Bioorg. Med. Chem. Lett. 41 (2021) 128030

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Isatin-derived azoles as new potential antimicrobial agents: Design, synthesis and biological evaluation

Vijai Kumar Reddy Tangadanchu [1,2], Yan-Fei Sui [2], Cheng-He Zhou [*]

Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Luminescence Analysis and Molecular Sensing, Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

ARTICLE INFO

Keywords:
Isatin
Azoles
Antimicrobial
DNA

ABSTRACT

Novel antibiotics are forced to be developed on account of multidrug-resistant bacteria with serious threats to human health. This work developed isatin-derived azoles as new potential antimicrobial agents. Bioactive assay revealed that isatin hybridized 1,2,4-triazole **7a** exhibited excellent inhibitory activity against *E. coli* ATCC 25,922 with an MIC value of 1 μg/mL, which was 8-fold more potent than reference drug norfloxacin. The active molecule **7a** possessed the ability to kill some bacteria and fungi as well as displayed low propensity to induce resistance towards *E. coli* ATCC25922. Preliminary mechanism investigation indicated that hybrid **7a** might block deoxyribonucleic acid (DNA) replication by intercalating with DNA and possibly interacting with DNA polymerase III, thus exerting its antimicrobial potency.

Isatin is present in Chinese herbal medicine *Indigo Naturalis*, *Folium Isatidis* and *Radix Isatidis*, being an endogenous biological regulator in brain, peripheral tissues, and other body fluids of human beings and animals. The versatility of molecular architecture has made it become an ideal platform for structural modification and derivatization. A large number of isatin derivatives were reported to demonstrate various pharmacological activities such as antibacterial,[1] antifungal,[2] anticancer,[3] antidepressant,[4] antiviral,[5] anthelmintic[6] and antimalarial[7] capabilities. Maremycin A and B[7] are microbial alkaloids of marine origin, and FDA approved drugs semaxanib (SU5416) as well as sunitinib (SU11248) are used for the treatment of various cancers (Fig. 1).[8] Based on the hydroxylation and alkenylation of *C*-3 carbonyl group in discovered pharmacodynamic agents and the antimicrobial work,[9] the similar modification was operated to investigate antibacterial and antifungal behaviors. Furthermore, substitution on *N*-1 position of isatin derived a mass of promising antimicrobial agents,[10] which prompted our keen interests to probe into *N*-substituted molecules as new potentiators against bacterial and fungal strains.

As privileged heterocyclic scaffolds, azoles are able to bind with the enzymes and receptors in organisms easily through non-covalent bond, thus possessing innumerable biological activities.[11] So far, plentiful azole antimicrobial drugs occupy an indispensable position in clinic such as antibacterial secnidazole and ornidazole[12] as well as antifungal fluconazole and voriconazole (Fig. 2).[13] However, the abuse of azole antibiotics has posed the emergence of drug resistance along with limited antimicrobial potency, which is a huge threat to human health.[14] For the sake of combating drug resistance, novel azole antimicrobial agents with high bioactivity and good bioavailability are forced to be developed (see Fig. 3).

Molecular hybridization as an emerging concept in drug discovery has recently gained increasing attention among medicinal community to circumvent growing serious drug resistance.[15] In consideration of biological potentiality of isatin and our previous findings of azole derivatives as for the antimicrobial activities, herein we combined isatin skeleton and azole rings including imidazoles, benzimidazoles, benzotriazole, 1,2,3-triazole and 1,2,4-triazole to generate a class of isatin azole hybrids. Further modification was performed at the 3-position of isatin through hydroxylation and alkenylation. All synthesized isatin-azoles were screened *in vitro* for their antibacterial and antifungal activities and the preliminary structure–activity relationships (SARs) were scrutinized. The discovered active molecule was further explored in bactericidal and fungicidal abilities, in addition, the propensity to induce drug resistance was also detected. Preliminary mechanism was studied on DNA, which presented initially explanation on the

* Corresponding author.
E-mail address: zhouch@swu.edu.cn (C.-H. Zhou).
[1] Postdoctoral fellow from CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, India.
[2] These authors contributed equally to this work.

https://doi.org/10.1016/j.bmcl.2021.128030
Received 19 January 2021; Received in revised form 3 April 2021; Accepted 5 April 2021
Available online 8 April 2021

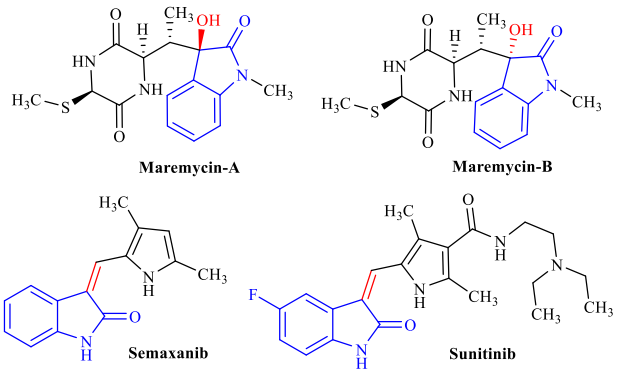


Fig. 1. Structures of biologically active isatin derivatives.

antimicrobial action mechanism. Furthermore, molecular simulation offered an important reference to elucidate the interaction with DNA.

