Accepted Manuscript

Discovery of 2-aminothiazolyl berberine derivatives as effectively antibacterial agents toward clinically drug-resistant Gram-negative *Acinetobacter baumanii*

Wei-Wei Gao, Lavanya Gopala, Rammohan R. Yadav Bheemanaboina, Guo-Biao Zhang, Shuo Li, Cheng-He Zhou

PII: S0223-5234(18)30051-5

DOI: 10.1016/j.ejmech.2018.01.038

Reference: EJMECH 10115

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 9 November 2017

Revised Date: 9 January 2018

Accepted Date: 11 January 2018

Please cite this article as: W.-W. Gao, L. Gopala, R.R. Yadav Bheemanaboina, G.-B. Zhang, S. Li, C.-H. Zhou, Discovery of 2-aminothiazolyl berberine derivatives as effectively antibacterial agents toward clinically drug-resistant Gram-negative *Acinetobacter baumanii*, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2018.01.038.

Graphical Abstract

Discovery of 2-aminothiazolyl berberine derivatives as effectively antibacterial agents toward clinically drug-resistant Gram-negative *Acinetobacter baumanii*

Wei-Wei Gao[1], Lavanya Gopala[1,†], Rammohan R. Yadav Bheemanaboina [1,‡], Guo-Biao Zhang[1], Shuo Li[2,*], Cheng-He Zhou[1,*]

[1] *Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Applied Chemistry of Chongqing Municipality, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China.*

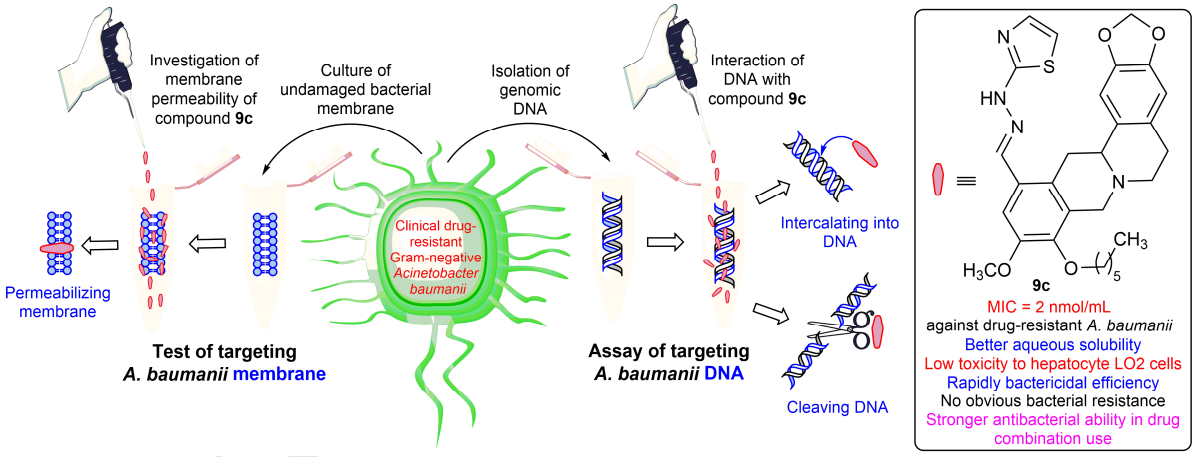
[2] *School of Chemical Engineering, Chongqing University of Technology, Chongqing 400054, P. R. China.*

[†] Postdoctoral researcher from Sri Venkateswara University, India

[‡] Postdoctoral researcher from CSIR-Indian Institute of Integrative Medicine (IIIM), India

* Corresponding Address:

Tel.: +86-23-68254967; fax: +86-23-68254967; E-mail: zhouch@swu.edu.cn (Cheng-He Zhou); lishuo@cqut.edu.cn (Shuo Li).



