, O*H*), 4.21−4.17 (m, 1H, Bim-N-C*H*$_2$CHOH), 4.08−4.04 (m, 1H, Bim-N-C*H*$_2$CHOH), 3.92 (s, 1H, C*H*OH), 2.85 (s, 4H, Pip-N-

(CH_2)$_2$), 2.43 (s, 4H, Pip-N-(CH_2)$_2$), 2.36 (d, J = 26.4 Hz, 3H, Bim-CH_3), 2.26 (dd, J = 11.5, 6.2 Hz, 2H, Pip-N-CH_2CHOH), 2.11 (s, 3H, COCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 144.9, 144.6, 144.0, 131.6, 130.6, 129.3, 123.8, 123.2, 119.2, 110.8, 110.7, 67.0, 61.3, 52.8, 48.8, 46.3, 24.6, 21.8 ppm.

N-(4-((4-(3-(1H-Benzo[d][1,2,3]triazol-1-yl)-2-hydroxypropyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (13c). Sulfonamide intermediate **13c** was prepared according to the similar process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), benzotriazole (0.21 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **13c** was acquired as yellow liquid. Yield: 31.1%; ^{1}H NMR (DMSO-d_6) δ:10.47 (s, 1H, NHCOCH$_3$), 7.87 (d, J = 8.5 Hz, 3H, Ph-2,6-H, benzotriazole 4-H), 7.78 (d, J = 8.4 Hz, 1H, benzotriazole 7-H), 7.64 (d, J = 8.8 Hz, 2H, Ph-3,5-H), 7.44 (t, J = 7.6 Hz, 1H, benzotriazole 6-H), 7.29 (t, J = 7.6 Hz, 1H, benzotriazole 5-H), 5.10 (d, J = 4.9 Hz, 1H, OH), 4.70 (dd, J = 14.2, 4.5 Hz, 1H, benzotriazole N-CH_2CHOH), 4.58 (dd, J = 14.2, 6.8 Hz, 1H, benzotriazole N-CH_2CHOH), 4.11−4.07 (m, 1H, CHOH), 2.71 (s, 4H, Pip-N-(CH_2)$_2$), 2.43 (s, 4H, Pip-N-(CH_2)$_2$), 2.40 (d, J = 6.9 Hz, 1H, Pip-N-CH_2CHOH), 2.33 (dd, J = 12.8, 5.6 Hz, 1H, Pip-N-CH_2CHOH), 2.12 (s, 3H, COCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 145.5, 144.0, 134.3, 129.3, 127.1, 123.9, 119.2, 111.7, 67.4, 61.7, 52.7, 49.1, 46.2, 24.6 ppm.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(1H-benzo[d]imidazol-1-yl)propan-2-ol (14a). Sulfonamide derivative **14a** was generated by the similar process depicted for compound **6a**. Compound **13a** (0.10 g, 0.22 mmol), and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **14a** was acquired as yellow solid. Yield: 71.1%; mp: 120−122 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 8.07 (s, 1H, Bim-2-H), 7.58 (d, J = 7.6 Hz, 1H, Bim-7-H), 7.50 (d, J = 7.7 Hz, 1H, Bim-4-H), 7.35 (d, J = 8.6 Hz, 2H, Ph-2,6-H), 7.19−7.13 (m, 2H, Ph-

3,5-*H*), 6.68 (d, *J* = 8.6 Hz, 2H, Bim-5,6-*H*), 6.08 (s, 2H, Ph-NH_2), 5.00 (d, *J* = 4.5 Hz, 1H, O*H*), 4.24 (dd, *J* = 14.3, 3.2 Hz, 1H, Bim-N-CH_2CHOH), 4.09 (dd, *J* = 14.3, 7.0 Hz, 1H, Bim-N-CH_2CHOH), 3.93 (s, 1H, C*H*OH), 2.79 (s, 4H, Pip-N-(CH_2)$_2$), 2.45 (d, *J* = 30.7 Hz, 4H, Pip-N-(CH_2)$_2$), 2.27 (d, *J* = 6.1 Hz, 2H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 153.7, 145.1, 143.7, 134.9, 130.1, 122.4, 121.6, 119.8, 113.2, 111.1, 67.0, 61.4, 52.9, 49.1, 46.3 ppm; HR-ESIMS m/z calcd for $C_{20}H_{25}N_5O_3S$ $[M + H]^+$, 416.1756; found, 416.1754.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(5-methyl-1H-benzo[d]imidazol-1-yl)propan-2-ol (14b). Sulfonamide derivative **14b** was generated by the similar process depicted for compound **6a**. Compound **13b** (0.10 g, 0.21 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **14b** was obtained as white solid. Yield: 70.9%; mp: 140−142 °C; ^{1}H NMR (DMSO-d_6) δ: 8.05 (d, *J* = 19.6 Hz, 1H, Bim-2-*H*), 7.48−7.38 (m, 2H, Bim-6,7-*H*), 7.36 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.00 (dd, *J* = 20.0, 8.2 Hz, 1H, Bim-4-*H*), 6.68 (dd, *J* = 8.6, 1.5 Hz, 2H, Ph-3,5-*H*), 6.11 (s, 2H, Ph-NH_2), 5.13 (s, 1H, O*H*), 4.24−4.19 (m, 1H, Bim-N-CH_2CHOH), 4.08 (dd, *J* = 14.4, 6.9 Hz, 1H, Bim-N-CH_2CHOH), 3.96 (s, 1H, C*H*OH), 3.07 (q, *J* = 7.3 Hz, 1H, Pip-N-CH_2CHOH), 2.84 (s, 4H, Pip-N-(CH_2)$_2$), 2.58 (s, 4H, Pip-N-(CH_2)$_2$), 2.38 (d, *J* = 15.2 Hz, 3H, Bim-CH_3), 2.35 (s, 1H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 153.8, 144.8, 144.5, 130.1, 124.0, 123.4, 119.1, 113.3, 110.9, 66.7, 61.0, 52.7, 49.1, 46.0, 21.8 ppm; HR-ESIMS m/z calcd for $C_{21}H_{27}N_5O_3S$ $[M + H]^+$, 430.1913; found, 430.1912.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(1H-benzo[d][1,2,3]triazol-1-yl)propan-2-ol (14c). Sulfonamide derivative **14c** was generated by the similar process depicted for compound **6a**. Compound **13c** (0.10 g, 0.22 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **14c** was acquired as brown solid. Yield: 56.4%;

mp: 248−250 °C; ^{1}H NMR (DMSO-d_6) δ: 7.97−7.78 (m, 2H, benzotriazole 4,7-*H*), 7.47 (s, 2H, benzotriazole 5,6-*H*), 7.34 (s, 2H, Ph-2,6-*H*), 6.68 (d, *J* = 8.6 Hz, 2H, Ph-2,6-*H*), 6.13 (s, 2H, Ph-NH_2), 5.17−5.05 (m, 1H, O*H*), 4.73 (dd, *J* = 14.2, 7.6 Hz, 1H, benzotriazole N-CH_2CHOH), 4.60 (dd, *J* = 14.2, 8.0 Hz, 1H, benzotriazole N-CH_2CHOH), 4.03 (d, *J* = 71.1 Hz, 1H, C*H*OH), 3.51 (d, *J* = 81.0 Hz, 2H, Pip-N-CH_2CHOH), 2.67 (s, 4H, Pip-N-(CH_2)$_2$), 2.43 (s, 4H, Pip-N-(CH_2)$_2$) ppm; ^{13}C NMR (DMSO-d_6) δ: 153.7, 145.5, 134.3, 130.1, 127.3, 124.0, 119.2, 113.2, 111.8, 71.4, 63.9, 55.4, 52.7, 46.1 ppm; HR-ESIMS m/z calcd for $C_{19}H_{24}N_6O_3S$ $[M + H]^+$, 417.1709; found, 417.1708.

N-(4-((4-(3-(9H-Carbazol-9-yl)-2-hydroxypropyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (15a). Sulfonamide intermediate **15a** was produced according to the similar process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), 9H-carbazole (0.30 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **15a** was acquired as light yellow liquid. Yield: 29.6%; ^{1}H NMR (DMSO-d_6) δ: 10.44 (s, 1H, N*H*COCH$_3$), 8.01 (d, *J* = 7.7 Hz, 2H, Cb-4,5-*H*), 7.88 (d, *J* = 8.7 Hz, 2H, Cb-1,8-*H*), 7.64 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.52 (d, *J* = 8.2 Hz, 2H, Ph-3,5-*H*), 7.33 (t, *J* = 7.7 Hz, 2H, Cb-2,7-*H*), 7.12 (t, *J* = 7.4 Hz, 2H, Cb-3,6-*H*), 4.96 (d, *J* = 4.9 Hz, 1H, O*H*), 4.36 (dd, *J* = 14.8, 4.8 Hz, 1H, Cb-N-CH_2CHOH), 4.24 (dd, *J* = 14.8, 6.5 Hz, 1H, Cb-N-CH_2CHOH), 4.00 (dd, *J* = 11.4, 5.9 Hz, 1H, C*H*OH), 2.69 (s, 4H, Pip-N-(CH_2)$_2$), 2.40 (dd, *J* = 12.5, 6.5 Hz, 4H, Pip-N-(CH_2)$_2$), 2.32 (dd, *J* = 12.7, 5.7 Hz, 2H, Pip-N-CH_2CHOH), 2.12 (s, 3H, COCH_3) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 144.0, 141.1, 129.3, 125.8, 122.5, 120.4, 119.2, 119.0, 110.2, 67.2, 62.1, 52.8, 49.1, 46.2, 24.6 ppm.