The isatin-azole hybrids **3–8** were efficiently synthesized *via* multi-step reactions starting from commercially available isatin. Intermediate **2** was obtained starting from isatin and 1,2-dibromoethane with sodium hydride as catalyst at 60 °C with a good yield of 71%. The *N*-alkylated precursor was then employed in the synthesis of desired isatin-azole hybrids as shown in Scheme 1. The targeted isatin azoles **3–5** were conveniently and efficiently obtained in 55–78% yields by the reactions of intermediate **2** with imidazoles, benzimidazoles, benzotriazole or triazoles in acetonitrile at 60 °C using potassium carbonate as base.

To investigate the efficacy of the *C*-3 carbonyl functionality in isatin backbone, isatin 1,2,4-triazoles **6–8** were developed from compound **5a** through appropriate derivatization of carbonyl group as depicted in Scheme 2. Initially, condensation of compound **5a** with a series of substituted carbonyl compounds in presence of diethyl amine provided

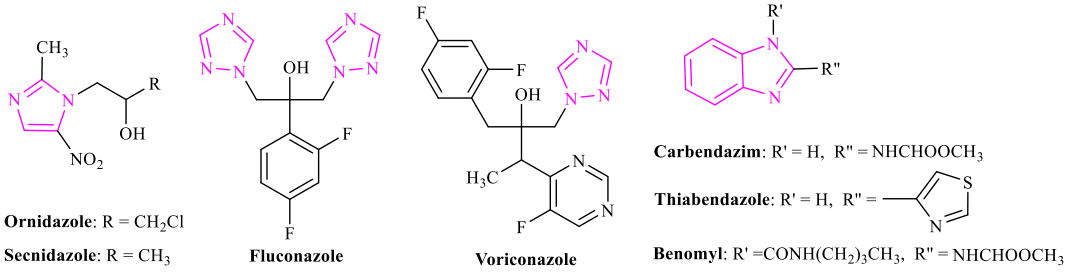


Fig. 2. Structures of antimicrobial azole drugs.

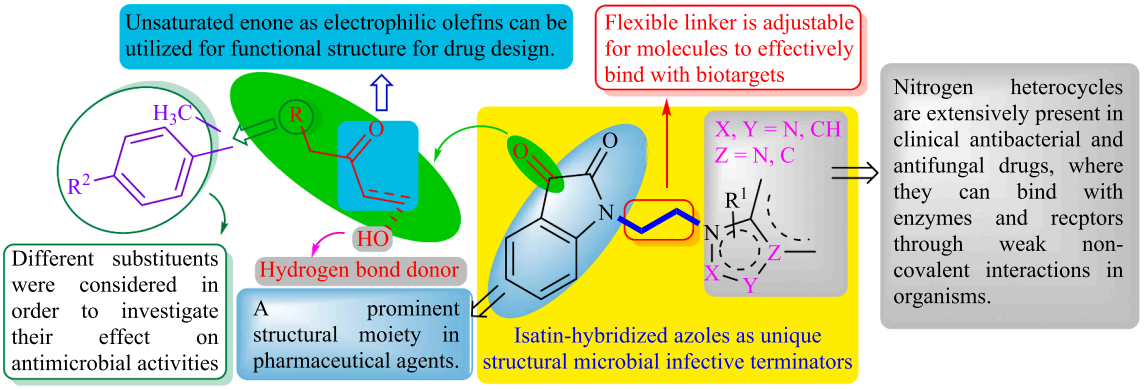
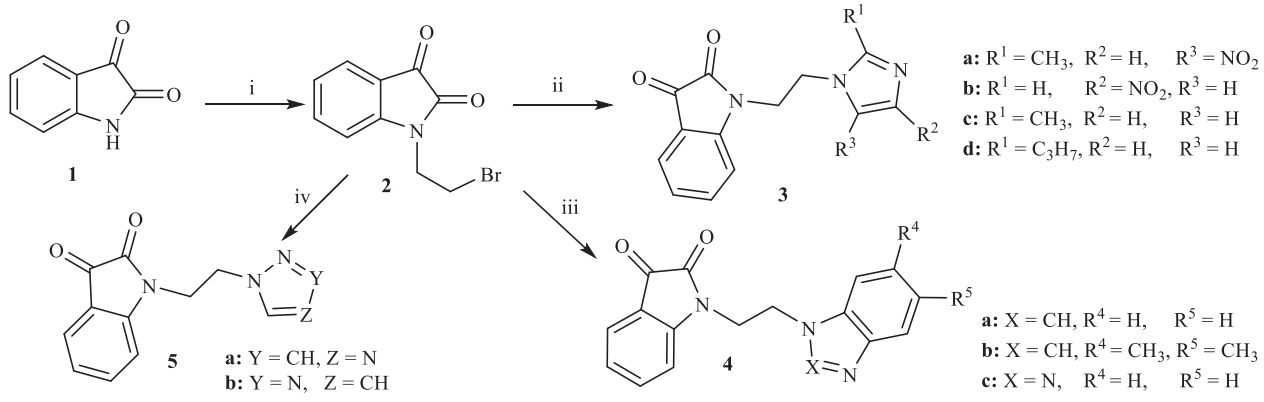
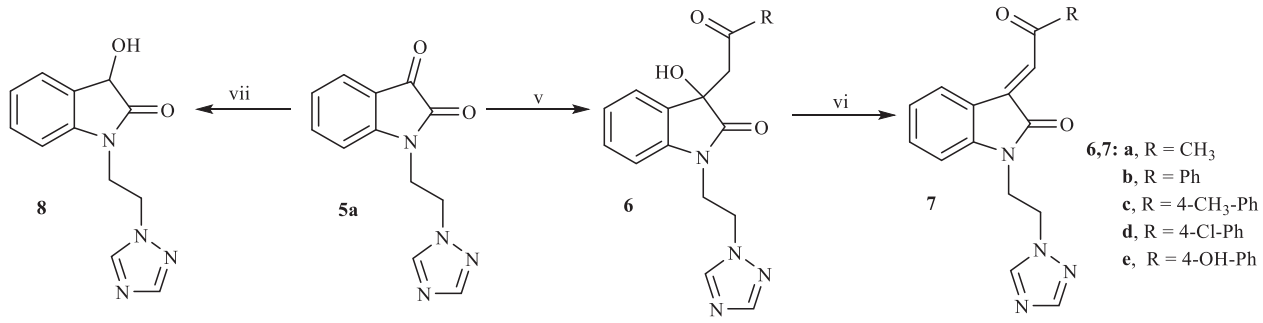


