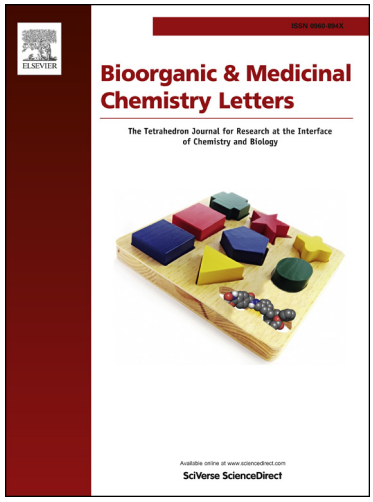

1,2,3-Triazole-derived naphthalimides as a novel type of potential antimicrobial agents: Synthesis, antimicrobial activity, interaction with calf thymus DNA and human serum albumin

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Graphical Abstract

1,2,3-Triazole-derived naphthalimides as a novel type of potential antimicrobial agents: Synthesis, antimicrobial activity, interaction with calf thymus DNA and human serum albumin

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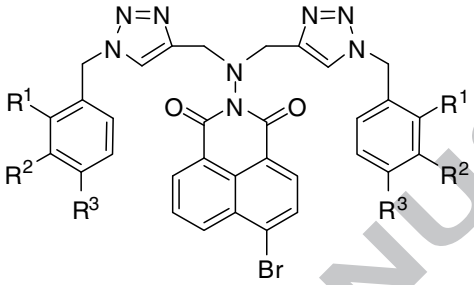
Jing-Song Lv[a,b], Xin-Mei Peng[a], Baathulaa Kishore[a,#], Cheng-He Zhou[a,*]



9e: R^1 = H, R^2 = Cl, R^3 = Cl
MIC = 1 μg/mL (*E. coli*)

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1,2,3-Triazole-derived naphthalimides as a novel type of potential antimicrobial agents: Synthesis, antimicrobial activity, interaction with calf thymus DNA and human serum albumin

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ABSTRACT

A series of 1,2,3-triazole-derived naphthalimides as a novel type of potential antimicrobial agents were synthesized and characterized by IR, NMR and HRMS spectra. All the new compounds were screened for their antimicrobial activity against four Gram-positive bacteria, four Gram-negative bacteria and three fungi. Bioactive assay manifested that 3,4-dichlorobenzyl compound **9e** and its corresponding hydrochloride **11e** showed better anti-*E. coli* activity than Norfloxacin and Chloromycin. Preliminary research revealed that compound **9e** could effectively intercalate into calf thymus DNA to form compound **9e**–DNA complex which might block DNA replication and thus exert antimicrobial activities. Human serum albumin could effectively store and carry compound **9e** by electrostatic interaction.

The increasing emergence of drug resistance, intractable pathogenic microorganisms and newly arising pathogens have become an increasingly serious and challenging problem for human health all over the world. This situation stimulates an urgent need to develop novel antimicrobial agents with completely different chemical structures possibly exerting different action mechanisms from current clinical drugs.[1] The related research has become a major topic worldwide.

Naphthalimides contain desirable π-conjugated backbone with double amide moieties. This type of unique structure can easily exert non-covalent forces such as π-π stacking, strong hydrophobicity, hydrogen bonds and so on. Naturally, naphthalimide-based compounds can readily interact with various active targets in biological system, and thus exhibit diverse biological activities.[2] Especially, naphthalimides as anticancer agents have been investigated very well and shown large clinical potentiality. A lot of work revealed that their anticancer mechanisms were possibly involved in the intercalation into deoxyribonucleic acid (DNA).[3] Recently, some researches have also found that naphthalimides exhibited quite large possibility in the treatment of antibacterial and antifungal infections.[4] This encourages our strong interest in investigating naphthalimide-based compounds as a novel type of potential antimicrobial agents.

With the introduction of 'click chemistry', 1,2,3-triazole derivatives have opened up a new opportunity to azole antimicrobial agents. The large dipole moment of 1,4-substituted 1,2,3-triazoles makes them to be served as hydrogen bond acceptor, which is favor for binding to biological target sites and improving solubility.[5] Moreover, 1,2,3-triazole could act as an attractive bridge group to connect two pharmacophores and/or biologically beneficial fragments into one molecule to generate innovative multifunctional compounds.[6] Many investigations showed that the incorporation of alkyl chains and/or variable aromatic substituents has an important effect on the antimicrobial activities.[7] In particular, halogen-containing aromatic compounds can efficiently improve the bioactivity due to the lipophilicity, inductive and mesomeric effects that affect the diffusion and interaction of these compounds with bacterial cells and tissues by enhancing solubility and membrane permeability.[8]

