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DOI: 10.1039/C6MD00357E

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ARTICLE



# Design, synthesis, biological evaluation of novel carbazole aminothiazoles as potential DNA-targeting antimicrobial agents†‡

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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A series of novel carbazole aminothiazoles as new type of antimicrobial agents were designed, synthesized and characterized by $^{1}$H NMR, $^{13}$C NMR, IR, MS and HRMS spectra. Some of the carbazole aminothiazoles exhibited good antimicrobial activities, particularly heptyl derived carbazole aminothiazole **4f** could effectively inhibit the growth of MRSA with MIC value of 4 μg/mL, which was superior to the reference drugs Chloromycin and Norfloxacin. Moreover, the cytotoxicity investigation indicated that bioactive compound **4f** did not exhibit cytotoxicity to Hep-2 cells within its MIC against MRSA. The preliminarily interactive investigation revealed that compound **4f** could effectively intercalate into calf thymus DNA to form **4f**–DNA complexes which might block DNA replication and thus exert antimicrobial activities. In addition, the binding behavior of compound **4f** to DNA revealed that compound **4f** could interact with DNA by hydrogen bonds and electrostatic interactions.

## 1. Introduction

Infectious diseases caused by bacteria and fungi have been causing serious health problems and high mortality especially in portions of South America, Indian subcontinent and tropical fraction of Africa. [1] A large number of antibiotics and synthetic drugs available for clinic play important roles in treatment of microbial infections. [2] However, many bacterial and fungal strains have become resistant to one or more drugs along with the abuse of antibiotics, among them the most challenging one is methicillin-resistant *Staphylococcus aureus* (MRSA), which make a number of first-line drugs decrease their effects or totally lose activities. [3] Thus, it is an urgent task to develop novel antimicrobial drugs with better treatment effectiveness.

Carbazole is an important type of naturally occurring and synthetic nitrogen-containing aromatic heterocycle. This special type of structure of carbazole enables its derivatives readily interact with various biological molecules such as enzymes and receptors, and thus display a broad spectrum of biological activities. [4] Carbazole ring is present in a variety of medicinally active substances including antimicrobial agents such as Carbazomycins and Murrayafoline A. [5] Moreover, recent research showed that carbazoles could non-covalently interact with DNA through intercalation, the minor groove binding or electrostatic interactions. [6] This encourages great interest in investigating carbazoles as a novel type of potential antimicrobial agents. [7] Our previous work showed that the introduction of five-membered azoles into the *N*-position of carbazole could effectively inhibit the growth of the tested bacteria and fungi which were even superior to the reference drugs Chloromycin and Norfloxacin. [8] In recent years, the modification of 3- and 6-positions of carbazole ring has become one of hot topics in the exploitation of novel antibacterial agents. [9]

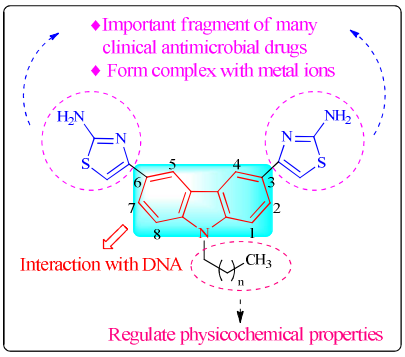


**Fig. 1** Design of novel carbazole aminothiazoles.

Aminothiazole as an important pharmacological fragment is prevalently present in quantities of clinical antimicrobial drugs, such as Cephalosporins, Tigemonam, Aztreonam, Carumonam, Sulfathiazole and Abafungin. Its special property motivates the

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‡ Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x.