Fig. 3. Design of novel potentially antimicrobial isatin-azole hybrids.



Scheme 1. Design of target isatin azoles **2–5**. Reagents and conditions: (i) 1,2-dibromoethane, NaH, DMF, 60 °C, 12 h; (ii) substituted 1*H*-imidazole, K_2CO_3, CH_3CN, 60 °C, 12 h; (iii) 1*H*-benzimidazole, substituted 1*H*-benzimidazole or 1*H*-benzotriazole, K_2CO_3, CH_3CN, 60 °C, 12 h; (iv)1*H*-1,2,4-triazole or 1*H*-1,2,3-triazole, K_2CO_3, CH_3CN, 60 °C, 10 h.



Scheme 2. Design of target isatin 1,2,4-triazole s **6–8**. Reagents and conditions: (v) carbonyl compound, diethyl amine, CH_3OH, rt, 24 h; (vi) Conc. HCl, glacial AcOH, C_2H_5OH, reflux, 3 h; (vii) CH_2Cl_2:C_2H_5OH, $NaBH_4$, 0 °C, 0.5 h.

Table 1
In vitro antibacterial data as MIC (μg/mL) for isatin-azole hybrids **3–8**.[a,b]

Compds	Gram-positive bacteria					Gram-negative bacteria					
	MRSA	*E. faecalis*	*S. aureus*	*S. aureus* ATCC 25923	*S. aureus* ATCC 29213	*K. pneumoniae*	*E. coli*	*E. coli* ATCC 25922	*P. aeruginosa*	*P. aeruginosa* ATCC 27853	*A. baumanii*
3a	128	128	128	256	128	64	128	128	64	256	128
3b	256	128	128	64	32	64	128	64	64	256	32
3c	128	128	128	128	256	64	256	128	128	128	32
3d	128	128	64	256	16	128	64	64	128	256	32
4a	256	256	64	256	256	128	256	256	512	256	128
4b	128	256	256	256	128	128	256	256	256	256	32
4c	128	512	256	256	256	256	128	128	256	256	128
5a	256	128	128	128	32	128	32	8	128	64	128
5b	256	128	32	256	256	256	256	128	128	128	32
6a	256	256	256	256	256	256	256	512	256	256	256
6b	128	256	128	256	256	256	256	512	512	512	64
6c	256	128	256	256	128	256	128	512	512	256	128
6d	128	256	128	256	128	256	128	512	512	128	64
6e	256	256	256	256	256	256	256	256	256	256	512
7a	128	32	64	128	64	32	64	1	32	32	64
7b	256	128	128	128	128	64	64	8	32	128	128
7c	128	256	64	8	256	256	64	256	128	512	32
7d	32	32	512	128	64	128	512	64	512	512	>512
7e	256	128	128	128	32	256	128	256	256	256	64
8	128	128	128	64	64	128	128	256	256	128	64
A	8	4	1	1	2	4	16	8	2	2	8

[a] Minimal inhibitory concentrations were determined by micro broth dilution method for microdilution plates.
[b] **A** = Norfloxacin, MRSA, *Methicillin-Resistant Staphylococcus aureus*; *E. faecalis*, *Enterococcus faecalis*; *S. aureus*, *Staphylococcus aureus*; *S. aureus* ATCC 25923, *Staphylococcus aureus* ATCC 25923; *S. aureus* ATCC 29213, *Staphylococcus aureus* ATCC 29213; *K. pneumonia*, *Klebsiella pneumonia*; *E. coli*, *Escherichia coli*; *E. coli* ATCC 25922, *Escherichia coli* ATCC ATCC 25922; *P. aeruginosa*, *Pseudomonas aeruginosa*; *P. aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* ATCC 27853; *A. baumanii*, *Acinetobacter baumanii*.