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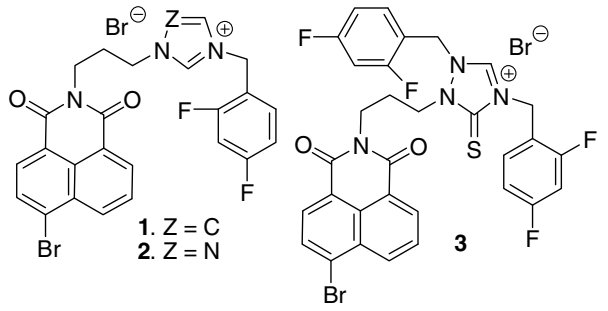
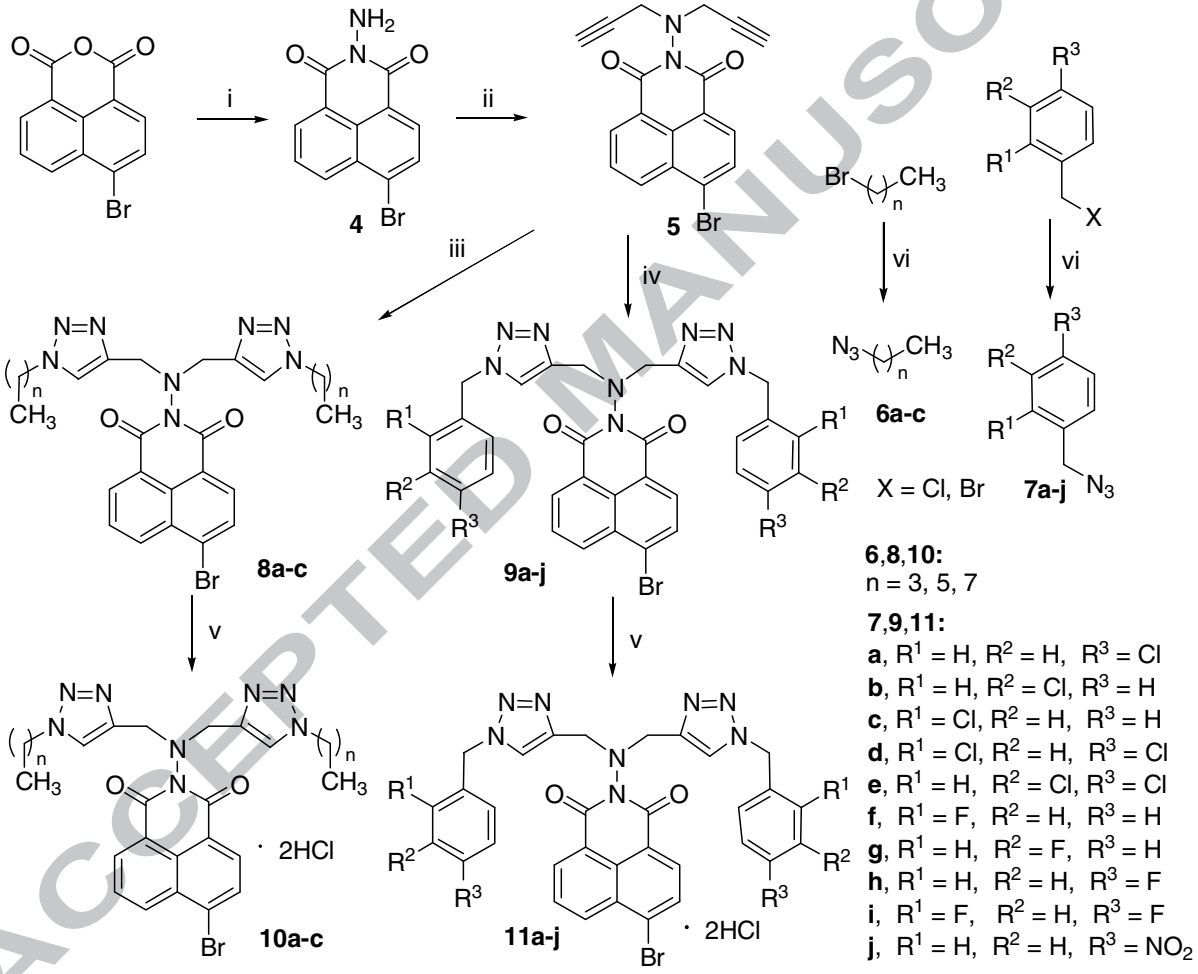


Our previous work revealed that the introduction of nitrogen-containing heterocyclic moieties into naphthalimide backbone is beneficial for antimicrobial activity. Imidazolyl naphthalimide (**1**), 1,2,4-triazolyl compound (**2**) and 5-thioxo-1,2,4-triazolyl derivative (**3**) all exhibited comparable or even superior antimicrobial activity to the standard drugs Norfloxacin, Chloromycin and Fluconazole.[9] However, to our best knowledge, the combination of 1,2,3-triazole with napthalimide skeleton has been seldom reported.

