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Original article

Novel potentially antibacterial naphthalimide-derived metronidazoles: Design, synthesis, biological evaluation and supramolecular interactions with DNA, human serum albumin and topoisomerase II

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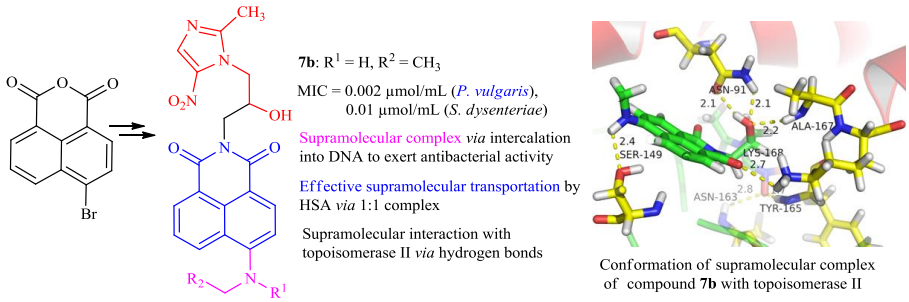
Graphical Abstract



A series of novel naphthalimide-derived metronidazoles as new antibacterial agents were developed. Relational supramolecular interactions with DNA, human serum albumin and topoisomerase II were investigated for the evaluation of antibacterial potentiality.

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ABSTRACT

A series of novel naphthalimide-derived metronidazoles as new type of antimicrobial agents were for the first time designed, synthesized and characterized by NMR, IR and HRMS spectra. Experimental results revealed that most of them displayed moderate to good antibacterial activity towards Gram-positive and negative bacteria. Especially, compound **7b** was able to not only exhibit effective inhibition towards the growth of *P. vulgaris* (MIC = 0.002 μmol/mL) and *S. dysenteriae* (MIC = 0.01 μmol/mL), but also have rapidly killing effect and prevent the development of bacterial resistance. Further research revealed that the highly active molecule **7b** could not only intercalate into calf thymus DNA to form a steady supramolecular complex and thus might block DNA replication to exert the powerful bioactivities, but also be effectively transported by human serum albumin (HSA) *via* the formation of the 1:1 supramolecular complex, in which hydrogen bonds and hydrophobic effect played important roles in the association of compound **7b** with HSA. Molecular docking indicated that the supramolecular interactions between **7b** and topoisomerase II were driven by hydrogen bonds.

1. Introduction

Metronidazole is a widely used clinical drug for the treatment of infectious diseases. Despite of its long term clinical use, there is less report about the incidence of its resistance. Therefore, much research has been directing towards its derivatives, so far many metronidazole derivatives as drugs like ornidazole, secnidazole, tinidazole and nimorazole have been successfully developed and extensively used in clinic [1]. This encourages continuous exploration to investigate metronidazole derivatives with potential medicinal application. It has been found that the formed reactive intermediates in microorganisms *via* the reduction of nitro group in metronidazole can covalently bind with DNA and trigger the adverse effects [2], so the sterical protection of nitro group is necessary to improve the metabolism and physicochemical property [3]. Our previous work revealed the introduction of a large structural fragment such as berberine and quinolone into metronidazole nucleus can not only exhibit good antimicrobial activities, but also broaden the antimicrobial spectrum [4]. However, to our best knowledge, the combination of metronidazole with napthalimide skeleton was seldom reported.

Naphthalimides have large π-deficient aromatic backbone with cyclic double imides and a naphthalene framework. The special structure makes naphthalimides readily interact with biological active sites *via* supramolecular interactions such as π-π stacking, cation-π, coordination bonds, hydrogen bonds, ion-dipole, hydrophobic effect, van der Waals and so on, thus exhibiting various biological activities [5]. In particular, some naphthalimide-based compounds like amonafide, mitonafide, elinafide and bisnafide have already shown large potentiality in the treatment of cancers by supramolecular interactions with biological molecule DNA or topoisomerase II (Top II) [6]. Recently, much literature showed that naphthalimides should also have quite large possibility as new type of antimicrobial agents [7]. Imidazolyl, 1,2,3-triazolyl and benzimidazolyl naphthalimides were found to display comparable or even superior antimicrobial activities to the standard drugs Norfloxacin, Chloromycin and Fluconazole [8]. Therefore, it is worth further studying naphthalimide derivatives as new antimicrobial agents.