1,2,4-triazole derivatives **6a–e** with good yields of 81–91%. Furthermore, these hybrids were dehydrated in the presence of hydrochloric acid and acetic acid, generating novel *α,β*-unsaturated ketones **7a–e** with good yields 67–81%. In addition, the *C*-3 carbonyl group in compound **5a** was reduced to hydroxyl fragment with sodium borohydride, which gave the desired target triazole derivative **8** with 60% yield.

Referring to National Committee for Clinical Laboratory Standards (NCCLS), the *in vitro* antimicrobial behavior of all the synthesized isatin azoles was screened for the determination of preliminary antibacterial and antifungal activities through the two folds serial dilution technique with the clinical drugs norfloxacin and fluconazole as positive control.[16] Minimal inhibitory concentration (MIC, μg/mL) was defined as the lowest concentration of the tested isatin azoles that completely inhibited the growth of strains. The antibacterial and antifungal abilities are summarized in Table 1 and Table S1, respectively.

Table 1 manifested that various azole-substituted isatins **3–5** possessed moderate to weak antimicrobial potentiality. Relatively, towards *A. baumanii*, 4-nitro imidazole isatin **3b** and 2-methyl imidazole isatin **3c** were more active than 2-methyl-5-nitro one **3a**, which might be the reason of electron cloud density. To explore the impact of longer aliphatic chain, 2-propyl imidazole isatin **3d** was obtained, which exhibited superior inhibitory action against *S. aureus* ATCC 29,213 (MIC = 16 μg/mL) and was equipotent to **3c** towards *A. baumanii*. The results revealed that the kinds and positions of substitutions on imidazole could affect antibacterial ability. Fallaciously, the antibacterial potency decreased when benzimidazoles and benzotriazole were introduced, suggesting that the enlargement of conjugate system of the azole ring was unfavorable for improving bacterial suppression. In comparison to 1,2,3-triazole isatin hybrid **5b**, 1,2,4-triazole derivative **5a** gave better antibacterial potency, especially towards *E. coli* ATCC 25,922 (MIC = 8 μg/mL), which might be due to the difference in electron cloud distribution.

Hybrid **5a** as a precursor was further explored through *C*-3 modification. The target hydroxylated 1,2,4-triazole isatins **6a–e** demonstrated undesirable inhibitory action towards the tested bacterial strains. Fortunately, enone derivatives suggested moderate to good inhibition, which might be because the enlarged conjugate systems. Conjugate **7a** exhibited remarkable anti-*E. coli* ATCC 25922 potency with MIC of 1 μg/mL, which was superior to reference drug norfloxacin (MIC = 8 μg/mL). Furthermore, *E. coli* ATCC 25922 was also quite sensitive to benzene

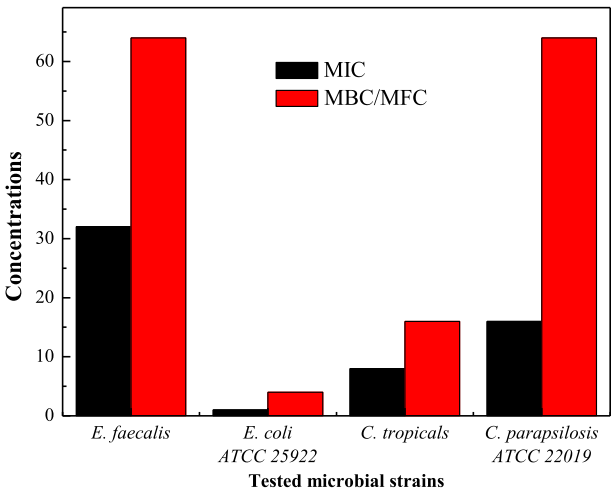


Fig. 4. Comparison of MIC and MBC/MFC values for compound **7a** against *E. faecalis*, *E. coli* ATCC 25922, *C. tropicals* and *C. parapsilosis* ATCC 22019.

derivative **7b** (MIC = 8 μg/mL), being equipotent to norfloxacin. Besides, 4-methyl benzene isatin conjugate **7c** could suppress the growth of *S. aureus* ATCC 25923 with MIC of 8 μg/mL. The results indicated that enone derivatives were favorable for enhancing antibacterial potency. Prominently, molecule **7a** endowed optimal antibacterial behavior with broad spectrum and special inhibition towards *E. coli* ATCC 25922.

Although the antifungal potency was not satisfactory, there is something worthy to be mentioned (Table S1). Various azole-substituted isatins **3–6** exhibited weak inhibition against the tested fungi as well, including antibacterial precursor **5a**. Surprisingly, the antifungal potency of active molecule **7a** was also desirable with MICs of 8–32 μg/mL, in which the *C. tropicals* was sensitive to **7a** comparably with fluconazole. Nevertheless, when methyl group was replaced with phenyl fragments, the antifungal ability of the phenyl conjugates **7b–e** reduced obviously. These results might be explained that the larger conjugate systems were unfavorable for enhancing antifungal potentiality. Hence, the antimicrobial evaluations selected an active molecule **7a** to be further studied.