Based on above considerations and as an extension of our continuous work,[9a,10] it is of great interest for us to investigate 1,2,3-triazole-derived naphthalimides as a novel type of potential antimicrobial agents with completely new structure from clinical drugs. Herein, we would like to report the synthesis of novel compounds **8a–c**, **9a–j** and their hydrochlorides with different lengths of alkyl chains or halogen-substituted aralkyl moieties *via* Cu(I) catalyzed 1,3-dipolar cycloaddition reaction. The prepared compounds were screened for their antibacterial and antifungal activities *in vitro*. With the aim to explore the preliminary mechanism of action, the strong active compound **9e** was further investigated for the interaction with calf thymus DNA. The transportation behavior of human serum albumin (HSA) to the highly active compound **9e** was also evaluated.



Scheme 1 Reagents and conditions: i) $NH_2NH_2 \cdot H_2O$, CH_3CH_2OH, r.t.; ii) DMF, 3-bromoprop-1-yne, 80 °C; iii) corresponding alkyl azides, 1%mol $CuSO_4 \cdot 5H_2O$, 2%mol sodium ascorbate, THF/H_2O (2: 1, V/V), 30 °C; iv) corresponding halogen-substituted aryl azides, 1%mol $CuSO_4 \cdot 5H_2O$, 2%mol sodium ascorbate, THF/H_2O (2: 1, V/V), 30 °C; v) hydrogen chloride saturated THF solution, r. t.; vi) NaN_3, benzene, THF, 70 °C.

The target triazole naphthalimides **8a–c** and **9a–j** were prepared *via* multi-step reactions and the synthetic process was outlined in Scheme 1. Commercially available 4-bromo-1,8-naphthalic anhydride was treated by hydrazine hydrate to give intermediate *N*-amino-naphthalimide **4** in 86% yield. The latter was reacted with 3-bromoprop-1-yne in DMF at 70 ºC to afford diprop-2-ynylamino-naphthalimide **5** in 95% yield and the crystal structure of compound **5** was obtained (**CCDC 956371,** see the supplementary information). Compounds **8a-c** and **9a-j** were easily synthesized in high yields ranging from 75% to 94% by the cycloaddition of compound **5** with alkyl azides **6a–c** or the substituted benzyl ones **7a–j**[11] using catalytic amount of copper sulfate and sodium ascorbate in THF/H_2O at room temperature. Compounds **8a–c** and **9a–i** were treated by hydrogen chloride saturated THF solution at room temperature to produce the corresponding hydrochlorides **10a–c** and **11a–i**. These new

compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and HRMS spectra (See the supplementary information).[12]

All the target compounds were evaluated for their antimicrobial activity *in vitro* against four Gram-positive bacteria, four Gram-negative bacteria and three fungi by two folds serial dilution technique (See the supplementary information).[13]

Table 1

In vitro antibacterial and antifungal activities as MIC[a,b,c] (μg/mL) for compounds **8–11**

Compds.	Gram-positive bacteria				Gram-negative bacteria				Fungi		
	S. aureus	MRSA	*B. subtilis*	*M. luteus*	*B. proteus*	*E. coli*	*P. aeruginosa*	*S. dysenteriae*	*C. abicans*	*A. flavus*	*B. yeast*
8a	> 512	> 512	> 512	> 512	> 512	> 512	> 512	> 512	> 512	64	> 512
8b	> 512	> 512	> 512	> 512	> 512	512	512	> 512	> 512	64	> 512
8c	> 512	> 512	> 512	> 512	> 512	512	512	> 512	> 512	16	> 512
9a	> 512	> 512	256	64	> 512	2	512	256	> 512	> 512	> 512
9b	256	> 512	256	> 512	> 512	2	64	> 512	64	> 512	64
9c	> 512	512	> 512	128	> 512	4	> 512	> 512	64	> 512	> 512
9d	> 512	> 512	> 512	256	> 512	8	> 512	> 512	> 512	32	> 512
9e	> 512	> 512	> 512	> 512	512	1	256	> 512	256	> 512	64
9f	> 512	> 512	> 512	256	> 512	32	> 512	> 512	> 512	64	> 512
9g	> 512	> 512	> 512	256	> 512	16	> 512	> 512	> 512	32	> 512
9h	> 512	> 512	> 512	512	> 512	32	> 512	> 512	> 512	> 512	> 512
9i	> 512	> 512	256	512	> 512	64	> 512	> 512	> 512	> 512	> 512
9j	> 512	> 512	256	512	> 512	256	> 512	> 512	> 512	> 512	> 512
10a	> 512	> 512	512	> 512	> 512	> 512	> 512	> 512	> 512	64	> 512
10b	> 512	> 512	> 512	512	> 512	512	512	> 512	> 512	32	> 512
10c	> 512	> 512	> 512	> 512	512	512	512	> 512	> 512	16	> 512
11a	256	512	256	32	> 512	2	512	256	> 512	256	> 512
11b	256	256	256	> 512	512	2	64	512	16	> 512	64
11c	> 512	512	> 512	128	> 512	4	> 512	> 512	64	> 512	> 512
11d	> 512	> 512	> 512	256	> 512	4	> 512	> 512	> 512	32	> 512
11e	256	256	512	512	256	0.5	256	> 512	256	> 512	32
11f	> 512	> 512	> 512	256	> 512	16	> 512	> 512	> 512	64	> 512
11g	> 512	> 512	> 512	256	> 512	16	> 512	> 512	> 512	32	> 512
11h	> 512	> 512	> 512	512	512	32	> 512	> 512	> 512	512	> 512
11i	> 512	> 512	128	512	> 512	32	> 512	> 512	512	> 512	> 512
A	16	16	32	16	32	16	4	32	—	—	—
B	4	1	2	1	2	2	1	2	—	—	—
C	—	—	—	—	—	—	—	—	2	256	4