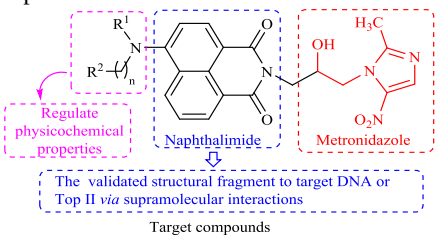


Fig. 1. Design of novel naphhtalimide-derived metronidazoles

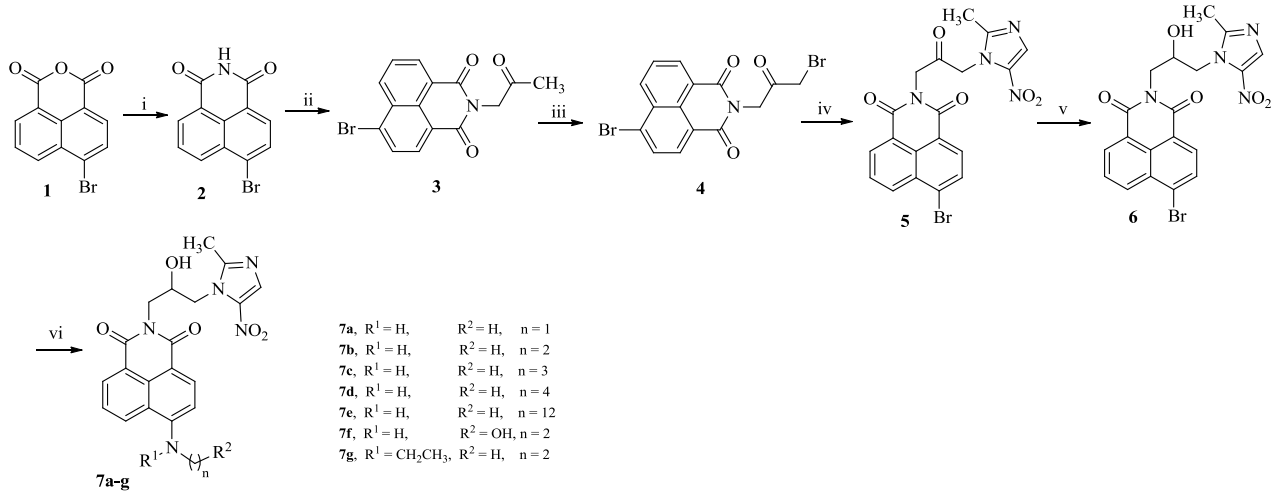
In view of the above observations, as an extension of our research on metronidazole-based derivatives as new antimicrobial agents, herein a series of novel naphthalimide-derived metronidazoles were designed for the first time (Fig. 1). The introduction of large structural fragment might not only be beneficial to the sterical protection of nitro group to produce the reactive intermediates in microorganisms *via* the reduction of nitro group in metronidazole, and to avoid triggering the adverse effects, thus overcoming drug resistance, but also be helpful to excellent antimicrobial activity *via* multiple binding of supramolecular interactions with DNA or Top II by the use of π-π stacking, hydrophobic effect, hydrogen bonds and so on. Furthermore, the amino group at the C-4 position of naphthalimide was capable to improve the affinity and water solubility of the target molecules by forming hydrogen bonds and coordination interaction *etc*. The aliphatic chain was reported to be capable of regulating physico-chemical properties, so a series of alkyl substituents were incorporated into the target compounds in order to investigate their effect on biological activities [9].

All the prepared compounds would be screened against Gram-positive bacterial, Gram-negative bacterial and fungal strains including Methicillin-resistant *Staphylococcus aureus* (MRSA). To further evaluate the prospective of the most active compound as new antibacterial drug, time-kill kinetic assay, and bacterial resistance development research would be done. Moreover, with an aim to explore the possible antibacterial mechanism of the prepared target compounds, the supramolecular interactions with DNA and Top II, which are the targets of many antibacterial drugs, would be performed towards the highly active compound. The supramolecular transportation behavior of the highly active compound by HSA would also be investigated to understand the distribution, deposition, metabolism and efficacy.

2. Results and discussion

2.1. Chemistry

The target compounds **7a–g** were synthesized *via* multi-step reactions from commercially available 4-bromo-1,8-naphthalic anhydride **1**. The synthetic process was outlined in Scheme 1. Commercial naphthalic anhydride **1** was treated with aqueous ammonia to produce intermediate **2** in excellent yield of 92%, and was further reacted with chloroacetone in DMF under the presence of potassium carbonate as base to afford compound **3** with a yield of 62%. The latter was brominated to generate the corresponding compound **4** in the yield of 53%. The prepared bromide **4** was coupled with 2-methyl-5-nitroimidazole in DMF at 40 °C using potassium carbonate as base to give the desired nitroimidazole **5** in the 64% yield. The reduction of compound **5** by sodium borohydride afforded the metronidazole derivative **6** in moderate yield. The further *N*-alkylation of compound **6** with various alkyl amines in dimethyl sulphoxide using potassium carbonate as base and copper(I) oxide as catalyst under nitrogen atmosphere produced a series of amino type of substituted naphthalimide metronidazoles **7a–g** in the moderate yields ranging from 40% to 60%. All the new compounds were confirmed by NMR, IR and HRMS spectra.