To investigate whether the active molecule **7a** exerted bactericidal and fungicidal ability, the minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) against bacteria (*E. faecalis*, *E. coli* ATCC 25922) and fungi (*C. tropicals*, *C. parapsilosis* ATCC 22019) were determined and subsequently compared to respective MIC values. According to CLSI, the ratio of MBC or MFC/MIC in the range of 1 to 2 is indicative of killing behavior, while a ratio ≥ 8 is contrastively determined as inhibitory action.[17] As shown in Fig. 4, compound **7a** not only demonstrated a weak bactericidal activity against clinical resistant *E. faecalis*, but also exerted fungi eradicating effect on *C. tropicals*. However, the highly suspectable *E. coli* ATCC 25922 could not be killed by hybrid **7a** in spite of the low suppressive concentration. Conjugate **7a** also presented a 4-fold difference between MIC and MFC against *C. parapsilosis* ATCC 22019, indicating a fungistatic behavior. These implied that isatin derivative **7a** had potentiality to be developed as bactericidal or fungicidal agent against some resistant strains.

Preventing bacteria from developing resistance is an important quality of potential antimicrobial agents.[18] Therefore, the drug resistance test was carried out to evaluate the probability of hybrid **7a** to elicit resistance towards *E. coli* ATCC 25922. Fig. S1 indicated that the susceptibility of *E. coli* ATCC 25922 to molecule **7a** remained nearly unchanged even after 10 passages, while the MIC values of norfloxacin towards *E. coli* ATCC 25922 got quickly enhanced after 6 passages. This consequence manifested that 1,2,4-triazole isatin **7a** developed undetectable resistance towards *E. coli* ATCC 25922.

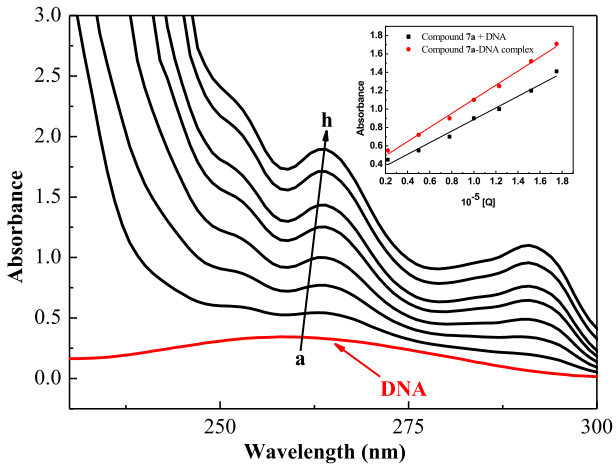


Fig. 5. UV absorption spectra of DNA with different concentrations of compound **7a** at pH 7.4 and room temperature. c(DNA) = 5 × 10^{-5} mol/L, and c (compound **7a**) = 0–2.0 × 10^{-5} mol/L for curves a–h respectively at an increment of 0.25 × 10^{-5} mol/L. Inset: comparison of absorption at 260 nm between the **7a**–DNA complex and the sum values of free DNA and free compound **7a**.

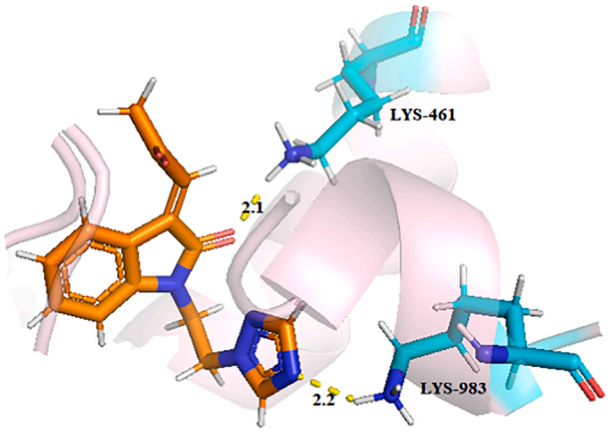


Fig. 6. Molecular modeling of compound **7a** and *E. coli* DNA polymerase III (PDB code: 5fkv).

DNA is widely utilized to rationally design and construct potential new antimicrobial agents, being a target with multiple active sites. Isatin is a planar structure, so it might be able to intercalate into DNA. *In vitro* binding mode of bioactive conjugate **7a** with DNA was studied through UV–Vis absorption spectroscopy, and calf thymus DNA was used as a model with medical importance, low cost and easy availability.[19] The maximum absorption peak of DNA at 260 nm in Fig. 5 got proportionally increased along with slightly red shift under the enhanced concentrations of 1,2,4-triazole isatin **7a**. Meanwhile, the measured absorbance of **7a**-DNA complex was higher than the simple sum of dissociative DNA and **7a**, which revealed a hyperchromism between **7a** and DNA. This consequence intrinsically demonstrated conformational changes in DNA duplex, where the DNA helix was quite possibly broken, and base-pairs were exposed. Based on the variations in the absorption spectra of DNA upon binding to **7a**, equation could be utilized to calculate the binding constant (K) (Fig. S2).