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

[b] A = Chloromycin, B = Norfloxacin, C = Fluconazole.

[c] *A. flavus*, *Aspergillus flavus*; *C. albicans*, *Candida albicans*; *M. luteus*, *Micrococcus luteus* ATCC 4698; MRSA, *Methicillin-Resistant Staphylococcus aureus* N315; *S. aureus*, *Staphylococcus aureus* ATCC25923; *P. aeruginosa*, *Pseudomonas aeruginosa*; *E. Coli, Escherichia coli* DH52; *S. dysenteriae*, *Shigella dysenteriae*; *B. subtilis*, *Bacillus subtilis*; *B. proteus*, *Bacillus proteus*; *B. typhi*, *Bacillus typhi. B. yeast*, *Beer yeast.*

As shown in Table 1, most of the target compounds were weak or inactive to the tested fungal strains. Interestingly, some prepared compounds displayed good activity against Fluconazole-insensitive *A. flavus.* Specially, compounds **8c** and **10c** showed high efficacy against *A. flavus* with MIC value of 16 μg/mL, which was 16-fold more potent than clinical drug Fluconazole. For antibacterial activity, all the target compounds also exhibited weak inhibition. However, to our surprise, compounds **9a–j** with differently substituted benzyl halides showed excellent activity against *E. coli* in comparison with other tested bacteria. Noticeably, 3,4-dichlorobenzyl derivative **9e** exhibited a quite low MIC value of 1 μg/mL, which was 16- and 2-fold more active than reference drugs Chloromycin (MIC = 16 μg/mL) and Norfloxacin (MIC = 2 μg/mL). The fluorobenzyl compounds **9f-i**, gave relatively low activity (MIC = 16–256 μg/mL). The preliminary structure activity relationship demonstrated that substitutents in 1,2,3-triazole ring exerted great influence on the antibacterial efficiencies. Alkyl 1,2,3-triazole derivatives **8a–c** obviously decreased antibacterial activities (MIC = 512 μg/mL) in comparison to aryl ones **9a–j** (MIC = 2–256 μg/mL). It was also observed that the introduction of halobenzyl moieties especially 3,4-dichlorobenzyl group could improve the antibacterial potency. Salts with improved water solubility have been proved to be favorable for enhancing the

bioactivity. It is remarkable that hydrochloride **11e** exhibited excellent anti-*E. coli* activity with MIC value of 0.5 μg/mL, which was 2–fold more potent than the corresponding precursor. This encouraged us with great interest to study the interactions of the high bioactive compound **9e** with DNA and human serum albumin (HSA) to further explore the possible preliminary action mechanism and transport behavior.

It is well known that DNA is an informational molecule encoding the genetic instructions used in the development and function of almost all the known living organisms.[14] Recently, DNA as a therapeutic target has attracted much attention in biomedical science.[15] There are several strategies currently used to inhibit DNA replication. One approach is to use DNA binding agents that can bind DNA so that it no longer acts as an effective substrate for the DNA polymerase. Binding studies between small molecules and DNA are important and helpful to develop novel and efficient drugs.[16] Here calf thymus DNA was selected as DNA model because of its medical importance, low cost and ready availability properties to investigate the possible antimicrobial action mechanism of the highly active compound **9e** at molecular level by UV-vis spectroscopic methods.[17]

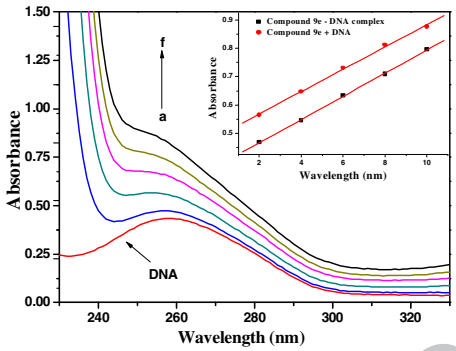


Figure 1. UV absorption spectra of DNA with different concentrations of compound **9e** (pH = 7.4, T = 288 K). Inset: comparison of absorption at 260 nm between the compound **9e**-DNA complex and the sum values of free DNA and free compound **9e**. c(DNA) = 6.55 × 10^{-5} mol L^{-1}, and c(compound **9e**) = 0, 0.50, 1.00, 1.50 and 2.00 × 10^{-5} mol L^{-1} for curves a–f, respectively.