Title page

Title:

Discovery of 2-aminothiazolyl berberine derivatives as effectively antibacterial agents toward clinically drug-resistant Gram-negative *Acinetobacter baumanii*

Author Names and Affiliations:

Wei-Wei Gao[1], Lavanya Gopala[1,†], Rammohan R. Yadav Bheemanaboina[1,‡], Guo-Biao Zhang[1], Shuo Li[2,*], Cheng-He Zhou[1,*]

[1] *Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Applied Chemistry of Chongqing Municipality, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China.*

[2] *School of Chemical Engineering, Chongqing University of Technology, Chongqing 400054, P. R. China.*

[†] Postdoctoral researcher from Sri Venkateswara University, India

[‡] Postdoctoral researcher from CSIR-Indian Institute of Integrative Medicine (IIIM), India

* Corresponding Address:

Tel.: +86-23-68254967; fax: +86-23-68254967; zhouch@swu.edu.cn (Cheng-He Zhou);

lishuo@cqut.edu.cn (Shuo Li).

Abstract:

Aminothiazolyl berberine derivatives as potentially antimicrobial agents were designed and synthesized in an effort to overcome drug resistance. The antimicrobial assay revealed that some target compounds exhibited significantly inhibitory efficiencies toward bacteria and fungi including drug-resistant pathogens, and the aminothiazole and Schiff base moieties were helpful structural fragments for aqueous solubility and antibacterial activity. Especially, aminothiazolyl 9-hexyl berberine **9c** and 2,4-dichlorobenzyl derivative **18a** exhibited good activities (MIC = 2 nmol/mL) against clinically drug-resistant Gram-negative *Acinetobacter baumanii* with low cytotoxicity to hepatocyte LO2 cells, rapidly bactericidal effects and quite slow development of bacterial resistance toward *A. baumanii*. Molecular modeling indicated that compounds **9c** and **18a** could bind with GLY-102, ARG-136 and/or ALA-100 residues of DNA gyrase through hydrogen bonds. It was found that compounds **9c** and **18a** were able to disturb the drug-resistant *A.*

baumanii membrane effectively, and molecule **9c** could not only intercalate but also cleave bacterial DNA isolated from resistant *A. baumanii*, which might be the preliminary antibacterial action mechanism of inhibiting the growth of *A. baumanii* strain. In particular, the combination use of compound **9c** with norfloxacin could enhance the antibacterial activity, broaden antibacterial spectrum and overcome the drug resistance.

***Keywords*:**

Antimicrobial; Cytotoxicity; Drug combination; DNA; Membrane permeabilization; Thiazole

1. Introduction

The drug resistance for antibiotics and clinically synthetic antibacterial agents is becoming a major global issue and threatens to overburden healthcare systems because of the increasing occurrence of multi-drug resistant (MDR) pathogens worldwide [1–4]. *Acinetobacter baumannii* as one of the ESKAPE pathogens is an important cause for severe infections, particularly in immunocompromised patients [5,6], and has acquired resistance to a wide spectrum of antibiotics used in clinical practice, which often makes treatment extremely difficult and the eradication of this bacterium from the healthcare environment almost impossible [7,8]. *A. baumannii* has been listed by the World Health Organization first on their global priority list of MDR pathogens currently threatening human health [9]. Therefore, an increasingly urgent need is to discover new antibacterial compounds with novel or multi-target activity against drug resistant strains. It is well-known that the discovery of new scaffolds derived from natural sources can open the way to alternative classes of antibiotics with probably new mechanisms of action.

Berberine is a natural isoquinoline quaternary alkaloid from many kinds of naturally medicinal plants such as *Coptis chinensis* (a traditional Chinese herb Huanglian), *Hydrastis canadensis*, *Berberis aristata*, *Coptis japonica*, *Phellondendron amurense etc* [10,11]. It has paid extensive attention for the large clinical potential in treating a number of diseases including infection, Alzheimer's disease, hyperlipidemia, diabetes, metabolic syndrome, obesity, fatty liver disease and so on [12,13]. In particular, berberine as a validated antibacterial agent against gastroenteritis, abdominal pain and diarrhea has been used in China for more than 2000 years [14,15]. Despite of the long term clinical use, the genotoxic, cytotoxic or mutagenic effects, and drug-resistance are seldom reported [16]. However, berberine is present at a very low level in blood and its bioavailability *in vivo* is less than 1% [17,18], which seriously limits its useful profile. It has been found that the structural changes such as disruption of the symmetry or molecular planarity would be efficient strategies to improve the intrinsic aqueous solubility [19,20]. Tetrahydroprotoberberrubine as one of the berberine derivatives can improve the molecular flexibility to a certain extent, which may be conducive to enhancing the water solubility and bioavailability, and is a valid structural alternative with widely medicinal potentiality [21]. Thus, it is important and worthy to further develop tetrahydroprotoberberrubine-based agents for their medicinal value with broader spectrum of antimicrobial activity and more effective for infectious treatments.