N-(4-((4-(3-(2-Bromo-9H-carbazol-9-yl)-2-hydroxypropyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (15b). Sulfonamide intermediate **15b** was produced according to the same process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), 2-bromo-9H-carbazole

(0.44 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **15b** was acquired as white solid. Yield: 25.6%; mp: > 250 °C; ^{1}H NMR (DMSO-d_6) δ: 10.42 (s, 1H, N*H*COCH$_3$), 8.05 (d, *J* = 7.7 Hz, 1H, Cb-4-*H*), 7.98 (d, *J* = 8.2 Hz, 1H, Cb-5-*H*), 7.87 (d, *J* = 8.8 Hz, 2H, Ph-2,6-*H*), 7.76 (s, 1H, Cb-1-*H*), 7.66 (d, *J* = 8.8 Hz, 2H, Ph-3,5-*H*), 7.56 (d, *J* = 8.3 Hz, 1H, Cb-8-*H*), 7.38 (t, *J* = 7.5 Hz, 1H, Cb-7-*H*), 7.24 (d, *J* = 8.3 Hz, 1H, Cb-3-*H*), 7.16 (t, *J* = 7.4 Hz, 1H, Cb-6-*H*), 5.02 (d, *J* = 5.0 Hz, 1H, O*H*), 4.36 (dd, *J* = 14.9, 4.2 Hz, 1H, Cb-N-C*H*$_2$CHOH), 4.23 (dd, *J* = 14.9, 6.2 Hz, 1H, Cb-N-C*H*$_2$CHOH), 3.99−3.94 (m, 1H, C*H*OH), 2.80 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.45 (d, *J* = 11.0 Hz, 4H, Pip-N-(C*H*$_2$)$_2$), 2.37 (d, *J* = 7.5 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.27 (dd, *J* = 12.6, 5.0 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.12 (s, 3H, COC*H*$_3$) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 144.1, 142.1, 141.4, 129.3, 128.7, 126.3, 122.0, 121.7, 120.6, 119.6, 119.2, 118.7, 113.2, 110.7, 67.4, 61.6, 52.9, 48.1, 46.3, 24.6 ppm.

N-(4-((4-(3-(3,6-Dibromo-9H-carbazol-9-yl)-2-hydroxypropyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (15c). Sulfonamide intermediate **15c** was produced according to the similar process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), 3,6-dibromo-9H-carbazole (0.59 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **15c** was acquired as white solid. Yield: 18.8%; mp: 175−177 °C; ^{1}H NMR (DMSO-d_6) δ: 10.45 (s, 1H, N*H*COCH$_3$), 8.34 (d, *J* = 1.0 Hz, 2H, Cb-4,5-*H*), 7.88 (d, *J* = 8.7 Hz, 2H, Cb-1,8-*H*), 7.66 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.53 (d, *J* = 8.8 Hz, 2H, Ph-3,5-*H*), 7.49 (dd, *J* = 8.7, 1.5 Hz, 2H, Cb-2,7-*H*), 4.95 (d, *J* = 3.7 Hz, 1H, O*H*), 4.34 (dd, *J* = 14.9, 4.0 Hz, 1H, Cb-N-C*H*$_2$CHOH), 4.24 (dd, *J* = 14.9, 6.7 Hz, 1H, Cb-N-C*H*$_2$CHOH), 3.96 (s, 1H, C*H*OH), 2.75 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.43 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.37 (d, *J* = 6.4 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.30 (dd, *J* = 12.6, 5.6 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.11 (s, 3H, COC*H*$_3$) ppm; ^{13}C NMR (DMSO-d_6)

δ: 169.6, 144.0, 140.2, 129.3, 128.9, 123.5, 123.3, 119.2, 112.7, 111.6, 67.2, 61.7, 52.8, 48.3, 46.3, 24.6 ppm.

N-(4-((4-(2-Hydroxy-3-(3-iodo-9H-carbazol-9-yl)propyl)piperazin-1-yl)sulfonyl)phenyl)acetamide(15d). Sulfonamide intermediate **15d** was produced according to the same process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), 3-iodo-9H-carbazole (0.53 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **15d** was acquired as white solid. Yield: 23.7%; mp: 186−188 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 10.43 (s, 1H, N*H*COCH$_3$), 8.42 (s, 1H, Cb-5-*H*), 8.06 (d, *J* = 7.7 Hz, 1H, Cb-4-*H*), 7.88 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 7.65 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 7.60 (d, *J* = 8.7 Hz, 1H, Cb-8-*H*), 7.53 (d, *J* = 8.3 Hz, 1H, Cb-2-*H*), 7.41 (d, *J* = 8.6 Hz, 1H, Cb-1-*H*), 7.36 (t, *J* = 7.6 Hz, 1H, Cb-7-*H*), 7.14 (t, *J* = 7.4 Hz, 1H, Cb-6-*H*), 4.94 (d, *J* = 4.7 Hz, 1H, O*H*), 4.34 (dd, *J* = 14.8, 4.3 Hz, 1H, Cb-N-C*H*$_2$CHOH), 4.22 (dd, *J* = 14.8, 6.6 Hz, 1H, Cb-N-C*H*$_2$CHOH), 3.98 (dd, *J* = 10.4, 5.1 Hz, 1H, C*H*OH), 2.73 (s, 4H, Pip-N-(C*H*$_2$)$_2$), 2.39 (d, *J* = 13.0 Hz, 4H, Pip-N-(C*H*$_2$)$_2$), 2.37 (s, 1H, Pip-N-C*H*$_2$CHOH), 2.31 (dd, *J* = 12.6, 5.5 Hz, 1H, Pip-N-C*H*$_2$CHOH), 2.12 (s, 3H, COC*H*$_3$) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.6, 144.0, 141.1, 140.3, 133.6, 129.3, 128.9, 126.5, 125.2, 121.3, 120.8, 119.5, 119.2, 112.9, 110.5, 81.8, 67.2, 61.9, 52.8, 48.2, 46.2, 24.6 ppm.

N-(4-((4-(3-(3,6-Di-tert-butyl-9H-carbazol-9-yl)-2-hydroxypropyl)piperazin-1-yl)sulfonyl)phenyl)acetamide (15e). Sulfonamide intermediate **15e** was produced according to the similar process like compound **5a**. Compound **4** (0.41 g, 1.20 mmol), 3,6-di-tert-butyl-9H-carbazole (0.50 g, 1.80 mmol) and TEA (1.40 mL, 10.00 mmol) were used starting materials. The pure product **15e** was acquired as white solid. Yield: 24.3%; mp: > 250 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 10.40 (s, 1H, N*H*COCH$_3$), 8.10 (s, 2H, Cb-4,5-*H*), 7.86 (d, *J* = 8.6 Hz, 2H, Ph-2,6-*H*), 7.68 (d, *J* = 8.6 Hz, 2H, Ph-3,5-*H*), 7.38 (q, *J* = 8.9 Hz, 4H, Cb-1,2,7,8-*H*), 4.88 (d, *J* =

4.8 Hz, 1H, O*H*), 4.28 (dd, *J* = 14.8, 4.0 Hz, 1H, Cb-N-CH_2CHOH), 4.15 (dd, *J* = 14.8, 6.9 Hz, 1H, Cb-N-CH_2CHOH), 3.97 (dd, *J* = 9.9, 4.7 Hz, 1H, C*H*OH), 2.84 (s, 4H, Pip-N-(CH_2)$_2$), 2.45 (s, 4H, Pip-N-(CH_2)$_2$), 2.38 (dd, *J* = 12.5, 6.3 Hz, 1H, Pip-N-CH_2CHOH), 2.32 (dd, *J* = 12.6, 5.9 Hz, 1H, Pip-N-CH_2CHOH), 2.09 (s, 3H, COCH_3), 1.39 (s, 18H, Cb-3,6-C(CH_3)$_3$) ppm; ^{13}C NMR (DMSO-d_6) δ: 169.5, 144.0, 141.3, 139.6, 129.3, 123.3, 122.5, 119.1, 116.4, 109.6, 67.7, 61.9, 52.9, 48.1, 46.4, 34.8, 32.4, 24.6 ppm.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(9H-carbazol-9-yl)propan-2-ol (16a). Sulfonamide derivative **16a** was generated by the similar process depicted for compound **6a**. Compound **15a** (0.10 g, 0.20 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **16a** was acquired as yellow solid. Yield: 73.5%; mp: > 250 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 8.14−8.03 (m, 2H, Cb-4,5-*H*), 7.63−7.52 (m, 2H, Cb-1,8-*H*), 7.35 (s, 4H, Ph-2,3,5,6-*H*), 7.20−7.12 (m, 2H, Cb-2,7-*H*), 6.70 (d, *J* = 6.7 Hz, 2H, Cb-3,6-*H*), 6.15 (s, 2H, Ph-NH_2), 4.98 (s, 1H, O*H*), 4.37 (d, *J* = 11.1 Hz, 1H, Cb-N-CH_2CHOH), 4.26 (d, *J* = 6.8 Hz, 1H, C*H*OH), 4.06 (d, *J* = 62.1 Hz, 1H, Cb-N-CH_2CHOH), 3.17 (s, 2H, Pip-N-CH_2CHOH), 2.67 (s, 4H, Pip-N-(CH_2)$_2$), 2.39 (s, 4H, Pip-N-(CH_2)$_2$) ppm; ^{13}C NMR (DMSO-d_6) δ: 141.1, 130.1, 125.8, 122.6, 120.4, 119.1, 113.3, 110.3, 67.3, 62.1, 52.9, 49.1, 46.2 ppm; HR-ESIMS m/z calcd for $C_{25}H_{28}N_4O_3S$ $[M + H]^+$, 465.1960; found, 465.1960.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(2-bromo-9H-carbazol-9-yl)propan-2-ol (16b). Sulfonamide derivative **16b** was generated by the same process depicted for compound **6a**. Compound **15b** (0.10 g, 0.17 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **16b** was acquired as brown solid. Yield: 72.2%; mp: 188−190 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 8.15 (d, *J* = 7.4 Hz, 1H, Cb-4-*H*), 8.09 (d, *J* = 8.0 Hz, 1H, Cb-5-*H*), 7.95 (s, 1H, Cb-1-*H*), 7.71 (d, *J* = 7.6 Hz, 1H, Cb-8-*H*), 7.47 (t, *J* = 7.3 Hz, 1H, Cb-7-