Hypochromism and hyperchromism are very important spectral features to distinguish the change of DNA double-helical structure in absorption spectroscopy. Due to the interaction between the electronic states of intercalating chromophore and that of the DNA base, the observed large hypochromism suggested a close proximity of the aromatic chromophore to the DNA bases.[18] With a fixed concentration of DNA, UV-vis absorption spectra were recorded with increasing amount of compound **9e**. As shown in Figure 1, UV-vis spectra displayed that the maximum absorption peak of DNA at 260 nm exhibited proportional increase and slight blue shift along with the increasing concentration of compound **9e**. Meanwhile the absorption value of simply sum of free DNA and free compound **9e** was greater than the measured value of DNA-compound **9e** complex. This meant that a strong hypochromic effect existed between DNA and compound **9e**. Based on the variations in these absorption spectra, equation (1) could be used to calculate the intrinsic binding constant (K):

$$\frac{A^0}{A-A^0}=\frac{\xi_C}{\xi_{D-C}-\xi_C}+\frac{\xi_C}{\xi_{D-C}-\xi_C}\frac{1}{K[9e]} \quad (1)$$

A^0 and A represent the absorbance of DNA in the absence and presence of compound **9e** at 260 nm, ξ_C and $\xi_{D\text{-}C}$ were the absorption coefficients of compound **9e** and DNA-**9e** complex respectively. The plot of $A^0/(A\text{-}A^0)$ versus 1/[compound **9e**] was constructed by using the absorption titration data and linear fitting, yielding the binding constant, K = 2.34×10^4 L/mol, R = 0.994, S.D. = 0.26 (R is the correlation coefficient. S.D. is standard deviation).

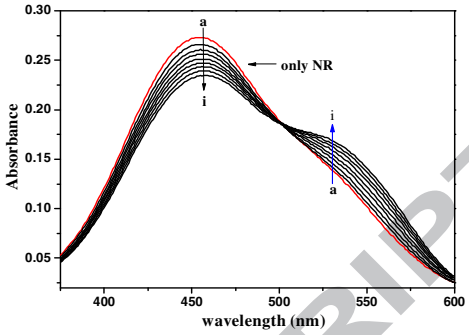


Figure 2. UV-vis absorption spectra of neutral red in the presence of DNA at pH 7.4 and room temperature. c(NR) = 2.0×10^{-5} mol L^{-1} and c(DNA) = 0, 0.48, 0.95, 1.43, 1.90, 2.38, 2.86, 3.33, and 3.81×10^{-5} mol L^{-1} for curves a–i, respectively.

To further understand the interaction between compound **9e** and DNA, the absorption spectra of competitive interaction of compound **9e** were investigated. In comparison with other common probes, neutral red (NR) shows lower toxicity, higher stability and more convenient application than other common probes. Furthermore, it has been sufficiently demonstrated by spectrophotometric and electrochemical techniques that NR can bind to DNA by an intercalative mode.[19] Therefore NR was employed as a spectral probe to investigate the binding mode of compound **9e** with DNA in our work. The absorption peak of the NR around 460 nm gradually decreased with increasing concentration of DNA, which suggested the formation of new DNA-NR complex (Figure 2). Figure 3 displayed the absorption spectra of a competitive binding between NR and compound **9e** with DNA. As gradually increasing the concentration of compound **9e**, an apparent intensity increase was observed around 460 nm. Compared to the absorption around 460 nm of NR-DNA complex, the absorbance at the same wavelength (inset of Figure 3) exhibited the reverse process. In addition to that, the absorbance at 282, 350 and 375 nm extensive broadening was also observed in the spectra. These various spectral changes were consistent with the intercalation of compound **9e** into DNA by substituting NR in the DNA-NR complex.

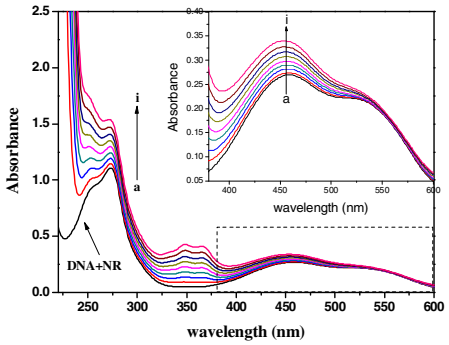


Figure 3. UV Absorption spectra of the competitive reaction between compound **9e** and neutral red with DNA. c(DNA) = 6.55 × 10^{-5} mol L^{-1}, c(NR) = 2.0 × 10^{-5} mol L^{-1}, and c(compound **9e**) = 0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 × 10^{-5} mol L^{-1} for curves a–i, respectively. (Inset) UV Absorption spectra of the system with the increasing concentration of **9e** in the wavelength range of 400–600 nm.