Neutral red (NR) NR, a planar dye with a confirmed intercalative binding mode with DNA, was used to probe into the action mode between **7a** and DNA.[20] The absorption spectrum (Fig. S3) manifested a competitive binding between NR and **7a** with DNA. With the increasing concentration of **7a**, the maximum absorption around 530 nm decreased, presenting a reverse process in comparison with the absorption of free NR in the presence of the increasing concentrations of

DNA. This suggested that molecule **7a** could intercalate into the double helix of DNA by competitively substituting NR in NR-DNA complex, which further blocked DNA replication and exerted antimicrobial activities.

Molecular docking study is widely employed in drug discovery to investigate the binding modes between small molecules and biomacromolecules.[21] In order to explore the influence on DNA polymerase of compound **7a**, *E. coli* DNA polymerase III (PDB ID: 5fkv) was selected to conduct the molecular docking. As shown in Fig. 6, the carbonyl fragment formed hydrogen bond with LYS-461 residue with a distance of 2.1 Å. Besides, the *N* atom at 4-position in the triazole ring also formed hydrogen bond with LYS-983 residue (d = 2.2 Å). These indicated that hybrid **7a** might interfere with the synthesis of *E. coli* DNA through binding with DNA polymerase III, thus displaying profound antibacterial potency.

To sum up, hybrids of isatin with a series of azoles were designed and efficiently synthesized from commercially available isatin. Bioactive assay manifested that 1,2,4-triazole isatin **7a** not only possessed excellent inhibitory activity against *E. coli* ATCC25922 with an MIC value of 1 μg/mL, which was superior to reference drug norfloxacin, but also suppressed the growth of *C. tropical* at a concentration of of 8 μg/mL, being comparable to standard drug fluconazole. The active hybrid **7a** was found with bacterial and fungal killing abilities against *E. faecalis* and *C. tropical* and hardly induced resistance towards *E. coli* ATCC 25922. Preliminary mechanism investigation indicated that active molecule **7a** might intercalate into DNA and potentially interact with DNA polymerase III, which could further block DNA replication, thus exerting its antimicrobial potency.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was partially supported by National Natural Science Foundation of China (No. 21971212), Chongqing Special Foundation for Postdoctoral Research Proposal (No. Xm2016039), and Program for Overseas Young Talents from State Administration of Foreign Experts Affairs, China (No. WQ2017XNDX047).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128030.

References

1 (a) Bhagat K, Bhagat J, Gupta MK, et al. Design, synthesis, antimicrobial evaluation, and molecular modeling studies of novel indolinedione–coumarin molecular hybrids. *ACS Omega*. 2019;4:8720–8730.(b) Wang R, Yin XY, Zhang YH, et al. Design, synthesis and antimicrobial evaluation of propylene-tethered ciprofloxacin-isatin hybrids. *Eur J Med Chem*. 2018;156:580–586.(c) Zhang XM, Guo H, Li ZS, et al. Synthesis and evaluation of isatin-β-thiosemicarbazones as novel agents against antibiotic-resistant Gram-positive bacterial species. *Eur J Med Chem*. 2015;101:419–430.

2 (a) Abo-Ashour MF, Eldehna WM, George RF, et al. Novel indole-thiazolidinone conjugates: Design, synthesis and whole-cell phenotypic evaluation as a novel class of antimicrobial agents. *Eur J Med Chem*. 2018;160:49–60.(b) Al-Wabli RI, Zakaria AS, Attia MI. Synthesis, spectroscopic characterization and antimicrobial potential of certain new isatin-indole molecular hybrids. *Molecules*. 2017;22:1958.(c) Tiwari S, Pathak P, Sagar R. Efficient synthesis of new 2,3-dihydrooxazole-spirooxindoles hybrids as antimicrobial agents. *Bioorg Med Chem Lett*. 2016;26:2513–2516.

3 (a) Meleddu R, Petrikaite V, Distinto S, et al. Investigating the anticancer activity of isatin/dihydropyrazole hybrids. *ACS Med Chem Lett*. 2019;10:571–576.(b) Aneja B, Khan NS, Khan P, et al. Design and development of isatin-triazole hydrazones as potential inhibitors of microtubule affinity-regulating kinase 4 for the therapeutic management of cell proliferation and metastasis. *Eur J Med Chem*. 2019;163:840–852.(c) Javid MT, Rahim F, Taha M, et al. Synthesis, SAR elucidations and molecular docking study of newly designed isatin based oxadiazole analogs as potent inhibitors of thymidine phosphorylase. *Bioorg Chem*. 2018;79:323–333.(d) Ammar YA, Fayed EA, Bayoumi AH, et al. New chalcones bearing isatin scaffold: synthesis, molecular modeling and biological evaluation as anticancer agents. *Res Chem Intermed*. 2017;43:6765–6786.