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DOI: 10.1039/C6MD00357E

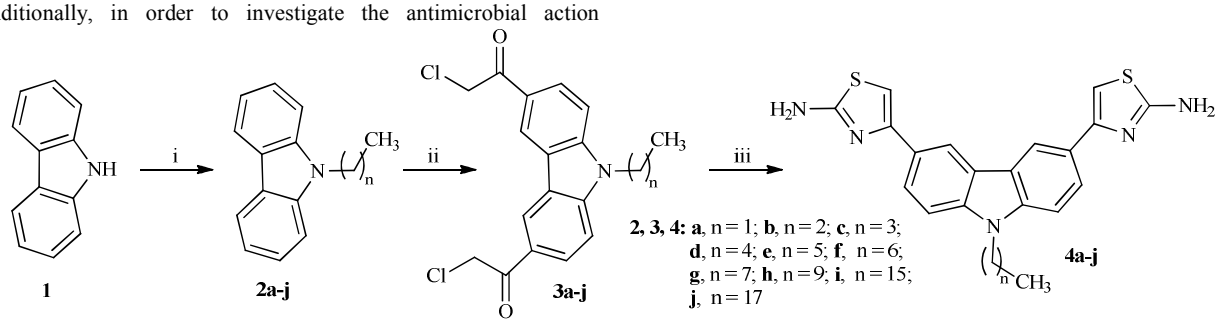



extensive studies to construct antimicrobial agents based on 2-aminothiazole. [10] Currently, structural modification of bioactive compounds by introducing 2-aminothiazole is one of the most convenient and rewarding methods to exploit new antimicrobial agents.

In view of the above considerations and our continuing efforts to develop carbazole-based antimicrobial agents, 2-aminothiazole was for the first time introduced into the 3- and 6-positions of carbazole backbone to investigate the contribution to antimicrobial activity. In addition, different alkyl groups were also introduced into the *N*-position of carbazole with the aim to study biological properties and understand structure-activity relationships. The design idea of novel carbazole aminothiazoles was shown in Fig. 1. Their antibacterial and antifungal activities were evaluated, and the preliminary antimicrobial mechanism was also investigated. Moreover, the highly bioactive carbazole aminothiazole derivatives were further examined for their cytotoxic properties on Human epidermoid cancer cells (Hep-2) by means of CKK-8 Kit. Additionally, in order to investigate the antimicrobial action mechanisms, the interaction of bioactive compound **4f** with calf thymus DNA was also carried out by UV–vis absorption spectroscopy and fluorescence spectra on molecular level.

## 2. Chemistry

The target *N*-alkylcarbazole aminothiazoles **4a–j** were synthesized *via* multi-step reactions from carbazole **1** and the synthetic routine was outlined in Scheme 1. Intermediates **2a**–**j** were prepared in yields of 96–98% by the *N*-alkylation of commercially available carbazole with various alkyl bromides under a nitrogen atmosphere, and then Friedel–Crafts reactions of *N*-alkylcarbazoles **2a**–**j** with chloroacetyl chloride in dichloromethane at room temperature produced carbazoles **3a–j** with yields of 30–36%. The further treatment of compounds **3a–j** with thiourea in refluxing absolute ethanol afforded the *N*-alkylcarbazole aminothiazoles **4a–j** in yields of 94–96%. All new compounds were confirmed by $^{1}$H NMR, $^{13}$C NMR, IR, MS and HRMS spectra (Supplementary Information).




**Scheme 1** Synthetic route of *N*-alkylcarbazole aminothiazoles. Conditions and reagents: i) corresponding alkyl bromide, NaH, dry DMF, r.t., 6 h; ii) $ClCH_2COCl$, $AlCl_3$, dry dichloromethane, r.t., 24 h; iii) thiourea, absolute ethanol, reflux, 1 h.

## 3. Results and discussion

### 3.1. Antimicrobial activities

The newly synthesized compounds were evaluated for their antimicrobial activities *in vitro* against four Gram-positive bacteria (*S. aureus* ATCC25923, *S. aureus* N315, *B. subtilis* ATCC6633 and *M. luteus* ATCC4698), four Gram-negative bacteria (*E. coli* JM109, *P. aeruginosa* ATCC27853, *E. typhosa* ATCC14028 and *B. proteus* ATCC13315) as well as five fungi (*C. utilis* ATCC9950, *A. flavus* ATCC204304, *B. yeast,* ATCC9763, *C. albicans* ATCC10231, *C. mycoderma* ATCC9888) using the standard two folds serial dilution method in 96-well microtest plates according to the National Committee for Clinical Laboratory Standards (NCCLS) (Supplementary Information). The biological tests were carried out in triplicate. Minimal inhibitory concentration (MIC, μg/mL) was defined as the lowest concentration of new compounds that completely inhibited the growth of microbes. Currently available antimicrobial drugs Chloromycin, Norfloxacin and Fluconazole were used as the positive control. The values of ClogP were calculated using ChemDraw Ultra 10.0 software. The antibacterial and antifungal data as well as ClogP values were depicted in Table 1, most of the prepared compounds gave weak to moderate antimicrobial activity against the tested strains *in vitro*. Preliminary active screening showed that carbazole aminothiazoles **4a–j** possessed better antibacterial efficacies than their precursors **3a–j**, which revealed that the 2-aminothiazole fragment was important in exerting antimicrobial activities.