HSA is a transport protein and the principal extracellular protein with a high concentration in blood plasma.[20] Thorough studies involving the interaction of drugs or bioactive small

molecules with serum albumins are not only beneficial to provide a proper understanding of the absorption, transportation, distribution, metabolism and excretion properties of drugs, but also significant to design, modify and screen of drug molecules. Therefore, it is important to study the interaction of compound **9e** with this protein.

In our experiment, the concentration of HSA solution was 1.0 × 10^{-5} M, and the concentrations of compound **9e** solution increased progressively from 0 to 1.8 × 10^{-5} M at an increment of 0.2 × 10^{-5} M. The effect of compound **9e** on fluorescence of HSA intensity at 298 K was shown in Figure 4. It was observed that a gradual decrease of the fluorescence intensity was caused by the increment of compound **9e**, accompanied with the slight blue shift of wavelengths in the albumin spectrum because of the increase of hydrophobicity. The purple line showed the only emission spectrum of compound **9e**, which indicated that compound **9e** did not possess significant fluorescence features, thus at the excitation wavelength (295 nm), the effect of compound **9e** on fluorescence of HSA could be negligible.

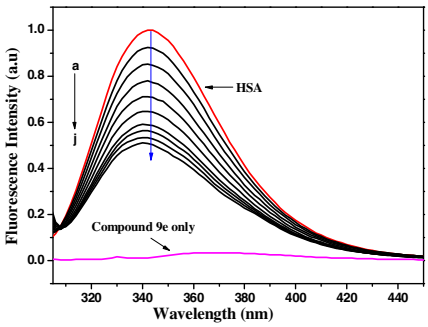


Figure 4. Emission spectra of HSA in the presence of various concentrations of compound **9e**. c(HSA) = 1.0 × 10^{-5} M; c(compound **9e**)/(10^{-5} M), a–j: from 0.0 to 1.8 at increments of 0.20; purple line shows the emission spectrum of compound **9e** only; T = 293 K, λex = 295 nm.

The decreased fluorescence intensity is usually described by the Stern-Volmer equation (2):[21]

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

In this equation, the F_0 and F represent steady-state fluorescence intensities in the absence and presence of compound **9e**, respectively. K_{SV} is the Stern-Volmer quenching constant, and [Q] is the concentration of compound **9e**. Hence, K_{SV} was calculated by linear regression of plot of F_0/F versus [Q] (See the supplementary information).

Quenching mechanisms are often classified as dynamic quenching, static quenching etc. depending on temperature and viscosity. Because higher temperatures result in larger diffusion coefficients, the quenching constants are expected to increase with gradually increasing temperature in dynamic quenching. However, the increase of temperature is likely to result in a smaller static quenching constant due to the dissociation of weakly bound complexes.[22]

Table 2
Stern-Volmer quenching constants for the interaction of compound **9e** with HSA at various temperatures

pH	T (K)	10^{-4} K_{SV} (L/mol)	R^a	S.D.[b]
7.4	296	5.38	0.998	0.030
	303	7.32	0.998	0.037
	310	7.39	0.996	0.054

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

Therefore, the K_{SV} was calculated from Stern-Volmer plots at each temperature. Table 2 demonstrated that the quenching constant K_{SV} in direct proportion to temperature, which indicated that the probable quenching mechanism of compound **9e**-HSA binding reaction was initiated by excited-state intermolecular collision and then inactivation by radiation (dynamic quenching).

In order to reconfirm that the probable fluorescence quenching mechanism of HSA by compound **9e** was mainly initiated by intermolecular collision, the difference absorption spectroscopy was employed. The UV-vis absorption spectrum of HSA and the difference absorption spectrum between HSA-compound **9e** (1:1) and compound **9e** at the same concentration could be almost superposed (Figure 5), the slight difference between the curve A and C suggested that the probable quenching mechanism of fluorescence of HSA by compound **9e** was mainly a dynamic quenching procedure.

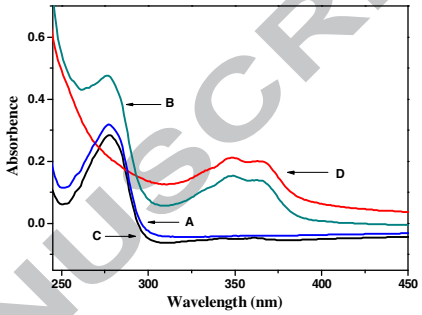


Figure 5. UV-vis spectra of HSA in the presence of compound **9e**: A, absorption spectrum of HSA only; B, absorption spectrum of compound **9e**/HSA 1:1; C, difference between absorption spectrum of compound **9e**/HSA 1:1 and compound **9e**; D, absorption spectrum of compound **5e** only. c(HSA) = c(compound **9e**) = 1.0 × 10^{-5} M.