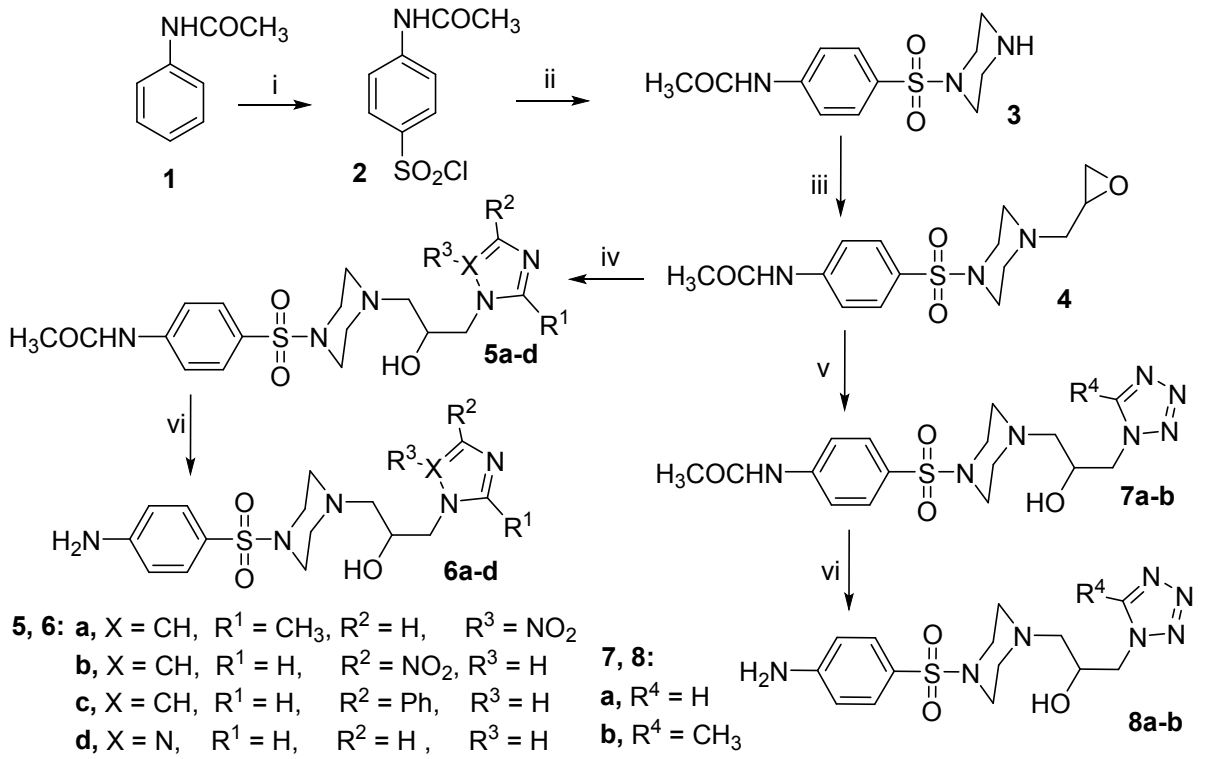
H), 7.37 (d, *J* = 8.6 Hz, 2H, Ph-2,6-*H*), 7.33 (d, *J* = 8.0 Hz, 1H, Cb-3-*H*), 7.23 (t, *J* = 7.3 Hz, 1H, Cb-6-*H*), 6.68 (d, *J* = 8.6 Hz, 2H, Ph-3,5-*H*), 6.21 (s, 2H, Ph-NH_2), 5.94 (s, 1H, O*H*), 4.45 (s, 1H, C*H*OH), 4.38 (d, *J* = 4.9 Hz, 2H, Cb-N-CH_2CHOH), 3.60 (d, *J* = 12.1 Hz, 4H, Pip-N-(CH_2)$_2$), 3.33−3.13 (m, 4H, Pip-N-(CH_2)$_2$), 2.73 (t, *J* = 11.1 Hz, 1H, Pip-N-CH_2CHOH), 2.67−2.59 (m, 1H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 154.2, 142.1, 141.3, 130.2, 126.6, 122.2, 121.9, 120.8, 120.0, 119.0, 113.3, 110.6, 65.0, 59.2, 52.2, 50.9, 47.5 ppm; HR-ESIMS m/z calcd for $C_{25}H_{27}BrN_4O_3S$ $[M + H]^+$, 543.1065; found, 543.1064.

1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(3,6-dibromo-9H-carbazol-9-yl)propan-2-ol (16c). Sulfonamide derivative **16c** was generated by the similar process depicted for compound **6a**. Compound **15c** (0.10 g, 0.15 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **16c** was acquired as white solid. Yield: 57.9%; mp: 180−182 °C; ^{1}H NMR (DMSO-d_6) δ: 8.47 (s, 2H, Cb-4,5-*H*), 7.68 (s, 2H, Cb-1,8-*H*), 7.60 (s, 2H, Cb-2,7-*H*), 7.36 (d, *J* = 7.8 Hz, 2H, Ph-2,6-*H*), 6.68 (d, *J* = 6.9 Hz, 2H, Ph-3,5-*H*), 6.19 (s, 2H, Ph-NH_2), 5.92 (s, 1H, O*H*), 4.39 (s, 3H, C*H*OH, Cb-N-CH_2CHOH), 3.58 (s, 4H, Pip-N-(CH_2)$_2$), 3.21 (s, 4H, Pip-N-CH_2), 2.71 (s, 2H, Pip-N-CH_2), 2.62 (s, 1H, Pip-N-CH_2CHOH), 2.39 (s, 1H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 140.2, 139.3, 130.2, 129.2, 123.7, 113.7, 113.3, 112.8, 111.4, 64.8, 59.0, 52.3, 49.1, 43.2 ppm; HR-ESIMS m/z calcd for $C_{25}H_{26}Br_2N_4O_3S$ $[M + H]^+$, 621.0171; found, 621.0170.

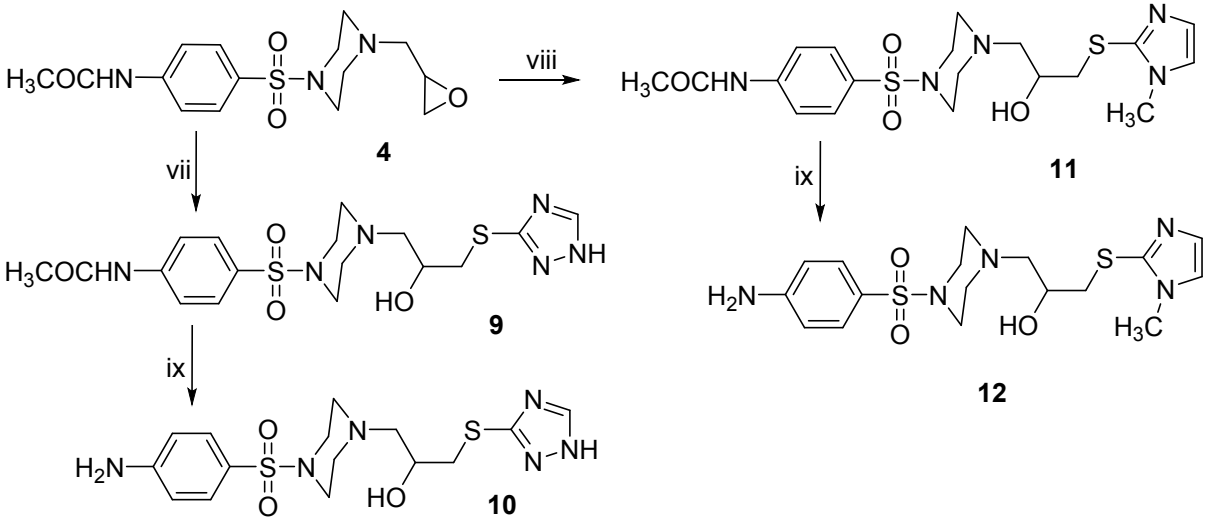
1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(3-iodo-9H-carbazol-9-yl)propan-2-ol (16d). Sulfonamide derivative **16d** was generated by the same process depicted for compound **6a**. Compound **15d** (0.10 g, 0.16 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **16d** was acquired as yellow solid. Yield: 58.1%; mp: 196−198 °C; ^{1}H NMR (DMSO-d_6) δ: 8.54 (s, 1H, Cb-5-*H*), 8.19 (d, *J* = 7.5 Hz, 1H, Cb-4-*H*),