As shown in Table 1, most of the target compounds were weak or inactive to the tested fungal strains. Interestingly, some prepared compounds exhibited good activities against Fluconazole-insensitive *Aspergillus flavus*. Specially, compounds **4h** and **4i** showed comparable efficacies (MIC = 128 μg/mL) against *A. flavus* to Fluconazole (MIC = 256 μg/mL). For antibacterial activity, the majority of target compounds also exhibited weak or moderate inhibition. However, to our surprise, some compounds with moderate length alkyl groups showed good activities against some tested bacteria. Specially, *N*-pentylcarbazole aminothiazole **4d** exhibited potent inhibition of *P. aeruginosa* with MIC value of 2 μg/mL, which was 8-fold more active than reference drug Chloromycin (MIC = 16 μg/mL). Among all the target compounds, the heptyl substituted compound **4f** with MIC value of 4 μg/mL gave 2-16 fold more potent antibacterial efficacy than Chloromycin and Norfloxacin against MRSA. The preliminary structure activity relationship demonstrated



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that the substituent on *N*-position of carbazole exerted great influence on the antibacterial efficacies. It could be observed that the prepared compounds with long hydrophobic alkyl chain such as pentyl and heptyl groups showed better antimicrobial activities than those of shorter ones, because the longer aliphatic group might improve the physiochemical properties which could beneficially enhance the absorption rate and transport of bioactive compound *in vivo*.

**Table 1** *In vitro* antimicrobial data as MIC values (μg/mL)[a, b, c, d] for compounds **3a–j** and **4a–j**.

| Compds | ClogP | Gram-negative bacteria | | | | Gram-positive bacteria | | | | Fungi | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *E. coli* | *B. proteus* | *P. aeruginosa* | *E. typhosa* | MRSA | *S. aureus* | *B. subtilis* | *M. luteus* | *C. utilis* | *A. flavus* | *B. yeast* | *C. albicans* | *C. mycoderma* |
| **3a** | 3.80 | 512 | 512 | 256 | 512 | 512 | 256 | 512 | 256 | 256 | 128 | 512 | 128 | |
| **3b** | 4.33 | 256 | 128 | 256 | 256 | 512 | 512 | 512 | 512 | 256 | 512 | 256 | 256 | 512 |
| **3c** | 4.86 | 512 | 256 | 512 | 512 | 256 | 256 | 256 | 256 | 128 | 256 | 512 | 512 | 128 |
| **3d** | 5.39 | 128 | 512 | 128 | 256 | 512 | 256 | 512 | 512 | 128 | 128 | 128 | 256 | 256 |
| **3e** | 5.29 | 512 | 16 | 16 | 256 | 512 | 512 | 512 | 256 | 256 | 512 | 512 | 512 | 256 |
| **3f** | 6.44 | 512 | 512 | 512 | 512 | 512 | 512 | 512 | 512 | 512 | 512 | 512 | 512 | 256 |
| **3g** | 6.98 | 512 | 512 | 256 | 512 | 512 | 512 | 512 | 512 | 512 | 512 | 256 | 512 | 512 |
| **3h** | 8.04 | 512 | 256 | 128 | 256 | 512 | 256 | 256 | 128 | 256 | 512 | 256 | 256 | 256 |
| **3i** | 10.15 | 256 | 256 | 256 | 512 | 256 | 128 | 256 | 256 | 256 | 256 | 512 | 512 | 256 |
| **3j** | 11.21 | 256 | 512 | 256 | 256 | 256 | 128 | 512 | 512 | 512 | 256 | 256 | 512 | 512 |
| **4a** | 4.88 | 128 | 256 | 256 | 256 | 512 | 256 | 256 | 128 | 128 | 256 | 256 | 64 | 64 |
| **4b** | 5.41 | 256 | 256 | 128 | 256 | 256 | 128 | 128 | 128 | 128 | 256 | 256 | 64 | 256 |
| **4c** | 5.94 | 128 | 128 | 64 | 128 | 256 | 128 | 256 | 128 | 256 | 256 | 128 | 128 | 64 |
| **4d** | 6.47 | 256 | 256 | 2 | 128 | 128 | 64 | 256 | 64 | 128 | 256 | 128 | 128 | 64 |
| **4e** | 6.70 | 128 | 256 | 32 | 256 | 64 | 32 | 512 | 256 | 256 | 512 | 256 | 256 | 128 |
| **4f** | 7.53 | 128 | 256 | 128 | 128 | 4 | 16 | 256 | 64 | 256 | 256 | 128 | 128 | 64 |
| **4g** | 8.06 | 512 | 128 | 512 | 512 | 32 | 64 | 512 | 512 | 512 | 256 | 512 | 512 | 512 |
| **4h** | 9.11 | 256 | 128 | 512 | 512 | 128 | 128 | 512 | 256 | 256 | 128 | 256 | 128 | 128 |
| **4i** | 11.23 | 128 | 128 | 512 | 512 | 256 | 256 | 512 | 256 | 128 | 128 | 256 | 512 | 512 |
| **4j** | 12.29 | 128 | 256 | 512 | 256 | 512 | 256 | 256 | 512 | 256 | 256 | 256 | 256 | 512 |
| **A** | -1.09 | 16 | 32 | 16 | 32 | 64 | 8 | 32 | 64 | - | - | - | - | - |
| **B** | -0.78 | 16 | 4 | 2 | 4 | 8 | 2 | 1 | 2 | - | - | - | - | - |
| **C** | - | - | - | - | - | - | - | - | - | 8 | 256 | 16 | 32 | 32 |