Scheme 1. Synthetic route of naphthalimide-derived metronidazoles. Reagents and conditions: (i) aqueous ammonia, 45 °C, 8 h; (ii) chloroacetone, potassium carbonate, DMF, 100 °C, 7 h; (iii) bromine, acetic acid, 60 °C, 3 h; (iv) 2-methyl-5-nitroimidazole, potassium carbonate, DMF, 40 °C, 5 h; (v) sodium borohydride, ethanol, 60 °C, 6 h; (vi) amines, copper(I) oxide, potassium carbonate, DMSO, 90 °C, 4 h.

2.2. Biological activity

As shown in Table S1 in Supporting information, most of the prepared compounds gave moderate to good antibacterial activity against the tested strains *in vitro*. The amino type of target compounds **7a–g** possessed the better antibacterial efficacy than intermediates **5** and bromo-substituted naphthalimide metronidazole **6**, which demonstrated that positive effect of amino group on biological activity. Especially, ethylamino deriavtive **7b** showed relatively good broad-spectrum and efficient antibacterial activities in comparison with other compounds. The anti-*P. vulgaris* potency of compound **7b** among the target compounds was the strongest with an excellent MIC value of 0.002 μmol/mL, which was 95-fold, 50-fold and 10-fold more potent than reference drugs Metronidazole, Chloromycin and Norfloxacin, respectively. Furthermore, *S. dysenteriae* and *E. coli* (DH52) were more sensitive to compound **7b** (MIC = 0.01 and 0.04 μmol/mL, respectively) than Chloromycin (MIC = 0.05 and 0.10 μmol/mL, respectively). Moreover, compound **7b** also exhibited better activity against MRSA, *S. aureus* and *B. typhi* than Chloromycin. Notably, derivative **7b** showed 5-fold inhibition potency against MRSA than Metronidazole with a MIC value of 0.04 μmol/mL. These indicated that naphthalimide metronidazole **7b** had large potentiality as a lead compound in the development of more effective board-spectrum antimicrobial agents. Continual studies found that the length of the alkyl chain also had a certain effect on the antibacterial efficacy. The replacement of ethyl group by dodecyl fragment, which yielded compound **7e**, resulted in low activity towards the tested strains. Moreover, the antibacterial activities of compounds **7a**, **7c** and **7d** were also weaker than those of the highly active molecule **7b**. These results suggested that the appropriate alkyl chain was beneficial for the biological activity of the target compounds. In comparison with compound **7b**, the introduction of hydroxyl group into the alkyl chain, which yielded hydroxyethyl amino compound **7f**, did not improve inhibition potency. The ethylation of secondary amino group in compound **7b** gave compound **7g** which also did not increase antibacterial potency, except for against *B. typhi* with the MIC value of 0.04 μmol/mL, which was 2.5-fold more potent than Chloromycin (MIC = 0.10 μmol/mL). The antifungal results (Table S2 in the Supporting information) showed that most of the target compounds had similar inhibitory tendency and intensity to their antibacterial activity. The antifungal activity of compound **7b** was stronger than other target compounds against all tested fungi with MICs between 0.01 and 0.04 μmol/mL. Particularly, compound **7b** showed 5-fold more active potency against *B. yeast* (MIC = 0.01 μmol/mL) than Fluconazole, and stronger activity against *C. utilis* (MIC = 0.02 μmol/mL) than Fluconazole. All naphthalimide-derived metronidazoles possessed better anti-*A. flavus* (MIC = 0.04**–**0.29 μmol/mL) efficiency than Fluconazole (MIC = 0.84 μmol/mL).

2.3. Resistance study

MRSA has been challenging the effectiveness of antibiotics including Norfloxacin [10]. Herein, the ability of MRSA to develop drug resistance against the most active compound **7b** was tested and clinical drug Norfloxacin was chosen as a positive control. As can be seen from Fig. 2, it indicated that the MIC for compound **7b** showed no obvious change, whereas that of Norfloxacin began to significantly increase after 5 passages against MRSA. It demonstrated that MRSA was more difficult to develop resistance against compound **7b** than clinical Norfloxacin.

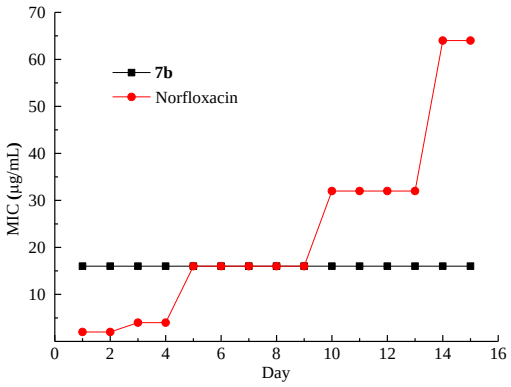


Fig. 2. Evaluation of resistance development against compound **7b** in bacterial strain MRSA.