4 Ma JY, Quan YC, Jin HG, et al. Practical synthesis, antidepressant, and anticonvulsant activity of 3-phenyliminoindolin-2-one derivatives. *Chem Biol Drug Des*. 2016;87:342–351.

5 (a) Meleddu R, Distinto S, Corona A, et al. (3Z)-3-(2-[4-(aryl)-1,3-thiazol-2-yl] hydrazin-1-ylidene)-2,3-dihydro-1H-indol-2-one derivatives as dual inhibitors of HIV-1 reverse transcriptase. *Eur J Med Chem*. 2015;93:452–460.(b) Mishra P, Kumar A, Mamidi P, et al. Inhibition of chikungunya virus replication by 1-[(2-methylbenzimidazol-1-yl) methyl]-2-oxo-Indolin-3-ylidene] amino] thiourea(MBZM-N-IBT). *Sci Rep*. 2016;6:20122.(c) Meleddu R, Distinto S, Corona A, et al. Isatin thiazoline hybrids as dual inhibitors of HIV-1 reverse transcriptase. *J Enzyme Inhib Med Chem*. 2017;32:130–136.(d) Devale TL, Parikh J, Miniyar P, et al. Dihydropyrimidinone-isatin hybrids as novel non-nucleoside HIV-1 reverse transcriptase inhibitors. *Bioorg Chem*. 2017;70:256–266.

6 Kumar S, Bains T, Kim ASW, et al. Highly potent 1*H*–1,2,3-triazole-tethered isatin-metronidazole conjugates against anaerobic foodborne, waterborne, and sexually-transmitted protozoal parasites. *Front Cell Infect Microbiol*. 2018;8:380.

7 Lan YX, Zou Y, Huang TT, et al. Indole methylation protects diketopiperazine configuration in the maremycin biosynthetic pathway. *Sci. China Chem*. 2016;59:1224–1228.

8 Hou YN, Shang CS, Wang H, et al. Isatin-azole hybrids and their anticancer activities. *Arch Pharm*. 2020;353:1900272.

9 Baharfar R, Asghari S, Rassi S, et al. Synthesis and evaluation of novel isatin and 5-isatinylidenerhodanine-based furan derivatives as antibacterial agents. *Res Chem Intermed*. 2015;41:6975–6984.

10 (a) Wang Y, Cheong WL, Liang ZG, et al. Hydrophobic substituents on isatin derivatives enhance their inhibition against bacterial peptidoglycan glycosyltransferase activity. *Bioorg Chem*. 2020;97, 103710.(b) Koshelev VN, Primerova OV, Vorobyev SV, et al. Synthesis, redox properties and antibacterial activity of hindered phenols linked to heterocycles. *Molecules*. 2020;25:2370.(c) Bhagat K, Bhagat J, Gupta MK, et al. Design, synthesis, antimicrobial evaluation, and molecular modeling studies of novel indolinedione coumarin molecular hybrids. *ACS Omega*. 2019;4:8720–8730.

11 (a) Zhou CH, Wang Y. Recent researches in triazole compounds as medicinal drugs. *Curr Med Chem*. 2012;19:239–280.(b) Hu CF, Zhang PL, Lv JS, et al. Ethylenic conjugated coumarin thiazolidinediones as new efficient antimicrobial modulators against clinical methicillin-resistant Staphylococcus aureus. *Bioor Chem*. 2020;94, 103434.(c) Sui YF, Li D, Wang J, et al. Design and biological evaluation of a novel type of potential multi-targeting antimicrobial sulfanilamide hybrids in combination of pyrimidine and azoles. *Bioorg Med Chem Lett*. 2020;30, 126982.

12 (a) Zhang L, Peng XM, Damu GLV, et al. Comprehensive review in current developments of imidazole-based medicinal chemistry. *Med Res Rev*. 2014;34:340–437.(b) Li ZZ, Tangadanchu VKR, Battini N, et al. Indole-nitroimidazole conjugates as efficient manipulators to decrease the genes expression of methicillin-resistant Staphylococcus aureus. *Eur J Med Chem*. 2019;179:723–735.(c) Zhang GB, Maddili SK, Tangadanchu VKR, et al. Discovery of natural berberine derived nitroimidazoles as potentially multi-targeting agents against drug-resistant Escherichia coli. *Sci. China Chem*. 2018;61:557–568.

13 (a) Zhang Y, Tangadanchu VKR, Bheemanaboina RRY, et al. Novel carbazole-triazole conjugates as DNA-targeting membrane active potentiators against clinical isolated fungi. *Eur J Med Chem*. 2018;155:579–589.(b) Fang XF, Li D, Tangadanchu VKR, et al. Novel potentially antifungal hybrids of 5-flucytosine and fluconazole: Design, synthesis and bioactive evaluation. *Bioorg Med Chem Lett*. 2017;27:4964–4969.

14 (a) Brown ED, Wright GD. Antibacterial drug discovery in the resistance era. *Nature*. 2016;529:336–343.(b) Stokes JM, MacNair CR, Ilyas B, et al. Pentamidine sensitizes Gram negative pathogens to antibiotics and overcomes acquired colistin resistance. *Nat Microbiol*. 2017;2:17028.