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

[b] MRSA, Methicillin-Resistant *Staphylococcus aureus* N315; *S. aureus, Staphylococcus aureus* ATCC25923; *B. subtilis, Bacillus subtilis* ATCC6633; *M. luteus, Micrococcus luteus* ATCC4698; *E. coli, Escherichia coli* JM109; *P. aeruginosa*, *Pseudomonas aeruginosa* ATCC27853; *B. proteus*, *Bacillus proteus* ATCC13315; *E. typhosa, Eberthella typhosa* ATCC14028; *C. utilis, Candida utilis* ATCC9950; *A. flavus, Aspergillus flavus* ATCC204304; *S. cervisea, Sacchromycese cervisea* ATCC9763; *C. albicans, Candida albicans* ATCC10231; *C. mycoderma*, *Candida mycoderma* ATCC9888.

[c] **A** = Chloromycin, **B** = Norfloxacin, **C** = Fluconazole.

[d] ClogP values were calculated by ChemDraw Ultra 10.0

The lipid/water partition of drugs plays an important role in exerting bioactivities by influencing the transportation, distribution, metabolism and excretion in organisms. [11] The ClogP values have been widely employed to predict transportation and bioactivities of drugs and it is well known that, for ideally pharmacokinetic and pharmacodynamic properties in a drug, the distribution coefficient is a moderate intermediate between hydrophilicity and hydrophobicity. [12] The calculated lipid/water partition coefficients (ClogP) of all prepared compounds were shown in Table 1. The ClogP of compounds **4a**–**j** increased with the increasing length of the alkyl chain, and an enhancement of the antimicrobial activities was observed in compounds **4a**–**f**, but decrease in compounds **4g**–**j**, these might explain the reason that higher lipophilic compounds were difficult to access the binding sites.

### 3.2. Cytotoxicity investigation

The highly bioactive carbazole aminothiazoles **4d** and **4f** were further examined for their cytotoxic properties on Human epidermoid cancer cells (Hep-2) by means of CKK-8 Kit. [13] Compounds **4d** and **4f** were dissolved in DMSO to prepare the stock solutions, and then the tested compounds were prepared in medium to obtain the required concentrations of 5, 10, 25, 50, 100 and 125 μg/mL. Cells were cultured for 24 h in these solutions. The cell viability was examined by microplate reader. Cytotoxicity results (Fig. 2) showed that the cell viability of the tested compounds was at least 85% within concentration of 125 μg/mL. It indicated that the tested compounds **4d** and **4f** did not exhibit cytotoxicity to Hep-2 cells within their MIC against *P. aeruginosa* and MRSA.