7.69 (d, *J* = 7.8 Hz, 2H, Cb-2,8-*H*), 7.56 (d, *J* = 8.1 Hz, 1H, Cb-1-*H*), 7.49−7.44 (m, 1H, Cb-7-*H*), 7.36 (d, *J* = 8.4 Hz, 2H, Ph-2,6-*H*), 7.22 (t, *J* = 7.4 Hz, 1H, Cb-6-*H*), 6.68 (d, *J* = 8.4 Hz, 2H, Ph-3,5-*H*), 6.20 (s, 2H, Ph-NH_2), 5.95 (s, 1H, O*H*), 4.43 (s, 1H, C*H*OH), 4.38 (s, 2H, Cb-N-CH_2CHOH), 3.63−3.47 (m, 4H, Pip-N-(CH_2)$_2$), 3.31−3.09 (m, 4H, Pip-N-(CH_2)$_2$), 2.74−2.67 (m, 1H, Pip-N-CH_2CHOH), 2.64−2.58 (m, 1H, Pip-N-CH_2CHOH) ppm; ^{13}C NMR (DMSO-d_6) δ: 154.2, 141.1, 140.3, 133.9, 130.2, 129.0, 126.8, 125.4, 121.5, 121.1, 119.9, 113.3, 113.0, 110.5, 82.3, 64.9, 52.2, 50.7, 49.1, 47.5 ppm; HR-ESIMS m/z calcd for $C_{25}H_{27}IN_4O_3S$ $[M + H]^+$, 591.0927; found, 591.0926.

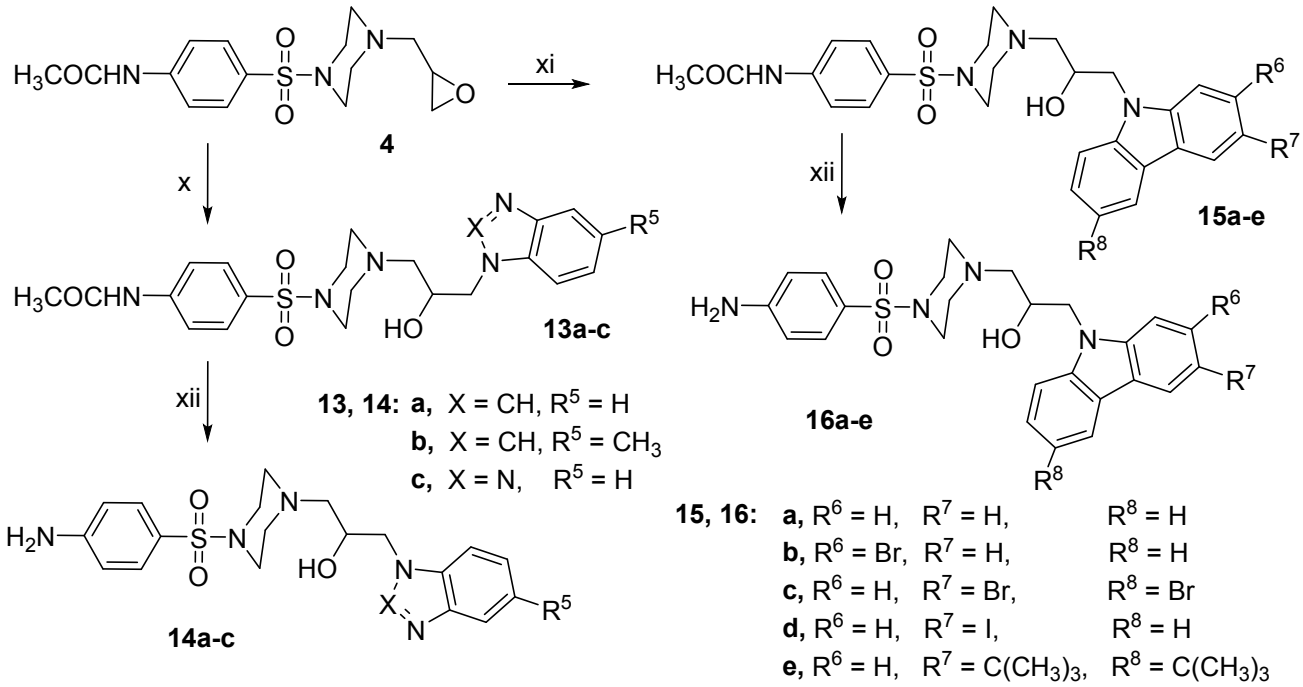
1-(4-((4-Aminophenyl)sulfonyl)piperazin-1-yl)-3-(3,6-di-tert-butyl-9H-carbazol-9-yl)propan-2-ol (16e). Sulfonamide derivative **16e** was generated by the similar process depicted for compound **6a**. Compound **15e** (0.10 g, 0.16 mmol) and hydrochloric acid solution (5 mL, 40%) were used starting materials. The pure product **16e** was acquired as white solid. Yield: 77.3%; mp: 204−206 °C; ^{1}H NMR (DMSO-d_6) δ: 8.12 (s, 2H, Cb-4,5-*H*), 7.40 (s, 4H, Cb-1,2,7,8-*H*), 7.36 (d, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 6.68 (d, *J* = 8.7 Hz, 2H, Ph-3,5-*H*), 6.09 (s, 2H, Ph-NH_2), 4.86 (s, 1H, O*H*), 4.29 (dd, *J* = 14.7, 3.8 Hz, 1H, Cb-N-CH_2CHOH), 4.16 (s, 1H, Cb-N-CH_2CHOH), 3.99 (s, 1H, C*H*OH), 2.81 (s, 4H, Pip-N-(CH_2)$_2$), 2.46 (s, 4H, Pip-N-(CH_2)$_2$), 2.39−2.32 (m, 2H, Pip-N-CH_2CHOH), 1.40 (s, 18H, Cb-3,6-C(CH_3)$_3$) ppm; ^{13}C NMR (DMSO-d_6) δ: 141.4, 139.6, 130.1, 123.4, 122.5, 116.5, 113.2, 109.6, 67.6, 61.9, 52.9, 49.1, 46.3, 34.8, 32.4 ppm; HR-ESIMS m/z calcd for $C_{33}H_{44}N_4O_3S$ $[M + H]^+$, 577.3212; found, 577.3211.



Scheme 1. Synthesis of five-membered azole sulfonamide hybrids. Reagents and conditions: (i) chlorosulfonic acid, 0 °C, 2 h; (ii) piperazine, dichloromethane, 0 °C, 1 h; (iii) potassium carbonate, epichlorohydrin, acetonitrile, 80 °C, 18 h; (iv) imidazoles or triazole, triethylamine, acetonitrile, 80 °C, 24 h; (v) tetrazoles, triethylamine, acetonitrile, 80 °C, 24 h; (vi) hydrochloric acid, ethanol, 78 °C, 1 h.



Scheme 2. Synthesis of thioether-bridged sulfonamide hybrids. Reagents and conditions: (vii) 1H-1,2,4-triazole-3-thiol, triethylamine, acetonitrile, 80 °C, 24 h; (viii) 1-methyl-1H-imidazole-2-thiol, triethylamine, acetonitrile, 80 °C, 24 h; (ix) hydrochloric acid, ethanol, 78 °C, 1 h.



Scheme 3. Synthesis of fused-azole sulfonamide hybrids. Reagents and conditions: (x) benzimidazoles or benzotriazole, triethylamine, acetonitrile, 80 °C, 24 h; (xi) carbazoles, triethylamine, acetonitrile, 80 °C, 24 h; (xii) hydrochloric acid, ethanol, 78 °C, 1 h.

Antibacterial assay. The prepared sulfonamide hybrids **4**−**16** were tested for their antibacterial activities against five Gram-positive bacteria (drug-resistant *Enterococcus faecalis* and *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* N315 (MRSA), *Staphylococcus aureus* ATCC 29213 and *Staphylococcus aureus* ATCC 25923) and six Gram-negative bacteria (drug-resistant *Pseudomonas aeruginosa, Escherichia coli*, *Acinetobacter baumanii*, and *Klebsiella pneumonia*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853). Detailed protocols were performed according to the literature report.[44] The minimum inhibitory concentrations (MICs) for compounds **4**−**16** were summarized in Table 1.

Antifungal assay. The synthesized sulfonamide hybrids **4**−**16** were also tested for their antifungal activities against five fungi (drug-resistant *Candida albicans*, *Candida tropical* and *Aspergillus fumigates*, *Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 90023). Detailed protocols were performed according to the literature report.[44] The MICs for compounds **4**−**16** were summarized in Table 2.

Bacterial membrane permeabilization assay. A 5 h grown culture (mid log phase) of *E. coli* was harvested (5 min, 3500 rpm), washed, and resuspended in glucose (5 mM) and HEPES buffer (5 mM) in 1:1 ratio (pH = 7.2). The tested compound **6a** (10 μL, 12 × MIC) was added to a cuvette containing the bacterial suspension (2 mL) and propidium iodide (PI) (10 μM). Fluorescence was monitored at 535 nm (excitation wavelength) and 617 nm (emission wavelength). PI, as a fluorescence dye for membrane permeability, was monitored by increasing fluorescence for 2 h.