The above experiments showed that compound **9e** could effectively interact with HSA, thereby causing hypochromic effect of ultraviolet spectroscopy. When the electronic transfer occurs between compound **9e** and HSA, it causes energy transfer without radiation, thereby resulting in the quenching of fluorescence spectrum. Further molecular electrostatic potentiality for the compound **9e** was investigated by full geometry optimizations of the studied systems which was performed by using the B3LYP functional[23] with 6-31G* basis set. Calculation presented in this work was carried out by the Gaussian 09[24] program package. The results manifested that the nucleophilic effect of carbonyl and 1,2,3-triazolyl groups (Figure 6, red region) in compound **9e** might induce an electrostatic effect with positive electricity of Lys199 in HSA. Therefore, compound **9e** and HSA might interact by electrostatic interactions.[25] This result was also evidenced by the value of enthalpy change (ΔH) and entropy change (ΔS) from the van't Hoff equation (found, ΔH = 0.118 kJ/mol ≈ 0, ΔS = 478.30 J/mol k), which was accordant with the literature (when $\Delta H \approx 0$, $\Delta S > 0$, the main force is electrostatic interaction).[26]

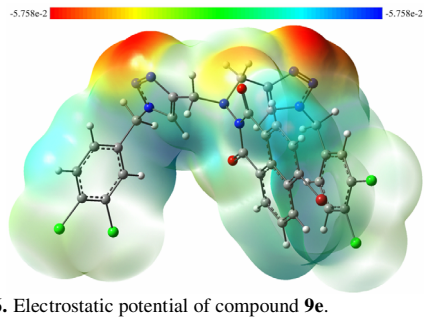


Figure 6. Electrostatic potential of compound **9e**.

In conclusion, a novel type of 1,2,3-triazole-derived naphthalimides and their corresponding hydrochlorides were

successfully synthesized for the first time *via* an easy, convenient and efficient synthetic procedure starting from commercial 4-bromo-1,8-naphthalic anhydride. The antimicrobial tests demonstrated that some prepared compounds exhibited good or even superior antibacterial and antifungal activities against the tested strains to reference drugs. Especially compounds **9a-e** and **11a-e** showed 2- to 16-fold more potent activity than Chloromycin against *E. coli* (MIC = 16 μg/mL). Hydrochlorides **11a-e** showed even better inhibitory activities and broad antibacterial spectrum in contrast to their precursors. The preliminary interactive investigations of compound **9e** with calf thymus DNA revealed that compound **9e** could effectively intercalate into DNA to form compound **9e**-DNA complex which might block DNA replication and thus exerting its antimicrobial activities. The binding research demonstrated that HSA could effectively store and carry compound **9e** by electrostatic interaction. All these results should be a promising starting point to optimize the structures of azole-derived naphthalimides to access potent antimicrobial agents. Further researches, including the extraction of *E. coli* DNA and its interaction with compound **9e**, the *in vivo* bioactive evaluation, the incorporation of different linkers (alkyl, aryl and heterocyclic moieties) and diverse azole heterocycle (pyrazole, oxazole, carbazole, benzimidazole, benzotriazole etc.) into naphthalimide backbone as well as various functional groups (ester, ketone, amino ones and metal, etc.) linked to azole ring, are now in progress in our group. All these will be discussed in future paper.

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12. Experimental: Melting points are determined on X-6 melting point apparatus and uncorrected. IR spectra were determined on a Bio-Rad FTS-185 spectrophotometer in the range of 400–4000 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AV 300 spectrometer using TMS as an internal standard. 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 following abbreviation was used to designate aryl group: NAPH, naphthalimidyl. The high-resolution mass spectra (HRMS) were recorded on an IonSpec FT-ICR mass spectrometer with ESI resource.

Synthesis of 2-amino-6-bromo-1H-benzo[de]isoquinoline-1,3(2H)-dione (***4***) A mixture of 6-bromobenzo[*de*]isochromene-1,3-dione (10.00 g, 36.1 mmol) and hydrazine hydrate (80%, 10 mL) in ethanol was stirred at room temperature over night. After the reaction came to the end (monitored by TLC, eluent, chloroform), the mixture was filtrated and dried to give crude product **4** (9.90 g) as khaki powder. Yield: 94%; mp: 219–220 °C

Synthesis of 6-bromo-2-(diprop-2-ynylamino)-1H-benzo[de]isoquinoline-1,3(2H) -dione (***5***) A mixture of compound **4** (1.98 g, 5.0 mmol), potassium carbonate (2.15 g, 15.0 mmol) and propargyl bromide (1.79 g, 15.0 mmol) in DMF (15 mL) was stirred at 80 °C for 48 h. After the reaction came to the end (monitored by TLC, eluent, chloroform), the mixture was cooled to room temperature and filtrated. The crude compound was further purified by silica gel column chromatography (eluent, chloroform/petroleum, 3/1, V/V) to give the desired compound **5** (2.30 g) as pale yellow solid. Yield: 76%; mp: 154–156 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, *J* = 7.2 Hz, 1H, NAPH-*H*), 8.61 (d, *J* = 8.4 Hz, 1H, NAPH-*H*), 8.46 (d, *J* = 7.8 Hz, 1H, NAPH-*H*), 8.07 (d, *J* = 7.8 Hz, 1H, NAPH-*H*), 7.88 (t, *J* = 7.8 Hz, 1H, NAPH-*H*), 4.25 (s, 4H, HC≡CCH_2), 2.15 (s, 2H, CH_2C≡C*H*).