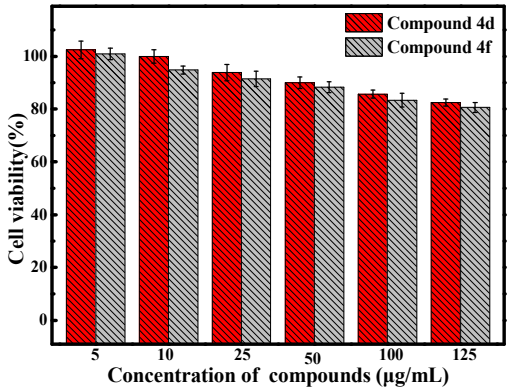


**Fig. 2** Cytotoxic assay of target compounds **4d** and **4f** on Hep-2 tested by CCK-8 Kit. Each data bar represents an average of three parallels, and error bars indicate one standard deviation from the mean.

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### 3.3. Interactions with calf thymus DNA

DNA is an informational molecule encoding the genetic information used in the development and function of almost all the known living organisms, which has been regarded as an important target for exploitation of novel antimicrobial drugs. [14] Here, the binding behavior of compound **4f** with calf thymus DNA, a DNA model that is medical important, low cost and readily available, was studied on molecular level *in vitro* using neutral red (NR) dye as a spectral probe by UV–vis absorption spectroscopy and fluorescence spectra. [15]

#### 3.3.1. Absorption spectra of DNA in the presence of compound 4f

Absorption spectroscopy is one of the most useful techniques used in DNA–binding studies. Hypochromism and hyperchromism are very important spectral features to distinguish the change of DNA double-helical structure in absorption spectroscopy. [16] Due to the strong interaction between the electronic states of intercalating chromophore and that of the DNA bases, the observed large hypochromism strongly displays a close proximity of the aromatic chromophore to the DNA bases. With a fixed concentration of DNA, the UV–vis absorption spectra were recorded with the increasing amount of compound **4f**. As shown in Fig. 3, UV–vis spectra suggested that the maximum absorption peak of DNA at 260 nm exhibited proportional increase and red shift with the increasing concentration of compound **4f**. Meanwhile the absorption value of simply sum of free DNA and free compound **4f** was a little greater than the measured value of **4f**–DNA complexes. This meant that a strong hypochromic effect existed between DNA and compound **4f**. Furthermore, the intercalation of the aromatic chromophore of compound **4f** into the helix and the strong overlap of $\pi$–$\pi^*$ states in the large $\pi$–conjugated system with the electronic states of DNA bases were consistent with the observed spectral changes. [17]

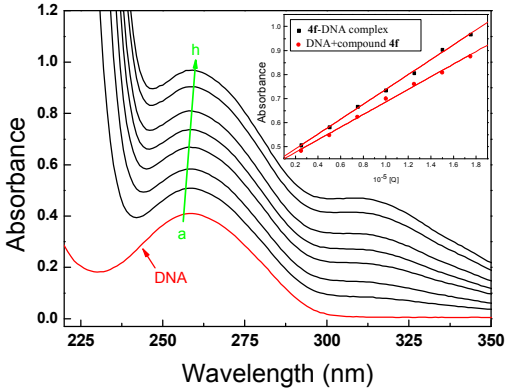


**Fig. 3** UV absorption spectra of DNA with different concentrations of compound **4f** (pH = 7.4, T = 290 K). Inset: comparison of absorption at 260 nm between the **4f**–DNA complex and the sum values of free DNA and free compound **4f**. c(DNA)= 5.08 × $10^{-5}$ mol/L, and c(compound **4f**) = 0–1.75 × $10^{-5}$ mol/L for curves *a*–*h* respectively at increment 0.25 × $10^{-5}$.

Based on the variations in the absorption spectra of DNA upon binding to compound **4f**, equation (1) can be utilized to calculate the binding constant ($K$).

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

$A^0$ and $A$ represent the absorbance of DNA in the absence and presence of compound **4f** at 260 nm, $\xi_C$ and $\xi_{D-C}$ are the absorption coefficients of compound **4f** and compound **4f**–DNA complexes respectively. The plot of $A^0/(A–A^0)$ versus 1/[compound **4f**] is constructed by using the absorption titration data and linear fitting (Supplementary Information: Fig. S1), yielding the binding constant, $K$ = 2.71 × $10^4$ L/mol, R = 0.9992, SD = 0.05248 (R is the correlation coefficient. SD is standard deviation).