Cytotoxicity assay. A standard MTT assay was applied to evaluate the cytotoxicity of compound **6a** against human breast cancer MCF-7 cells. Cells were seeded in a 96-well plate at a density of about 5×10^4 and cultured in a DMEM culture medium (10% serum and 1% penicillin/streptomycin) in an incubator for 24 h (37 °C, 5% CO_2). Samples of different concentrations were then added into different wells and incubated with cells for another 48 h. Afterwards, each well was added the MTT stock solution (25 μL, 5 mg/mL in PBS). After incubation for another 4 h, the DMEM medium was removed and DMSO (150 μL) was added. The optical density per well at 570 nm was measured by a microplate reader (Bio-Rad 680). The absorbance values were normalized to wells in which cells were not treated with compound **6a**. Data were presented as the average values with standard deviations.

Resistance study. The most active metronidazole derivative **6a** was further investigated for the bacterial resistance. The detailed protocols were performed according to the literature.[4]

Bactericidal kinetic assay. The rate of bactericidal activity, namely, the rate at which the compounds killed bacteria was evaluated by performing time-kill kinetics. Briefly, *E. coli* strains grew for 6 hours in a suitable medium at 37 ^{o}C and were diluted in the appropriate medium. Metronidazole derivative **6a** and norfloxacin were added to the bacterial solution (*E. coli* of approximately 5.0×10^4 CFU/mL) at concentrations of 4 × MIC in a 96-well plate and incubated at 37 ^{o}C. At different time intervals, aliquots from the solution (30 μL) were taken out and 10-fold diluted in 0.9% saline. And then the dilutions (30 μL) were plated on respective agar plates and incubated at 37 ^{o}C for 24 h. The results after counting bacterial colonies represented in logarithmic scale: log (CFU/mL) *vs* time (in hour).

Drug combination assay. The drug combination studies between the most active metronidazole derivative **6a** and standard drugs norfloxacin and fluconazole were investigated by 2-fold dilution checkerboard assay method with concentration values from 1/64 to 4-fold the MIC value of each molecule. The drug combination effect is usually expressed using fractional inhibitory concentration (FIC) index. The FIC value may be calculated as FIC = MIC of molecule A in mixture/MIC of molecule A alone + MIC of molecule B in mixture/MIC of molecule B alone. Using this method, FIC ≤ 0.5 represents synergism, FIC > 0.5 and ≤ 1.0 represents additivism, FIC > 1 and ≤ 2 represents an indifferent effect, and FIC > 2 represents antagonism.[45]

Isolating genomic DNA from drug-resistant *E. coli* strain. The genomic DNA of *E. coli* was extracted according to the literature.[44]

RESULTS AND DISCUSSION

Antibacterial activity. The antibacterial screening *in vitro* demonstrated that few of the target compounds displayed good efficacy as shown in Table 1. Remarkably, metronidazole hybrid **6a** was the most active molecule against clinical drug-resistant *Escherichia coli*, which could be further investigated as a potential antibacterial agent.

In the series of imidazole sulfonamides, hybrids **5a**−**b** and **6a**−**c** exhibited moderate antibacterial activities against Gram-negative *P. aeruginosa*, *A. baumanii* and *E. coli* as well as Gram-positive *E. faecalis* and *S. aureus*, of which compounds **5a** (MIC = 0.069 mM) and **5b** (MIC = 0.071 mM) showed better activity against *E. coli*. Nitroimidazole derivative **6b** displayed weak inhibition against *E. faecalis* (MIC = 0.156 mM), interestingly it improved the potency against *E. coli* (MIC = 0.039 mM). Typically, metronidazole sulfonamide **6a** displayed broad antibacterial spectrum, which had MIC values ranging from 0.038 to 0.075 mM toward *P. aeruginosa, E. faecalis* and *A. baumanii*, and 42, 11, 11-fold greater in comparison to reference drug norfloxacin (MIC = 1.605 or 0.803 mM), respectively. It was noticeable that derivative **6a** highlighted superior activity in the inhibition of *E. coli* strains (MIC = 0.019 mM), which was 84-fold more potent than norfloxacin (MIC = 1.605 mM). However, triazole compounds **5d** (MIC = 0.078 mM) and **6d** (MIC = 0.087 mM) were primarily susceptive to *E. coli*. While tetrazole sulfonamides **7a-b** and **8a-b** were sensitive toward some tested bacterial strains. These results dramatically manifested that the imidazole hybrids were more favorable for the antibacterial activity. Especially, derivative **6a** could have great potential to become an *E. coli* inhibitor, therefore we selected this target compound for further studies. Nevertheless, the triazole and tetrazole sulfonamides were almost not conducive to the inhibiting ability of bacteria other than *E. coli*.

In terms of thioether-bridged sulfonamides, triazole hybrids **9** and **10** were not susceptive to most of the tested bacterial strains. But imidazole ones **11** (MIC = 0.071 mM) and **12** (MIC = 0.078 mM) had quite better selectivity to *E. coli*. The results revealed that imidazolyl thio-ether fragment could effectively improve the antibacterial activities.

In addition, the antibacterial activities of benzimidazole and benzotriazole analogs possessing larger conjugated systems were also evaluated. In the benzimidazole hybrids, molecules **13a** and **13b** along with their deacetylated products **14a** and **14b** showed inferior inhibitory effects and the benzotriazole sulfonamides also displayed no significant inhibition. These results disclosed that benzimidazole and benzotriazole derivatives were unfavorable for the antibacterial efficiency.

Table 1. MIC (mM) for sulfonamide hybrids **4**−**16** against bacteria *in vitro*[a, b, c]

Compds	Gram-positive bacteria					Gram-negative bacteria					
	MRSA	*E. F.*	*S. A.*	*S. A.* 25923	*S. A.* 29213	*K. P.*	*E. C.*	*P. A.*	*A. B.*	*P. A.* 27853	*E. C.* 25922
4	0.754	0.754	0.189	0.377	0.189	0.754	0.189	0.752	0.377	0.189	0.377
5a	0.549	0.274	0.137	0.274	0.137	0.274	0.069	0.274	0.274	0.137	0.274
5b	0.566	0.283	0.283	0.566	0.566	0.566	0.071	0.283	0.283	0.141	0.566
5c	0.529	0.132	0.265	0.529	0.529	0.265	0.265	0.529	0.265	0.132	0.529
5d	0.627	0.157	0.157	0.313	0.313	0.627	0.078	0.313	0.157	0.157	0.313
6a	0.302	0.075	0.302	0.302	0.603	0.603	0.019	0.038	0.075	0.151	0.302
6b	0.312	0.156	0.312	0.312	0.624	0.624	0.039	0.156	0.156	0.156	0.624
6c	0.290	0.290	0.290	0.580	0.580	0.580	0.145	0.290	0.290	0.290	0.290
6d	0.699	0.175	0.175	0.175	0.699	0.699	0.087	0.349	0.175	0.349	0.349
7a	0.625	0.156	0.625	0.313	0.313	0.313	0.313	0.625	0.313	0.156	0.625
7b	0.604	0.302	0.604	0.604	0.604	0.302	0.302	0.604	0.604	0.604	0.302
8a	0.697	0.174	0.348	0.348	0.697	0.697	0.174	0.697	0.348	0.697	0.348
8b	0.671	0.336	0.671	0.671	0.336	0.336	0.336	0.336	0.671	0.671	0.671
9	0.581	0.291	0.581	0.581	0.291	0.291	0.291	0.291	0.291	0.291	0.581
10	0.321	0.321	0.161	0.321	0.642	0.642	0.161	0.642	0.321	0.321	0.642
11	0.564	0.282	0.564	0.282	0.282	0.282	0.071	0.282	0.564	0.282	0.564
12	0.622	0.311	0.311	0.311	0.622	0.622	0.078	0.311	0.622	0.156	0.311
13a	0.559	0.140	0.559	0.280	0.140	0.559	0.140	0.280	0.559	0.280	0.280
13b	0.543	0.271	0.543	0.543	0.543	0.271	0.271	0.543	0.271	0.136	0.543
13c	0.558	0.140	0.279	0.279	0.558	0.558	0.279	0.279	0.279	0.279	0.558
14a	0.616	0.308	0.308	0.308	0.616	0.616	0.308	0.308	0.308	0.308	0.308

14b	0.596	0.298	0.298	0.298	0.596	0.596	0.298	0.298	0.298	0.298	0.298
14c	0.615	0.307	0.307	0.615	0.615	0.615	0.307	0.307	0.154	0.615	0.154
15a	0.505	0.253	0.253	0.505	0.126	0.505	0.253	0.253	0.505	0.253	0.505
15b	0.437	0.055	0.437	0.219	0.437	0.874	0.109	0.109	0.109	0.219	0.874
15c	0.771	0.096	0.771	0.771	0.096	0.771	0.048	0.096	0.193	0.096	0.771
15d	0.405	0.202	0.405	0.809	0.202	0.405	0.101	0.405	0.202	0.202	0.809
15e	0.207	0.104	0.207	0.104	0.207	0.414	0.104	0.207	0.207	0.207	0.414
16a	1.102	0.551	0.551	1.102	1.102	0.551	0.276	0.551	1.102	1.102	0.551
16b	0.942	0.118	0.118	0.942	0.471	0.471	0.118	0.942	0.236	0.942	0.236
16c	0.823	0.051	0.051	0.206	0.823	0.206	0.026	0.411	0.206	0.411	0.103
16d	0.867	0.217	0.108	0.867	0.867	0.434	0.108	0.217	0.434	0.434	0.217
16e	0.444	0.111	0.055	0.444	0.444	0.222	0.111	0.111	0.111	0.444	0.222
A	0.025	0.803	0.025	0.003	0.003	0.803	1.605	1.605	0.803	0.050	0.025

[a]Minimum inhibitory concentrations were measured by micro-broth dilution method.