Azole compounds like imidazoles [22], triazoles [23,24], tetrazoles [25,26], thiazoles [27,28] and benzene-fused derivatives benzimidazoles [29,30], benzotriazoles [31] and benzothiazoles [32,33] are one of the most important kinds of *N*-heterocycles with various bioactivities and have been extensively employed in the development of antimicrobial agents, especially the aminothiazole derivatives. Aminothiazole moiety is present in a variety of clinical drugs, such as antibacterial sulfathiazole and cephalosporins cefodizime, cefmenoxime, anticancer dasatinib, and antiinflammatory meloxicam [34–36]. The successful development of many clinical aminothiazole-based drugs has been motivating more and

more efforts to develop new bioactive molecules on the basis of this fragment [37]. The structural modifications of clinical drugs by 2-aminothiazole is one of the most convenient and rewarding methods to exploit new medicinal agents [38]. Very recently, the introduction of 2-aminothiazole moiety into the 3-position of antibacterial quinolone skeleton was found to not only improve antimicrobial potency but also afford multi-targeting agents with both membrane active and DNA intercalating potency [39–41]. Aminothiazole fragment can easily interact with DNA, enzymes and other biomacromolecules through noncovalent interactions such as hydrogen bonds, π–π stacking, coordination, ion dipole, hydrophobic effect as well as van der Waals force, because of the unique structural fragment aminothiazole with both electron-donating groups (-NH- or -NH_2, -S-) and the electron-accepting C=N group [42,43]. Therefore, the aminothiazole modified derivatives were endowed to possess multi-targeting binding with multiple biological active sites in biological system, thus possibly overcoming the severe resistance and exhibiting strong bioactivity [44].

Our previous research revealed that the introduction of azoles such as imidazoles [45], benzimidazoles [46,47] and triazoles [48] into the natural berberine scaffold not only improved biological potencies but also broadened the antimicrobial spectrum, including against methicillin-resistant *Staphylococcus aureus* (MRSA). However, to our surprise, aminothiazole, an important structural fragment which is extensively present in a large number of clinical drugs and candidates, so far has not been observed to modify the naturally antibacterial berberine. As continuous efforts in discovering and developing novel small molecule compounds with new or multiple targets effectively inhibiting resistant bacterial strains, herein we have overwhelming interest in combining the naturally antibacterial berberine backbone and the important antibacterial structural aminothiazole to develop a novel series of potentially antibacterial hybrids. The new hybrids of aminothiazole and berberine derivative are expected to exert multi-targeting properties including the validated berberine targeting DNA and membrane activity *via* multiple binding to biological system by using electron-donating groups (-NH- or -NH_2, -S-), electron-accepting bond (C=N) and aromatic moiety in aminothiazole and berberine fragments. More importantly, the aminothiazole functional group may also be helpful for enhancing the water solubility of target molecules *via* forming hydrogen bonds by using its amino and imino groups. Reasonably, the designed target compounds may have larger potential in treating antimicrobial infections including clinically drug-resistant strains because of potential multi-targeting. Therefore, in this work, a series of aminothiazolyl berberine derivatives were constructed. In order to investigate the structure-activity relationships, various substituents, including halophenyl groups and aliphatic chains like alkyl, hydroxyalkyl, alkenyl, alkynyl and cyano ones for regulating the molecular rigidity and flexibility with the validated remarkable influence in biological activities, were introduced to modify the aminothiazolyl berberine backbone. The Schiff base moiety was employed as a bridge linker between aminothiazolyl fragment and berberine backbone because it is a validated fragment to beneficially improve the water solubility [49,50] (Fig. 1).