#### 3.3.2 Absorption spectra of NR interactions with DNA

It was apparent that the absorption peak of the NR around 460 nm displayed gradual decrease with the increasing concentration of DNA, and a new band around 530 nm developed. This was attributed to the formation of the new DNA-NR complexes. An isosbestic point at about 504 nm also provided evidence of DNA-NR complexes formation. To further understand the interactions between compound **4f** and DNA, the absorption spectra of competitive interaction of compound **4f** were investigated. NR is a planar phenazine dye, which is structurally similar to other planar dyes like thiazines and xanthenes with higher stability, lower toxicity and more convenient application. In recent years, it has been demonstrated that the binding of NR with DNA is an intercalation mode. [18] Therefore, NR was used as a spectral probe to investigate the binding mode of **4f** with DNA in this work. [19] The absorption spectra of the NR dye upon the addition of DNA were displayed in Fig. 4. The research results showed that the absorption peak of the NR around 460 nm exhibited gradual decrease with the increasing concentration of DNA, and a new band around 530 nm developed. This was attributed to the formation of the new DNA–NR complexes.

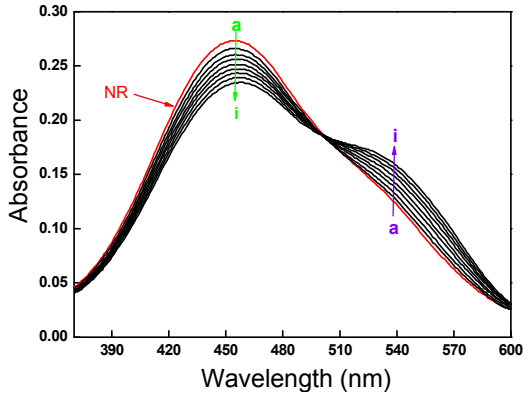


**Fig. 4** UV absorption spectra of NR in the presence of DNA at pH 7.4 and room temperature. $c$(NR) = 2 × $10^{-5}$ mol/L, and $c$(DNA) = 0–3.81 × $10^{-5}$ mol/L for curves *a*–*i* respectively at increment 0.48 × $10^{-5}$.

#### 3.3.3 Absorption spectra of competitive interactions of compound 4f and NR with DNA

The absorption spectra of a competitive binding between NR and compound **4f** with DNA were showed in Fig. 5, with the gradually increasing concentration of **4f**, an apparent maximum absorption around 460 nm of the DNA-NR complexes

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increased. Compared with the absorption band around 460 nm of the free NR in the presence of the increasing concentration of DNA (Fig. 4), the absorbance at the same wavelength exhibited a reverse process (inset of Fig. 5). The results suggested that compound **4f** intercalated into the double helix of DNA by substituting for NR in the DNA-NR complexes. In addition, the increase of absorbance at 276 nm and fluorescence quenching spectra also provided evidence for intercalation of compound **4f** into DNA (Supplementary Information: Fig. S2).

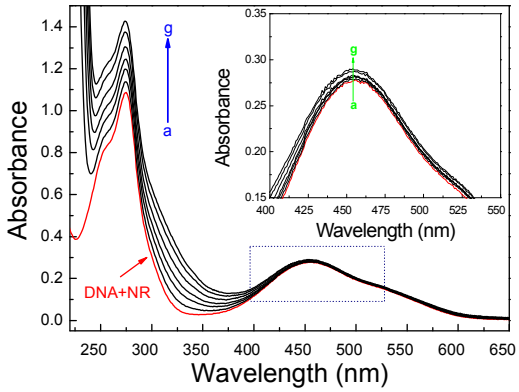


**Fig. 5** UV absorption spectra of the competitive reaction between **4f** and neutral red with DNA. c(DNA) = $4.35 \times 10^{-5}$ mol/L, c(NR) = $2 \times 10^{-5}$ mol/L, and c(compound **4f**) = 0–$1.5 \times 10^{-5}$ mol/L for curves *a*–*g* respectively at increment $0.25 \times 10^{-5}$. Inset: absorption spectra of the system with the increasing concentration of **4f** in the wavelength range of 350–600 nm absorption spectra of competitive reaction between compound **4f** and NR with DNA.