[b]MRSA, Methicillin-Resistant *Staphylococcus aureus* N315; *E. F.*, *Enterococcus faecalis*; *S. A.*, *Staphylococcus aureus*; *S. A.* 25923, *Staphylococcus aureus* ATCC 25923; *S. A.* 29213, *Staphylococcus aureus* ATCC 29213; *K. P.*, *Klebsiella pneumonia*; *E. C.*, *Escherichia coli*; *P. A.*, *Pseudomonas aeruginosa*; *A. B.*, *Acinetobacter baumanii*; *P. A.* 27853, *Pseudomonas aeruginosa* ATCC 27853; *E. C.* 25922, *Escherichia coli* ATCC 25922.

[c]A = Norfloxacin.

Notably, carbazoles with arresting π-conjugated backbone were also investigated for their effects on antibacterial activities. Herein, carbazole sulfonamides **15a**−**e** and **16a**−**e** possessed moderate to better antibacterial activities against most of the tested strains, of which derivatives **15b** and **16b** having one bromine showed slightly potent anti-*E. coli* ability with MIC values of 0.109 and 0.118 mM, respectively. In contrast with the mono-bromo compounds **15b** and **16b**, di-bromides **15c** (MIC = 0.048 mM) and **16c** (MIC = 0.026 mM) increased the potency against *E. coli*, 33, 62-fold more potent than norfloxacin (MIC = 1.605 mM). However, mono-iodo compounds **15d** and **16d** and di-*tert*-butyl compounds **15e** and **16e** exhibited a little weaker activity against *E. coli* with MIC values of 0.101−0.111 mM. These results exposed that the electron-withdrawing group like bromine atom on carbazole possessed remarkable effect on

antibacterial activity. This might be explained by the improved lipophilicity, making it easy to penetrate the cell membrane and promote the antibacterial activity. Furthermore, the iodine atom and *tert*-butyl group on account of their larger space resistance had a big impact on the conjugated system and co-planarity of the molecules, and resulted in them unfavorable for being delivered to the binding sites.

Antifungal activity. The antifungal data of all the sulfonamide derivatives in Table 2 disclosed that most of the derivatives displayed weak inhibitory activities against *C. albicans* and *C. tropicalis* with MIC values of 0.103−0.754 mM, inferior to reference drug fluconazole (MIC = 0.013 or 0.026 mM). It was found that the majority of hybrids possessed much better anti-*A. fumigatus* activity with MIC values of 0.054−0.625 mM, manifested that these sulfonamides were highly selective for drug-resistant *A. fumigates.* Particularly, compounds **6a**, **6b**, **16d** and **16e** exhibited excellent inhibition toward drug-resistant *A. fumigates* with MIC values of 0.054−0.078 mM, 15, 15, 11, 11-fold more powerful in comparison with fluconazole (MIC = 0.836 mM). Additionally, imidazole hybrid **6a** also displayed better activities against *C. parapsilosis* ATCC 22019 and *C. albicans* ATCC 90023 with each MIC value of 0.075 mM. These results demonstrated that imidazole ring was the most significant for the enhancement of antifungal efficiency and imidazole sulfonamides deserved to be further explored as potential antifungal agents.

Table 2. MIC (mM) for sulfonamide hybrids **4**−**16** against fungi *in vitro*[a, b]

Compds	Fungi					Compds	Fungi				
	C. A.	*C. T.*	*A. F.*	*C. A.* 90023	*C. P.* 22019		*C. A.*	*C. T.*	*A. F.*	*C. A.* 90023	*C. P.* 22019
4	0.189	0.754	0.377	0.377	0.377	**13a**	0.280	0.280	0.280	0.559	0.280
5a	0.137	0.137	0.137	0.549	0.549	**13b**	0.543	0.271	0.271	0.543	0.271
5b	0.141	0.141	0.141	0.566	0.566	**13c**	0.279	0.558	0.558	0.279	1.117
5c	0.132	0.265	0.265	1.059	1.059	**14a**	0.616	0.154	0.154	0.308	1.232
5d	0.313	0.313	0.313	0.313	0.627	**14b**	0.596	0.149	0.149	0.298	1.192
6a	0.302	0.302	0.075	0.075	0.075	**14c**	0.615	0.615	0.154	0.307	0.615

6b	0.312	0.312	0.078	0.156	0.156	**15a**	0.126	0.253	0.505	0.505	0.253
6c	0.616	0.308	0.308	0.308	0.616	**15b**	0.109	0.219	0.437	0.219	0.874
6d	0.349	0.699	0.175	0.349	0.699	**15c**	0.193	0.193	0.385	0.385	0.771
7a	0.156	0.625	0.625	0.625	0.625	**15d**	0.202	0.202	0.405	0.809	0.809
7b	0.302	0.604	0.604	0.604	0.604	**15e**	0.207	0.207	0.207	0.104	0.414
8a	0.348	0.348	0.174	0.348	0.348	**16a**	0.551	0.551	0.276	1.102	0.551
8b	0.671	0.336	0.336	0.336	0.671	**16b**	0.471	0.236	0.236	0.471	0.236
9	0.291	0.291	0.291	0.581	0.581	**16c**	0.411	0.103	0.206	0.206	0.103
10	0.642	0.642	0.161	0.642	0.642	**16d**	0.434	0.108	0.054	0.217	0.108
11	0.141	0.564	0.282	0.282	0.564	**16e**	0.222	0.222	0.055	0.222	0.111
12	0.622	0.622	0.156	0.622	0.311	**B**	0.013	0.026	0.836	0.003	0.007

[a] *C. A., Candida albicans; C. T., Candida tropicalis; A. F., Aspergillus fumigatus; C. A.* 90023, *Candida albicans* ATCC 90023*; C. P.* 22019*, Candida parapsilosis* ATCC 22019.

[b] B = Fluconazole.

Analysis of ClogP values. The lipophilic/hydrophilic properties of bioactive molecules have remarkable influence on various biological processes containing distribution, transportation and metabolism. The theoretically calculated value of partition coefficient (ClogP), as a measurable criteria of lipophilic property of drugs, can contribute to the ideal pharmacokinetic and pharmacodynamic characteristics of drugs if it is in appropriate value. Apparently, most of target compounds were lipophilic with ClogP values from 0.04 to 10.17 (Figure 2).

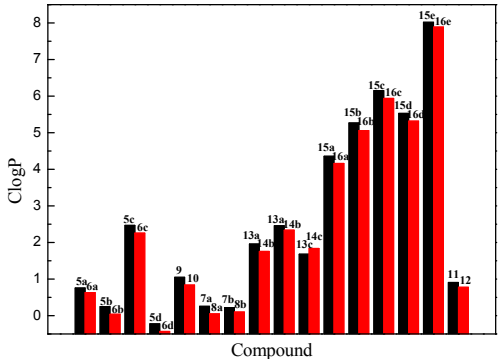


Figure 2. CLogP values of sulfonamide hybrids **5**−**16** were obtained by ChemDraw Ultra 14.0.

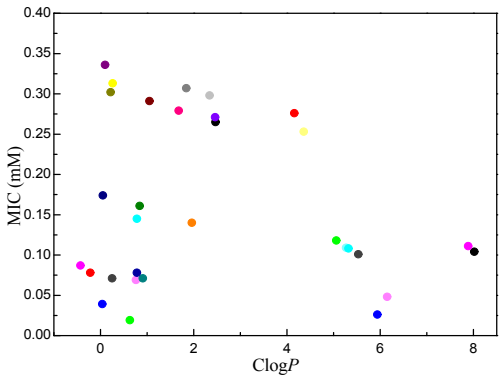


Figure 3. CLogP values of sulfonamide hybrids **5**−**16** *vs* their antibacterial activity against drug-resistant *E. coli*.