2.4. Bactericidal kinetic assay

To determine the bactericidal potency of the synthesized compounds, the viability of exponentially growing MRSA against the most active **7b** was investigated by time–kill kinetics experiment [11]. The data revealed more than 3 log (CFU/mL) reduction in the number of viable bacteria in one hour at a concentration of 4 × MIC (refer to Fig. S1 in Supporting information). The experimental result manifested that compound **7b** had rapidly killing effect against MRSA.

2.5. Cytotoxicity

The *in vitro* cytotoxic activity of compound **7b** was evaluated in MCF-7 (human breast adenocarcinoma) cells by Cell Counting Kit-8 (CCK8) method. As shown in Fig. S2 in Supporting information, when the concentration of compound **7b** was 6.25 μg/mL, the cell viability was 70.4%. With the increasing concentration of compound **7b**, the toxicity gradually became high and the IC_{50} value was 8.51 μg/mL, which suggested that it might be a promising candidate to treat cancer.

2.6. Supramolecular interactions with calf thymus DNA

The investigation of supramolecular interactions is a prevalent active field, especially in developing supramolecular drugs [12]. DNA is a main cellular target for antimicrobial agents. There is considerable attention in investigating the supramolecular interactions of small molecules with DNA for the rational design of new and efficient DNA targeting drugs [13]. To explore the preliminary mechanism of antimicrobial action, calf thymus DNA was often employed as DNA model [14]. Neutral Red (NR) is a convenient planar phenazine dye which has been demonstrated that the binding mode of NR with DNA is intercalation [15]. Therefore, NR was selected as a spectral probe to explore the binding model between the biological active compound **7b** and calf thymus DNA on molecular level *in vitro* by UV–vis spectroscopy (Supporting information).

The absorption spectra of supramolecular interactions of the most active compound **7b** with DNA were shown Fig 3. With the increasing concentration of **7b**, an apparent intensity increase was observed in the developing band around 460 nm. In comparison to the absorption around 460 nm of free NR in the presence of the increasing concentrations of DNA (Fig. S4 in Supporting information), the absorbance at the same wavelength exhibited the reverse process (inset of Fig. 3). The results suggested that compound **7b** should intercalate into the double helix of DNA by substituting for NR in the DNA–NR complex. In addition, the increase of absorbance at 276 nm provided evidence for intercalation of compound **7b** into DNA.

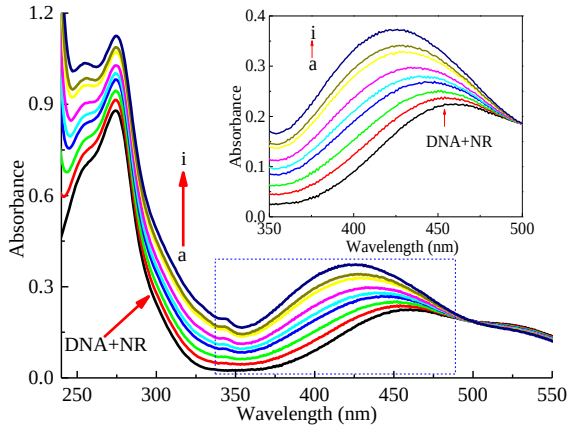


Fig. 3. UV absorption spectra of supramolecular interactions of the active compound **7b** with DNA. c(DNA) = 4.32 × 10^{-5} mol/L, c(NR) = 2 × 10^{-5} mol/L, and c(compound **7b**) = 0–2.25 × 10^{-5} mol/L for curves *a*–*i*, respectively, at increment 0.25 × 10^{-5}.

*2.7. Supramolecular interactions of compound **7b** with HSA*

HSA is a main carrier protein for a variety of endogenous and exogenous substances in the body, therefore assists in their distribution and deposition [16]. It has been well accepted that the overall distribution, metabolism, and efficacy of drugs can be changed by their affinity to HSA. So the investigation of supramolecular interactions with HSA can provide a better understanding of the absorption, transportation, distribution, metabolism and excretion properties of drug molecules [17]. The experimental results (Table 1) indicated that compound **7b** could spontaneously interact with HSA to form 1:1 stable supermolecular complex *via* hydrophobic effect and hydrogen bonds (Supporting information).

Table 1 Binding constants and sites of **7b**–HSA supramolecular system

T (K)	Scatchard Method			
	$10^{-4}K_b$ (L/mol)	*R*	S.D.	*n*
286	8.34	0.992	0.029	1.05
298	7.57	0.999	0.005	1.04
310	6.95	0.996	0.020	1.06

*2.8. Supramolecular interactions of compound **7b** with topoisomerase II*

To rationalize the observed antibacterial activity and understand the possible mechanism, the supramolecular docking investigation of compound **7b** with topoisomerase II receptor (PDB code: 1ZXN) was undertaken [18]. The mimetic results showed that compound **7b** possessed a high total score (9.02) against topoisomerase II. Supramolecular interactions of compound **7b** with topoisomerase II receptor was shown in Fig. 4, the amido groups could interact with Ser149 and Lys418 residues of Top II through hydrogen bonds with the distance of 2.4 Å and 2.7 Å, respectively. Furthermore, the hydroxyl group could form hydrogen bonds with Ala167 and Asn91 residues in nearly 2.1 Å distance. There were also hydrogen bonds between nitro group and residues of Tyr165 and Asn163 with the distance of 1.7 Å and 2.8 Å, respectively. These multi-site bindings might be favorable to stabilize the compound **7b**–topoisomerase II supramolecular complex, which might be responsible for the good inhibitory efficacy of compound **7b** against the tested strains.