15 (a) Ivasiv V, Albertini C, Gonçalves AE, et al. Molecular hybridization as a tool for designing multitarget drug candidates for complex diseases. *Curr Top Med Chem*. 2019;19:1694–1711.(b) Hu YY, Wang J, Li TJ, et al. An unexpected discovery toward novel membrane active sulfonyl thiazoles as potential MRSA DNA intercalators. *Future Med Chem*. 2020;12:1709–1727.(c) Cui SF, Addla D, Zhou CH. Novel 3-aminothiazolquinolones: Design, synthesis, bioactive evaluation, SARs, and preliminary antibacterial mechanism. *J Med Chem*. 2016;59(59):4488–4510.(d) Wang LL, Battini N, Bheemanaboina RRY, et al. A new exploration towards aminothiazolquinolone oximes as potentially multi-targeting antibacterial agents: design, synthesis and evaluation acting on microbes, DNA, HSA and topoisomerase IV. *Eur J Med Chem*. 2019;179:166–181.(e) Peng XM, Damu GLV, Zhou CH. Current developments of coumarin compounds in medicinal chemistry. *Curr Pharm Des*. 2013;19:3884–3930.

16 (a) Sun H, Ansari MF, Battini N, et al. Novel potential artificial MRSA DNA intercalators: Synthesis and biological evaluation of berberine-derived thiazolidinediones. *Org Chem Front*. 2019;6:319–334.(b) Hu YY, Bheemanaboina RRY, Battini N, et al. Sulfonamide-derived four-component molecular hybrids as novel DNA-targeting membrane active potentiators against clinical Escherichia coli. *Mol Pharm*. 2019;16:1036–1052.

17 (a) Kaul M, Parhi AK, Zhang Y, et al. A bactericidal guanidinomethyl biaryl that alters the dynamics of bacterial FtsZ polymerization. *J Med Chem*. 2012;55:10160–10176.(b) Rostom SAF, Faidallah HM, Radwan MF, et al. Bifunctional ethyl 2-amino-4-methylthiazole-5-carboxylate derivatives: Synthesis and in vitro

biological evaluation as antimicrobial and anticancer agents. *Eur J Med Chem*. 2014; 76:170–181.

18 (a) Wang LL, Battini N, Bheemanaboina RRY, et al. Design and synthesis of aminothiazolyl norfloxacin analogues as potential antimicrobial agents and their biological evaluation. *Eur J Med Chem*. 2019;167:105–123.(b) Li ZZ, Gopala L, Tangadanchu VKR, et al. Discovery of novel nitroimidazole enols as Pseudomonas aeruginosa DNA cleavage agents. *Bioorg Med Chem*. 2017;25:6511–6522.(c) Chen JP, Battini N, Ansari MF, et al. Membrane active 7-thiazoxime quinolones as novel DNA binding agents to decrease the genes expression and exert potent anti-methicillin-resistant Staphylococcus aureus activity. *Eur J Med Chem*. 2021;217, 113340.

19 (a) Kuzmenko A, Oguienko A, Esyunina D, et al. DNA targeting and interference by a bacterial Argonaute nuclease. *Nature*. 2020;587:632–637.(b) Wang YN, Bheemanaboina RRY, Cai GX, et al. Novel purine benzimidazoles as antimicrobial agents by regulating ROS generation and targeting clinically resistant Staphylococcus aureus DNA groove. *Bioorg Med Chem Lett*. 2018;28:1621–1628.

20 (a) Xu LJ, Wang JN, Sun N, et al. Neutral red as a specific light-up fluorescent probe for i-motif DNA. *Chem Commun*. 2016;52:14330–14333.(b) Li D, Bheemanaboina RRY, Battini N, et al. Novel organophosphorus aminopyrimidines as unique structural DNA-targeting membrane active inhibitors towards drug-resistant methicillin-resistant Staphylococcus aureus. *Med Chem Commun*. 2018;9:1529–1537.

21 (a) Wang J, Battini N, Ansari MF, et al. Synthesis and biological evaluation of quinazolonethiazoles as new potential conquerors towards Pseudomonas aeruginosa. *Chin J Chem*. 2020;39. https://doi.org/10.1002/cjoc.202000627.(b) Zhang Y, Tangadanchu VKR, Cheng Y, et al. Potential antimicrobial isopropanol-conjugated carbazole azoles as dual targeting inhibitors of Enterococcus faecalis. *ACS Med Chem Lett*. 2018;9:244–249.(c) Wang J, Ansari MF, Zhou CH. Unique para-aminobenzenesulfonyl oxadiazoles as novel structural potential membrane active antibacterial agents towards drug-resistant methicillin resistant Staphylococcus aureus. *Bioorg Med Chem Lett*. 2021. https://doi.org/10.1016/j.bmcl.2021.127995. (d) Sui YF, Ansari MF, Zhou CH. Pyrimidinetrione-imidazoles as a unique structural type of potential agents towards Candida albicans: Design, synthesis and biological evaluation. *Chem Asian J*. 2021. https://doi.org/10.1002/asia.202100146.