In the series of sulfonamide derivatives **5**−**16**, ClogP values were related to the conjugated system. The ClogP values of the tested hybrid compounds followed this order: carbazolyl > benzimidazolyl > benzotriazolyl > tetrazolyl > triazolyl > imidazolyl hybrid. By contrast, carbazole derivatives **15** and **16** had the optimal lipophilicity (ClogP = 4.16−8.02), but most of them did not exhibit preconceived antibacterial activity toward drug-resistant *E. coli* (Figure 3). For instance, *tert*-butyl derivative **15e** (ClogP = 8.02) even with the best lipophilic property did not exhibit prospective inhibitory performance due to its large space resistance. In addition, benzimidazole and benzotriazole hybrids **13** and **14** with moderate lipophilicity exhibited weak efficiency against *E. coli* (ClogP = 1.68−2.46). Interestingly, in the series of imidazole derivatives (ClogP = 0.04−2.47), metronidazole hybrid **6a** (ClogP = 0.63) was the most potent inhibitor against *E. coli* and other imidazoles also showed better anti-*E. coli* activity. Meanwhile, triazole and tetrazole hybrids showed slightly better activity toward *E. coli* with mild lipophilicity, where ClogP values ranged from 0.05 to 1.05. These results displayed that the larger conjugated system could not improve the antibacterial ability effectively, although it was beneficial to enhance the capacity of lipophilicity. Therefore, the proper lipophilic compounds

with ClogP values in the range of 0.04 to 0.78 have shown superior antibacterial activity, for which could easily interact with binding sites.[46]

Bacterial membrane permeabilization study. The quest for bacterial membrane active agents is a method having a profound impact on dealing with the problem of bacterial resistance, and the nature of membrane-active antibacterial agents is less likely to lead to bacterial resistance. Thus, it is essential to assess the membrane permeabilization of active molecule **6a** against drug-resistant *E. coli* using propidium iodide (PI) dye and fluorescent spectra by forming the PI-DNA complex. It was obvious that fluorescence intensity increased significantly within 60 minutes (Figure 4). This phenomenon was attributed to the presence of compound **6a** with a MIC value of 0.228 mM (12 × MIC), which caused the bacterial membrane to be damaged. The result indicated that compound **6a** was able to interact with the membrane and might effectively permeate the membrane of drug-resistant *E. coli*.[47]

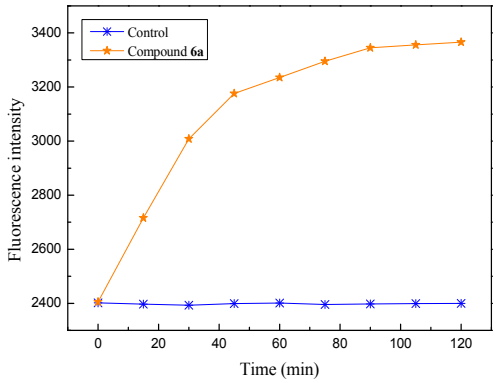


Figure 4. Membrane permeabilization of drug-resistant *E. coli* for metronidazole hybrid **6a** (12 × MIC).

Cytotoxicity. Cytotoxicity assessment of target compounds is an indispensable part of drug discovery. The highly active compound **6a** was further evaluated for its toxicity toward human breast cancer MCF-7 cells by MTT assay. The cytotoxic result (Figure 5) indicated that the cell viability of molecule **6a** remained over 75% even if cultivated in 500 μg/mL, which suggested

that this hybrid highlighted good selectivity over human breast cancer MCF-7 cells for *E. coli*. Thus, the good tolerance of compound **6a** revealed its promising therapeutic potential.[48]

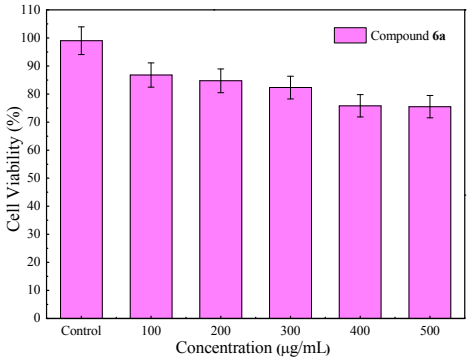


Figure 5. Cytotoxic assay of metronidazole hybrid **6a** on human breast cancer MCF-7 cells tested by MTT methodology.

Resistance. The increasing resistance of antibiotics has made it a serious and global challenge to potentially antimicrobial agents. The mutagenicity of bacteria increased resistance even if the smaller bacterial population was exposed to low concentration, which could facilitate the ease of selecting low-level resistance mutation. Hence, taking the reference drug norfloxacin as the positive control, the probability of drug resistance of metronidazole hybrid **6a** against susceptible pathogenic bacteria *E. coli* was studied. Results in Figure 6 revealed that even after 16 passages, the MIC values of compound **6a** did not change obviously, while MIC values of norfloxacin gave 16-fold increase against *E. coli* after 12 passages. It could be drawn that hybrid **6a** did not easily give rise to drug resistance in *E. coli* as norfloxacin did.[49]

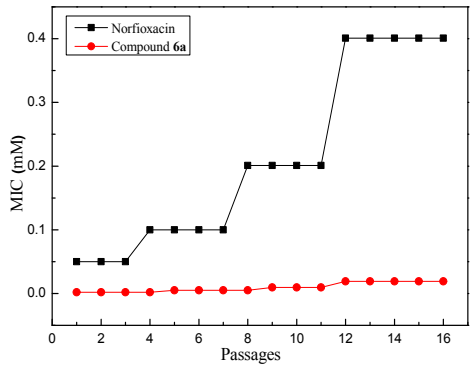


Figure 6. Resistance study for metronidazole hybrid **6a** against drug-resistant *E. coli*.

Bactericidal kinetic study. The bactericidal efficiency for the highly active molecule **6a** was evaluated against *E. coli* by using time-kill kinetic experiment. It could be seen that at the concentration of 4-times MIC, the number of viable bacteria decreased by more than 10^3 CFU/mL within 2 h. This result showed that molecule **6a** had rapidly killing effect against *E. coli*.

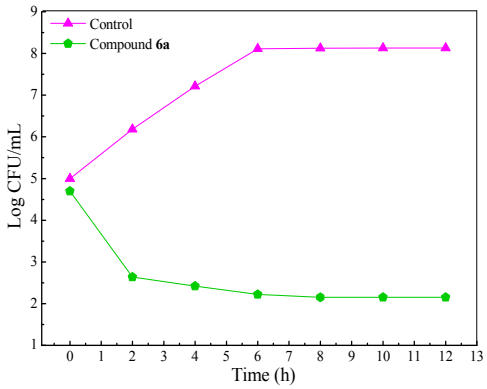


Figure 7. Bactericidal kinetics of metronidazole hybrid **6a** at 4 × MIC against *E. coli*.

Drug combination study. Combination therapy has been regarded as a substitute method in improving antimicrobial curative effect, broadening active spectrum, and combating serious drug resistance. It is a boon for treating mixed diseases that can not be cured only by one single drug.[17] The drug combination of the most active compound **6a** and clinical norfloxacin was studied (Table 3). It could be seen that the tested strains detected by combined drug could effectively improve its antibacterial effect compared with the single use. The combination of compound **6a** with norfloxacin against *S. aureus* was detected as the most efficient potentiation, which produced the synergism effect (FIC = 0.26). In addition, the combination of compound **6a** with norfloxacin against *A. baumanii* had an additivism effect (FIC = 0.56). Unfortunately, the combination of hybrid **6a** with norfloxacin against *E. coli, E. faecalis* and *P. aeruginosa*

exhibited indifferent effect. Additionally, the combination of compound **6a** with fluconazole against *A. fumigatus* produced the synergism effect (FIC = 0.28) (Table 4). Besides, the combination of fluconazole with derivative **6a** showed additivism effect against fungi *C. albicans* and *C. tropicalis*. These results indicated that the combinations of metronidazole hybrid **6a** with antibacterial and antifungal drugs could prominently enhance their antimicrobial activity and overcome drug resistance with less dose. This synergy of compound **6a** provided a promising opportunity to strengthen the limited selection of antimicrobial antibiotics currently available. Furthermore, the availability of drug combinations needs deeper studies to improve the antimicrobial efficiencies, and more comprehensive elucidation of synergistic effects requires deep mechanistic study.

Table 3. Drug combinations of metronidazole hybrid **6a** with antibacterial drug norfloxacin[a]

Bacteria	Norfloxacin		
	Compound **6a** MIC/μg·mL^{-1}	Effect	FIC index
E. faecalis	0.25	indifference	1.13
S. aureus	0.50	synergism	0.26
E. coli	0.03	indifference	1.02
P. aeruginosa	0.06	indifference	1.03
A. baumanii	0.13	additivism	0.56

[a]*E. faecalis*, *Enterococcus faecalis*; *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *A. baumanii*, *Acinetobacter baumanii*.

Table 4. Drug combinations of metronidazole hybrid **6a** with antifungal drug fluconazole[a]

Fungi	Fluconazole		
	Compound **6a** MIC/μg·mL^{-1}	Effect	FIC index
C. albicans	1	additivism	0.52

C. tropicalis	1	additivism	0.53
A. fumigatus	0.06	synergism	0.28

[a]*C. albicans, Candida albicans; C. tropicalis, Candida tropicalis; A. fumigatus, Aspergillus fumigatus.*

Molecular docking study. Molecular docking study is widespreadly employed to investigate the binding modes.[50] Here, a docking evaluation was conducted to explore the inhibitory effects of highly active molecule **6a** against DNA hexamer duplex and human carbonic anhydrase isozyme II by using "Autodock 4.2". DNA hexamer duplex played a very important role in DNA replication and was also essential for cell viability. Carbonic anhydrases (CAs) were responsible for catalyzing a simple but critical reaction to all organisms, namely, the reversible hydration of carbon dioxide to bicarbonate and protons. Because the abnormal levels or activities of human CAs were often connected with diverse human diseases, the inhibition of these enzymes may be exploited pharmacologically.[51]

The docking evaluation manifested good binding energy (−7.61 and −7.51 kcal/mol) for sulfonamide hybrid **6a** against DNA hexamer duplex and human carbonic anhydrase isozyme II, respectively. As shown in Figure 8 for DNA hexamer duplex, the hydrogen atom of amino group on the phenyl ring of molecule **6a** was adjacent to DT-4 by the formation of a hydrogen bond in a distance of 2.0 Å. The oxygen atom of nitro group in the imidazole and DG-2 also formed hydrogen bonds with distances of 1.8 and 1.9 Å, respectively. Besides, the hydrogen atom and oxygen atom of hydroxyl group on compound **6a** were adjacent to DT-4 and DA-3, forming hydrogen bonds with distances of 2.2 and 1.9 Å, respectively. For human carbonic anhydrase isozyme II showed in Figure 9,[52] hydrogen bond was formed between the hydrogen atom of amino group on the phenyl ring and residue THR-199, the oxygen atom of hydroxyl group and

residue HIS-64, as well as the oxygen atom of sulfonyl group and residue GLN-92 with a distance of 2.2 Å. All the hydrogen bonds might be helpful for the stability of sulfonamide hybrid-enzyme complexes, which might provide a basis for the good antibacterial activity of molecule **6a**.