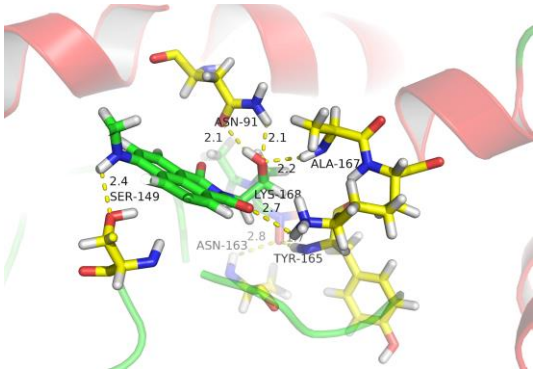


Fig. 4. Stereoview of conformation of compound **7b**–topoisomerase II supramolecular complex

3. Conclusion

A series of novel naphthalimide-derived metronidazoles were for the first time designed and successfully synthesized as potential antibacterial agents through a convenient and efficient procedure. All the target compounds were confirmed by NMR, IR and HRMS spectra. The *in vitro* antimicrobial evaluation revealed that some of the target compounds could effectively inhibit the growth of some tested strains. Structure–activity relationship suggested that aliphatic chains at the C-4 position of naphthalimide exerted a certain impact on biological activity. The most active compound **7b** with the ethylamino group could effectively inhibit the growth of *P. vulgaris* and *S. dysenteriae* with MIC values of 0.002 and 0.01 μmol/mL, respectively. Importantly, compound **7b** had the ability to rapidly kill the tested strains and did not trigger development of bacterial resistance. Moreover, the most active molecule **7b** could not only intercalate into calf thymus DNA to form a steady supramolecular complex which might block DNA replication to display the antibacterial activity, but also be effectively transported by HSA *via* the formation of the 1:1 biological supramolecular complex, in which hydrogen bonds and hydrophobic effect played important roles in the association of compound **7b** with HSA. Molecular docking indicated that the supramolecular interactions between **7b** and topoisomerase II were driven by hydrogen bonds. Additionally, compound **7b** showed concentration-dependent toxicity to MCF-7 cells. Further research studies including *in vivo* bioactivities, structural modification alicyclic amines (morpholine, pyrrolidine, piperidine and piperazine, *etc.*) and heterocyclic azole rings (triazole, benzimidazole, their derivatives, *etc.*) into the naphthalimide backbone as well as their corresponding metal supramolecular complexes are now in progress. All these will be discussed in the full paper.

4. Experimental

4.1. General procedures for synthesis of compounds.

*4.1.1. General procedures for synthesis of compounds **2**–**6** have been provided in Supporting information.*

*4.1.2. Synthesis of 6-(ethylamino)-2-(2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (**7b**)*

A mixture of compound **6** (4.59 g, 10 mmol), ethylamine (4.51 g, 100 mmol), copper(I) oxide (0.29 g, 2 mmol), potassium carbonate (0.69 g, 5 mmol) in dimethyl sulfoxide (10 mL) was stirred at 90 ºC for 4 h (monitored by TLC, eluent, chloroform/methanol, 40:1, v/v). After the solvent was evaporated under reduced pressure, and the resulting residue was extracted with chloroform (3 × 30 mL), the organic layers were combined, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography eluting with chloroform/methanol (50:1, V/V) to give the compound **7b** (2.46 g) as the yellow solid. Yield: 58%; mp: > 250 ºC; IR (KBr, cm^{-1}) *v*: 3377 (O-H), 3137 (N-H), 1675 (C=O), 1637,1580, 1500, 1456 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.70 (d, 1H, *J* = 8.1 Hz, NAPH-*H*), 8.45 (d, 1H, *J* = 7.0 Hz, NAPH-*H*), 8.28 (d, 2H, *J* = 8.5 Hz, NAPH-*H*, Im-4-*H*), 7.69 (dd, 2H, *J* = 17.4, 9.7 Hz, NAPH-*H*, -N*H*CH_2), 6.78 (d, 1H, *J* = 8.3 Hz, NAPH-*H*), 5.45 (d, 1H, *J* = 4.3 Hz, O*H*), 4.13 (tdd, 4H, *J* = 17.1, 11.8, 5.4 Hz, NAPH-CH_2, Im-CH_2), 3.95 (dd, 1H, *J* = 13.7, 8.9 Hz, OH-C*H*), 3.43 (m, 2H, -NHCH_2), 2.35 (s, 3H, Im-CH_3), 1.31 (t, 3H, *J* = 6.6 Hz, -CH_2CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ 164.7, 163.8, 151.1, 146.1, 145.7, 134.8, 131.2, 130.2, 129.0, 124.7, 123.1, 122.6, 120.7, 108.3, 104.2, 68.1, 51.3, 43.5, 38.0, 14.1, 13.4; HRMS (ESI) calcd. for $C_{21}H_{21}N_5O_5$ $[M+Na]^+$, 446.1440; found, 446.1443.