### 3.4 Molecular modeling

To rationalize the observed antibacterial activity and investigate the action mechanism of carbazole aminothiazole **4f**, a flexible ligand receptor docking investigation was undertaken. [20] In this work, molecular docking study was performed between compound **4f** and bacterial DNA (PDB code: 2XCS) to understand their binding modes.

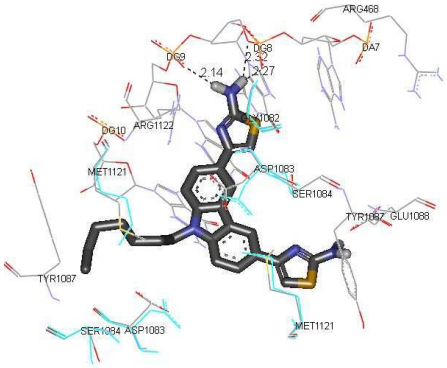


**Fig. 6** Three-dimensional conformation of compound **4f** docked in DNA.

The docking mode with the lowest binding free energy (-10.48 kJ/mol) was shown in Fig. 6 (Supplementary Information: Fig. S3). The docking result of compound **4f** with DNA might rationalize the possible binding behaviors. The amino group of aminothiazole was in close proximity to the residue DG 9 of DNA through a hydrogen bond with distance of 2.14 Å. Moreover, the amino group in **4f** could also form two hydrogen bonds with DNA residue DG 8 with distance of 2.27 and 2.32 Å. These results indicated that the importance of the aminothiazole fragment in interaction with DNA. In addition, electrostatic interactions also existed between compound **4f** with DA7 and DG10 in DNA. These hydrogen bonds and electrostatic interactions might be beneficial to stabilize the compound–DNA complexes, which might prevent the formation of hydrogen bonds between DNA base pairs to further influence the stability of DNA, thus inhibiting its physiological function.

## 4. Conclusions

In conclusion, a series of *N*-alkylcarbazole aminothiazoles were designed and synthesized for the first time *via* an easy, convenient and efficient synthetic route starting from commercial carbazole. All the new compounds were confirmed by NMR, IR and HRMS spectra. The *in vitro* antimicrobial evaluation revealed that some target compounds could effectively inhibit the growth of some tested strains. Structure-activity relationships suggested that the length of alkyl chain could significantly affect the antimicrobial efficacies, and the aminothiazole fragment was important to exert biological activities. Noticeably, carbazole aminothiazole **4f** displayed potent inhibition ability against MRSA (MIC = 4 μg/mL), which was better than reference drugs. Further cell toxicity assay indicated that compound **4f** did not exhibit cytotoxicity to Hep-2 cells within concentration of 125 μg/mL, which was far higher than its MIC against MRSA. The interactive investigations by UV–vis spectroscopic and fluorescence methods revealed that compound **4f** could effectively intercalate into calf thymus DNA to form **4f**–DNA complexes which might further block DNA replication, and thus exerted the antimicrobial activities. Molecular docking indicated that compound **4f** could bind with DNA through hydrogen bonds and electrostatic interactions. All of these indicated that compound **4f** was a promising antimicrobial candidate with good curative effect and low cell toxicity. Further research, including the introduction of aralkyl group (*eg*. *p*-fluorobenzyl, *p*-chlorobenzyl, 2,4-difluorobenzene benzyl) into *N*-position of carbazole and interactions with metal ions as well as *in vivo* biological studies are currently in progress, and all of these will be discussed in the future paper.

## Acknowledgements

This work was partially supported by National Natural Science Foundation of China (No. 21372186, 21172181), the Research Fund for International Young Scientists from International (Regional) Cooperation and Exchange Program of NSFC (No. 81450110451), and the Specialized Research Fund for the Doctoral Program of Higher Education of China (SRFDP 20110182110007) and Fundamental Research Funds for the Central Universities (No. XDJK2016E059).

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View Article Online
DOI: 10.1039/C6MD00357E

# Design, synthesis, biological evaluation of novel carbazole aminothiazoles as potential DNA-targeting antimicrobial agents

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Synthesis of a series of carbazole aminothiazoles as a new type of potential antimicrobial agents, and preliminary interactions with DNA indicated a possible intercalation mechanism.