Synthesis of 2-(bis((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-6-bromo-1H-benzo[de]isoquinoline-1,3(2H) dione (***9a***) To a solution of compound **5** (0.51 g, 1.41 mmol) in mixture (20 mL, 1/1, v/v) of THF and H_2O was added chlorobenzyl azide (0.50 g, 3.00 mmol). Hereafter sodium ascorbate (2% mmol) and copper (II) sulfate pentahydrate (1% mmol) was added successively, and the mixture was stirred for 3 h at room temperature. The resulting mixture was diluted with water (10 mL) and extracted by $CHCl_3$ (3 × 20 mL). The organic phase was dried over anhydrous sodium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, chloroform/methanol, 30/1, V/V) to give compound **9a** as yellow solid. Yield: 75%; mp 193–194 °C. IR (KBr, cm^{-1}): 3129, 2934, 2866, 1675, 1631, 1588, 1570, 1492, 1456, 1400, 1238, 851, 750, 646; ^1H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, 1H, *J* = 8.4 Hz, NAPH-*H*), 8.40 (d, 1H, *J* = 7.1 Hz, NAPH-*H*), 8.17 (d, 1H, *J* = 7.9 Hz, NAPH-*H*), 7.99 (d, 1H, *J* = 7.9 Hz, NAPH-*H*), 7.79 (t, 1H, *J* = 7.9 Hz, NAPH-*H*), 7.62 (s, 2H, Tri-*H*), 7.14 (d, 4H, *J* = 8.3 Hz, 4-ClCH_2Ph 2,6-*H*), 6.98 (d, 4H, *J* = 8.3 Hz, 4-ClCH_2Ph 3,5-*H*), 5.35 (s, 4H, 4-ClPh-CH_2), 4.74 (s, 4H, Tri C^4-CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.57, 144.48, 134.37, 133.49, 133.22, 132.26, 131.39, 131.01, 130.57, 130.51, 129.00, 128.95, 128.53, 127.99, 123.37, 122.97, 122.12, 53.13, 49.57; HRMS (ESI) calcd. for: $C_{32}H_{23}BrCl_2N_8O_2$ $[M+Na]^+$, 723.0397; found, 723.0392.

Synthesis of 2-(bis((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-6-bromo-1H-benzo[de]isoquinoline-1,3(2H)-dione (***11a***) To a solution of compound **9a** (0.51 g, 1.41 mmol) in mixture (20 mL, 1/1, v/v) of THF and H_2O was added anhydrous HCl in THF (1 mL), the mixture was stirred for 3 h at room temperature. After the reaction came to the end (monitored by TLC, eluent, chloroform),

solvents were removed to give compound **11a** as bright yellow solid. Yield: 98%; mp > 200 °C. IR (KBr, cm^{-1}): 3149, 2934, 2866, 1666, 1632, 1587, 1571, 1492, 1456, 1400, 1229, 839, 747, 649; ^{1}H NMR (300 MHz, DMSO-*d6*) δ 8.54 (d, 1H, J = 8.1 Hz, 1H, NAPH-*H*), 8.42 (d, 1H, J = 7.1 Hz, NAPH-*H*), 8.19 (s, 2H, NAPH-*H*), 8.06 (s, 2H, Tri-*H*), 7.98 (t, 1H, J = 7.7 Hz, NAPH-*H*), 7.21 (d, 4H, J = 8.2 Hz, 4-ClCH_2Ph 3,5-*H*), 7.02 (d, 4H, J = 8.2 Hz, 4-ClCH_2Ph 3,5-*H*), 5.48 (s, 4H, 4-ClPh-CH_2), 4.64 (s, 4H, Tri C4-CH_2); ^{13}C NMR (75 MHz, DMSO-d6) δ 163.30, 143.60, 135.56, 133.15, 132.90, 132.25, 131.78, 131.58, 130.21, 129.71, 129.57, 129.23, 128.85, 128.32, 124.90, 123.40, 122.61, 52.08, 48.77; HRMS (ESI) calcd. for: $C_{32}H_{25}BrCl_4N_8O_2$ $[M\text{-}2Cl\text{-}2H+Na]^+$, 723.0402; found, 723.0404.

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