*4.1.3. Synthesis of compounds **7a** and **7c-g** according to the procedure described for compound **7b***

4.2. General methods for biological assays.

The antimicrobial activity *in vitro* for all the target compounds were evaluated for four Gram-positive bacteria (*Staphylococcus aureus* ATCC25923, *Methicillin-Resistant Staphylococcus aureus* N315 (MRSA), *Bacillus subtilis* ATCC6633 and *Micrococcus luteus* ATCC4698), six Gram-negative bacteria (*Escherichia coli* DH52, *Escherichia coli* JM109, *Pseudomonas aeruginosa* ATCC27853, *Shigella dysenteriae*, *Proteus vulgaris* and *Bacillus typhi*) and five fungi (*Candida albicans* ATCC76615, *Candida mycoderma*, *Candida utilis*, *Aspergillus flavus* and *Beer yeast*) using two folds serial dilution technique in 96–well micro-test plates recommended by National Committee for Clinical Laboratory Standards (NCCLS) with the positive control of clinically antimicrobial drugs Chloromycin, Norfloxacin, Metronidazole and Fluconazole.

4.3. General methods for resistance, bactericidal kinetic assays have been provided in Supporting information.

4.4. General methods for supramolecular interactions with DNA, HSA, and topoisomerase II have been provided in Supporting information.

Acknowledgment

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References

[1] (a) L. Zhang, X.M. Peng, G.L.V. Damu, R.X. Geng, C.H. Zhou, Comprehensive review in current developments of imidazole-based medicinal chemistry, Med. Res. Rev. 34 (2014) 340-437;
(b) K. Lewis, Platforms for antibiotic discovery, Nat. Rev. Drug Discovery 12 (2013) 371-387;
(c) X.M. Peng, G.X. Cai, C.H. Zhou, Recent developments in azole compounds as antibacterial and antifungal agents, Curr. Top. Med. Chem. 13 (2013) 1963-2010;
(d) L.P. Peng, S. Nagarajan, S. Rasheed, C.H. Zhou, Synthesis and biological evaluation of a new class of quinazolinone azoles as potential antimicrobial agents and their interactions with calf thymus DNA and human serum albumin, Med. Chem. Commun. 6 (2015) 222-229.

[2] (a) H.S. Sutherland, A. Blaser, I. Kmentova, et al., Synthesis and structure-activity relationships of antitubercular 2-nitroimidazooxazines bearing heterocyclic side chains, J. Med. Chem. 53 (2010) 855-866;
(b) L. Zhang, K.V. Kumar, S. Rasheed, R.X. Geng, C.H. Zhou, Design, synthesis, and antimicrobial evaluation of novel quinolone imidazoles and interactions with MRSA DNA, Chem. Biol. Drug Des. 86 (2015) 648-655.

[3] (a) L. Zhang, K.V. Kumar, R.X. Geng, C.H. Zhou, Design and biological evaluation of novel quinolone-based metronidazole derivatives as potent Cu^{2+} mediated DNA-targeting antibacterial agents, Bioorg. Med. Chem. Lett. 25 (2015) 3699-3705;
(b) M.N. Trivedi, S.Y. Gabhe, U.D. Vachhani, R.B. Patel, C.P. Shah, Synthesis of some 2-methyl-5-nitroimidazole derivatives as potential antimicrobial agents, J. Chem. Pharm. Res. 3 (2011) 313-319.

[4] (a) L. Zhang, J.J. Chang, S.L. Zhang, et al., Synthesis and bioactive evaluation of novel hybrids of metronidazole and berberine as new type of antimicrobial agents and their transportation behavior by human serum albumin, Bioorg. Med. Chem. 21 (2013) 4158-4169;
(b) S.F. Cui, L.P. Peng, H.Z. Zhang, et al., Novel hybrids of metronidazole and quinolones: Synthesis, bioactive evaluation, cytotoxicity, preliminary antimicrobial mechanism and effect of metal ions on their transportation by human serum albumin, Eur. J. Med. Chem. 86 (2014) 318-334;
(c) X.M. Peng, G.L.V. Damu, C.H. Zhou, Current developments of coumarin compounds in medicinal chemistry, Curr. Pharm. Des. 19 (2013) 3884-3930;
(d) X.M. Peng, K.V. Kumar, G.L.V. Damu, C.H. Zhou, Coumarin-derived azolyl ethanols: synthesis, antimicrobial evaluation and preliminary action mechanism, Sci. China Chem. 59 (2016) 878-894.