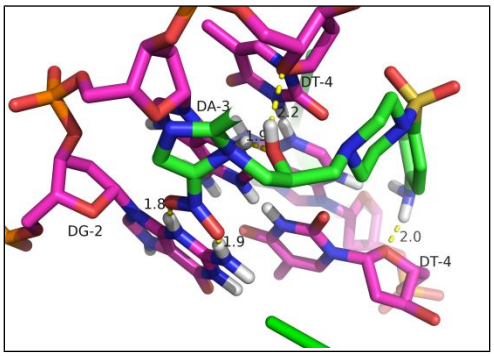


Figure 8. 3D conformation of metronidazole hybrid **6a** docked in DNA hexamer duplex (PDB code: 3FT6).

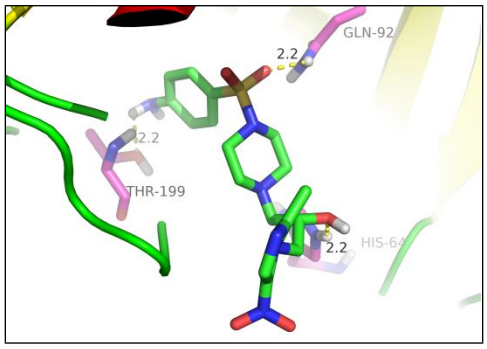


Figure 9. 3D conformation of metronidazole hybrid **6a** docked in human carbonic anhydrase isozyme II (PDB code: 4Q6E).

Interaction with *E. coli* DNA. DNA is an important drug target and has been widely used in the reasonable design and construction of new high-efficiency antibacterial drugs. Sulfonamides can interact effectively with DNA in a variety of binding modes. Here, *E. coli* DNA was selected as the model to explore the possible antibacterial action mechanism. Therefore, the interactions

between the most active metronidazole hybrid **6a** and *E. coli* DNA *in vitro* were studied by UV-vis absorption spectroscopy.[53,54]

Absorption spectrometry is one of the most significant techniques in DNA-binding studies. Hypochromism and hyperchromism detected by absorption spectrometry are key spectral characteristics to identify the variation of DNA double-helical structure. The large hypochromism observed in the interaction between the different DNA base and intercalating chromophore strongly indicates the close proximity between them. At the fixed concentration of DNA, UV-vis absorption spectra were recorded as the content of sulfonamide hybrid **6a** increased. As shown in Figure 10, the maximum absorption peak of DNA at 260 nm showed a little red shift and increased proportionally with the increasing concentration of hybrid **6a**. Furthermore, the absorption value of simple addition of free DNA and molecule **6a** was a little higher than that of **6a**-DNA complex. Thereby, a weak hypochromic effect existed between metronidazole hybrid **6a** and DNA, which could be a result of the chromatin fragment of compound **6a** inserted into the DNA helix and the π-π* states of the aromatic skeleton strongly overlap with DNA bases.

According to the variations of the DNA absorption spectra after binding to molecule **6a**, intrinsic binding constant (K) was calculated by using the following equation (1).[55]

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

A and A^0 denotes the DNA absorbance in the presence and absence of hybrid **6a** at 260 nm, ξ_{D-C} and ξ_C are the absorption coefficients of hybrid **6a**-DNA complex and hybrid **6a**, respectively. The absorption titration data and linear fitting (ESI, Figure S1) were used to draw the plot of $A^0/(A-A^0)$ versus 1/[compound **6a**] and get the binding constant, K = 1.92 × 10^4 L/mol, standard deviation, SD = 0.05, correlation coefficient, R = 0.999.

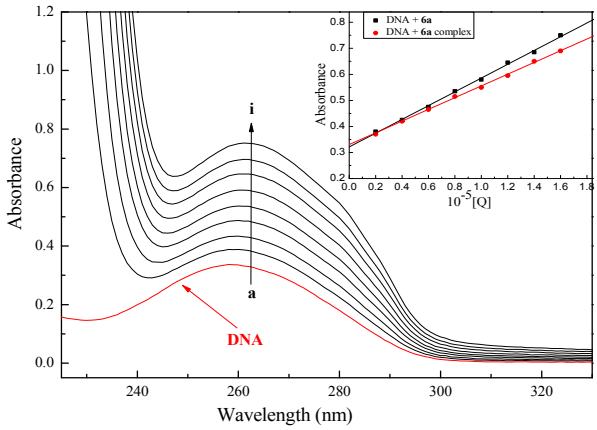


Figure 10. Different concentrations of metronidazole hybrid **6a** and DNA (T = 298 K, pH = 7.4). Inset: absorption of **6a**-DNA complex at 260nm compared with the sum of free hybrid **6a** and DNA. c (DNA) =1.23 × 10^{-5} mol/L and c (hybrid **6a**) = 0–2.00 × 10^{-5} mol/L with an increment of 0.25 × 10^{-5} for curves a-i, respectively.

Neutral red (NR) dye was employed as a spectral probe to further elaborate the interaction between metronidazole hybrid **6a** and *E. coli* DNA because of its higher stability and lower toxicity. The absorption spectra of NR dye added to DNA were shown in Figure S2 (ESI), with the increase of DNA concentration, the absorption peak of NR decreased gradually at about 460 nm and a new band appeared around 530 nm, which might be due to the newly formed DNA−NR complex. The isosbestic point at 500 nm also offered evidence for new DNA−NR complex.[56,57]

As shown in Figure 11, the intensity was significantly increased in the 275 nm development band with the increasing concentration of hybrid **6a**. Comparatively, with the increase of DNA concentration, the absorption of free NR at about 275 nm presented an opposite process at the same wavelength. The results suggested that molecule **6a** could further block DNA replication and exert its strong antibacterial activity by competing with NR to insert into DNA at the insertion site.[58]

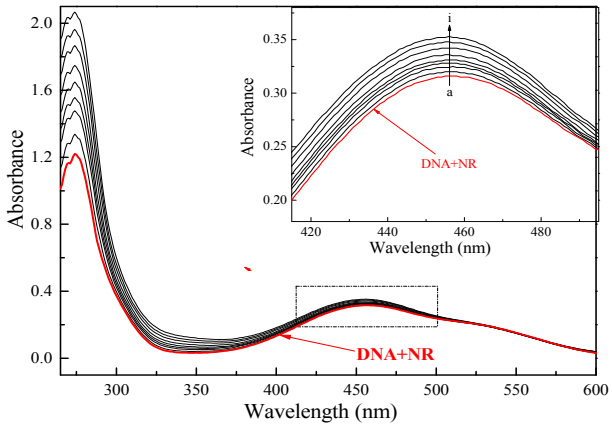


Figure 11. The competitive reaction between NR with DNA and metronidazole hybrid **6a**. c (DNA) = 1.23 × 10^{-5} mol/L, c (NR) = 2 × 10^{-5} mol/L, and c (hybrid **6a**) = 0–4.0 × 10^{-5} mol/L with an increment of 0.5 × 10^{-5} for curves a-i, respectively. Inset: with the increase of compound (**6a**) concentration, the absorption spectra of the system in 410–500 nm range of competitive reaction between NR with DNA and hybrid **6a**.

Conclusions

In this work, a series of novel sulfonamide-based four-component hybrids have been prepared from commercially avaliable acetanilide *via* convenient procedures. The *in vitro* antimicrobial evaluation revealed that the introduction of different azole rings to sulfonamide had significant effects on improving the antimicrobial efficiency of target compounds. Especially, metronidazole hybrid **6a** exhibited excellently inhibiting ability against drug-resistant *E. coli* with a low MIC value of 0.019 mM and excellent interference toward the growth of drug-resistant *A. fumigates* with MIC value of 0.075 mM, and also displayed no obvious toxicity toward human breast cancer MCF-7 cells. ClogP values ranging from 0.04 to 0.78 resulted in the best balance between lipophicity and antibacterial activity. Synergistic combinations with clinically antibacterial or antifungal drugs also improved the therapeutic effect. Molecular docking showed that hydrogen

bonds existed among DNA hexamer duplex, human carbonic anhydrase isozyme II and metronidazolyl hybrid **6a**. Further antibacterial mechanism indicated that the most active hybrid **6a** might effectively inserted into DNA of drug-resistant *E. coli* by the formation of **6a**-DNA complex which could block DNA replication to employ its powerful bioactivity. From the above, it might be concluded that molecule **6a** would have great potential in new bactericidal drug development, and exert potent antimicrobial potencies with a multi-targeting action mechanism.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

E. coli, *Escherichia coli*; MRSA, methicillin resistant *Staphylococcus aureus*; DMSO, dimethyl sulfoxide; TLC, thin layer chromatography; ClogP, partition coefficient; MICs, minimum inhibitory concentrations; FIC, fractional inhibitory concentration.

SUPPORTING INFORMATION

1. Experimental Protocols
2. Some Representative Spectra

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