[5] (a) H.H. Gong, D. Addla, J.S. Lv, C.H. Zhou, Heterocyclic naphthalimides as new skeleton structure of compounds with increasingly expanding relational medicinal applications, Curr. Top. Med. Chem. 16 (2016) 3303-3364;
(b) G.L.V. Damu, Q.P. Wang, H.Z. Zhang, et al., A series of naphthalimide azoles: Design, synthesis and bioactive evaluation as potential antimicrobial agents, Sci. China Chem. 56 (2013) 952-969;
(c) S. Banerjee, E.B. Veale, C.M. Phelan, et al., Recent advances in the development of 1,8-naphthalimide based DNA targeting binders, anticancer and fluorescent cellular imaging agents, Chem. Soc. Rev. 42 (2013) 1601-1618;
(d) Z. Chen, L. Han, M.H. Xu, et al., Rationally designed multitarget anticancer agents, Curr. Med. Chem. 20 (2013) 1694-1714.
[6] (a) M.F. Brana, M. Cacho, A. Gradillas, et al., Intercalators as anticancer drugs, Curr. Pharm. Des. 7 (2001) 1745-1780;
(b) S.Y. Tan, D.H. Sun, J.K. Lyu, et al., Antiproliferative and apoptosis-inducing activities of novel naphthalimide–cyclam conjugates through dual topoisomerase (topo) I/II inhibition, Bioorg. Med. Chem. Lett. 23 (2015) 5672-5680.
[7] (a) Y.Y. Zhang, J.L. Mi, C.H. Zhou, X.D. Zhou, Synthesis of novel fluconazoliums and their evaluation for antibacterial and antifungal activities, Eur. J. Med. Chem. 46 (2011) 4391-4402;
(b) Y.Y. Zhang, C.H. Zhou, Synthesis and activities of naphthalimide azoles as a new type of antibacterial and antifungal agents, Bioorg. Med. Chem. Lett. 21 (2011) 4349-4352;
(c) A.S. El-Azab, A.M. Alanazi, N.I. Abdel-Aziz, et al., Synthesis, molecular modeling study, preliminary antibacterial, and antitumor evaluation of N-substituted naphthalimides and their structural analogues, Med. Chem. Res. 22 (2013) 2360-2375.
[8] (a) H.H. Gong, K. Baathulaa, J.S. Lv, G.X. Cai, C.H. Zhou, Synthesis and biological evaluation of Schiff base-linked imidazolyl naphthalimides as novel potential anti-MRSA agents, Med. Chem. Commun. 7 (2016) 924-931;
(b) Y.L. Luo, B. Kishore, K.V. Kumar, C.H. Zhou, G.X. Cai, Novel benzimidazole derived naphthalimide triazoles: synthesis, antimicrobial activity and interactions with calf thymus DNA, Sci. China Chem. 58 (2015) 483-494;
(c) J.S. Lv, X.M. Peng, B. Kishore, C.H. Zhou, 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, Bioorg. Med. Chem. Lett. 24 (2014) 308-313.
[9] (a) M. Verma, V. Luxami, K. Paul, Synthesis, *in vitro* evaluation and molecular modelling of naphthalimide analogue as anticancer agents, Eur. J. Med. Chem. 68 (2013) 352-360;
(b) H.Z. Zhang, G.L.V. Damu, G.X. Cai, C.H. Zhou, Design, synthesis and antimicrobial evaluation of novel benzimidazole type of fluconazole analogues and their synergistic effects with chloromycin, norfloxacin and fluconazole, Eur. J. Med. Chem. 64 (2013) 329-344.
[10] (a) N. Sunduru, L. Gupta, K. Chauhan, et al., Synthesis and antibacterial evaluation of novel 8-fluoro norfloxacin derivatives as potential probes for methicillin and vancomycin-resistant *Staphylococcus aureus*, Eur. J. Med. Chem. 46 (2011) 1232-1244;
(b) Y. Cheng, S.R. Avula, W.W. Gao, et al., Multi-targeting exploration of new 2-aminothiazolyl quinolones: Synthesis, antimicrobial evaluation, interaction with DNA, combination with topoisomerase IV and penetrability into cells, Eur. J. Med. Chem. 124 (2016) 935-945;
(c) W.W. Gao, S. Rasheed, V.K.R. Tangadanchu, et al., Design, synthesis and biological evaluation of amino organophosphorus imidazoles as a new type of potential antimicrobial agents, Sci. China Chem. doi: 10.1007/s11426-016-9009-6.
[11] L. Zhang, D. Addla, P. Jeyakkumar, et al., Discovery of membrane active benzimidazole quinolones-based topoisomerase inhibitors as potential DNA-binding antimicrobial agents, Eur. J. Med. Chem. 111 (2016) 160-182.
[12] (a) C.H. Zhou, L.L. Gan, Y.Y. Zhang, Review on supermolecules as chemical drugs, Sci. China Ser. B-Chem. 52 (2009) 415-458;
(b) C.H. Zhou, F.F. Zhang, L.L. Gan, Y.Y. Zhang, R.X. Geng, Research in supramolecular chemical drugs, Sci. China Ser. B-Chem. 39 (2009) 208-252 (in Chinese);
(c) Y. Cheng, H. Wang, D. Addla, C.H. Zhou, Current researches and applications of azole-based supermolecules as medicinal agents, Chin. J. Org. Chem. 36 (2016) 1-42 (in Chinese);
(d) H. Wang, P. Jeyakkumar, S. Nagarajan, J.P. Meng, C.H. Zhou, Current researches and applications of perylene compounds, Prog. Chem. 27 (2015) 704–743 (in Chinese).
[13] (a) B.K. Paul, N. Guchhait, Exploring the strength, mode, dynamics, and kinetics of binding interaction of a cationic biological photosensitizer with DNA: Implication on dissociation of the drug DNA complex *via* detergent sequestration, J. Phys. Chem. B. 115 (2011) 11938-11949;
(b) X.M. Peng, L.P. Peng, S. Li, Quinazolinone azolyl ethanols: Potential lead antimicrobial agents with dual action modes targeting MRSA DNA, Future Med. Chem. 8 (2016) 1927-1940;
(c) J. Wen, Y.L. Luo, H.Z. Zhang, et al., A green and convenient approach toward benzimidazole derivatives and their antimicrobial activity, Chin. Chem. Lett. 27 (2016) 391-394;
(d) X.L. Li, Y.J. Lin, Q.Q. Wang, et al., The novel anti-tumor agents of 4-triazol-1,8-naphthalimides: Synthesis, cytotoxicity, DNA intercalation and photocleavage, Eur. J. Med. Chem. 46 (2011) 1274-1279.
[14] (a) S. Narva, S. Chitti, B.R. Bala, et al., Synthesis and biological evaluation of pyrrolo[2,3-b]pyridine analogues as antiproliferative agents and their interaction with calf thymus DNA, Eur. J. Med. Chem. 114 (2016) 220-231;
(b) P. Jeyakkumar, L. Zhang, S.R. Avula, C.H. Zhou, Design, synthesis and biological evaluation of berberine-benzimidazole hybrids as new type of potentially DNA-targeting antimicrobial agents, Eur. J. Med. Chem. 122 (2016) 205-215.
[15] (a) G.W. Zhang, P. Fu, L. Wang, M.M. Hu, Molecular spectroscopic studies of farrerol interaction with calf thymus DNA, J. Agric. Food Chem. 59 (2011) 8944-8952;
(b) S.Q. Wen, P. Jeyakkumar, S.R. Avula, L. Zhang, C.H. Zhou, Discovery of novel berberine imidazoles as safe antimicrobial agents by down regulating ROS generation, Bioorg. Med. Chem. Lett. 26 (2016) 2768-2773.
[16] (a) S.M.S. Abdullah, S. Fatma, G. Rabbani, J.M. Ashraf, A spectroscopic and molecular docking approach on the binding of tinzaparin sodium with human serum albumin, J. Mol. Struct. 1127 (2017) 283-288;
(b) J.R. Duan, H.B. Liu, P. Jeyakkumar, et al., Design, synthesis and biological evaluation of novel Schiff base-bridged tetrahydroproberberine triazoles as new type of potential antimicrobial agents, Med. Chem. Commun. doi: 10.1039/c6md00688d;
(c) B.T. Yin, C.Y. Yan, X.M. Peng, et al., Synthesis and biological evaluation of α-triazolyl chalcones as a new type of potential antimicrobial agents and their interaction with calf thymus DNA and human serum albumin, Eur. J. Med. Chem. 71 (2014) 148-159.
[17] (a) S.F. Cui, D. Addla, C.H. Zhou, Novel 3-aminothiazolquinolones: Design, synthesis, bioactive evaluation, SARs, and preliminary antibacterial mechanism, J. Med. Chem. 59 (2016) 4488-4510;
(b) H.Z. Zhang, P. Jeyakkumar, K.V. Kumar, C.H. Zhou, Synthesis of novel sulfonamide azoles via C-N cleavage of sulfonamides by azole ring and relational antimicrobial study, New J. Chem. 39 (2015) 5776-5796.
[18] A. Kamal, M. Sathish, L. Nayak, et al., Design and synthesis of dithiocarbamate linked β-carboline derivatives: DNA topoisomerase II inhibition with DNA binding and apoptosis inducing ability, Bioorg. Med. Chem. 23 (2015) 5511-5526.