Fig. 1

The antimicrobial activities *in vitro* for the target 2-aminothiazolyl berberine derivatives and their new precursor compounds were evaluated against clinically Gram-positive and Gram-negative bacteria and fungi including drug-resistant strains. The aqueous solubility of compounds was investigated to helpfully explain the differences in antimicrobial activities. To identify the safety profile, the cytotoxic test for the highly active compound was done against normal human cells. The further experimental studies including bactericidal kinetic assay, drug resistance development, bacterial membrane permeabilization and theoretical exploration of molecular modeling was also performed to verify the research values and foreground of the derivatives. Moreover, the genomic DNA was isolated from the sensitive resistant strains in order to explore the possible antibacterial mechanism by the use of UV-visible absorption spectra and along with Agarose gel electrophoresis. Finally, the combination use of the most active molecule with clinically antibacterial drugs was evaluated to further excavate its potential in enhancing the antimicrobial efficiency and overcoming drug resistance.

2. Results and discussion

2.1. Chemistry

The target 2-aminothiazolyl berberine derivatives were conveniently synthesized from natural berberine according to the routes outlined in Schemes 1–3. Commercially available berberine chloride **1** was easily converted into tetrahydroprotoberberrubine-12-carbaldehyde **4** through demethylation, reduction and formylation in an excellent yield of 82.0% (Scheme 1). The condensation of compound **4** with hydrazinecarbothioamide in anhydrous alcohol conveniently afforded carbothioamide derivative **5**, and then further cyclization with 2-chloroacetaldehyde efficiently produced hydroxyl aminothiazolyl berberine derivative **6** in 45.0% yield.

Scheme 1

The desired 2-aminothiazolyl berberine derivative **6** was further structurally modified with an attempt to investigate the effects of various substituents on bioactivities. Much effort failed in modifying the 9-hydroxyl group of compound **6**. An alternative strategy had to start from 9-hydroxyl berberine-derived aldehyde **4**. The synthetic route was shown in Scheme 2.

Scheme 2

The substitution of compound **4** with a series of aliphatic bromides or chlorides in *N*,*N*-dimethylformamide (DMF) at 80 ℃ using potassium carbonate as the base gave the 9-position substituted berberine derivatives including alkyl one **7**, alkenyl one **10**, alkynyl or cyano one **13**. These prepared intermediates were further condensed with hydrazinecarbothioamide to afford aliphatic carbothioamide precursors **8**, **11** and **14**, respectively, which were further reacted with 2-chloroacetaldehyde in anhydrous ethanol at 80 ℃ to produce the desirable aliphatic aminothiazolyl

berberine derivatives **9**, **12** and **15** in 30.1–45.8% yields, respectively.

The target phenyl series of aminothiazolyl berberine derivative **18** was also obtained in yields ranging from 33.3% to 42.1% under the similar reaction condition starting from intermediate **4**, the substituted benzyl chlorides, hydrazinecarbothioamide and 2-chloroacetaldehyde (Scheme 3).

Scheme 3

The structures of all the new compounds were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS spectra. The spectral analyses were in accordance with the assigned structures, and all the spectral data were listed in the Experimental section. The HRMS for each new target compounds gave a major fragment of $[M + H]^+$ according to their molecular formula.

2.2. Biological Activity

All the new compounds including berberine thioamide intermediates (**5**, **8a-g**, **11**, **14a-b** and **17a-i**) and target 2-aminothiazolyl berberine derivatives (**6**, **9a-g**, **12**, **15a-b** and **18a-i**) were evaluated for biological activities *in vitro* toward a panel of bacteria (standard *S. aureus* ATCC 25923, *B. subtilis* ATCC 21216, *M. luteus* ATCC 4698, *E. coli* DH52, *P. aeruginosa* ATCC 27853; MRSA (Methicillin-resistant *Staphylococcus. aureus* (N315), drug-resistant *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumonia*, *A. baumanii*) and fungi (standard *C. albicans* ATCC 76615, *C. utilis* ATCC 9950, *C. mycoderma* ATCC 96918, *A. flavus* ATCC 204304, *S. cerevisiae* ATCC 9763, *C. parapsilosis* ATCC 22019; drug-resistant *C. albicans C. tropicals*, *A. fumigates*). The MIC (minimum inhibitory concentration) values were determined by using a protocol according to the Clinical and Laboratory Standard Institute (CLSI) guidelines [51].

2.2.1. Antibacterial activity

The antibacterial data in Tables S1 and S2 revealed that some intermediates berberine thioamide derivatives displayed better inhibitory activity against the tested standard and clinically drug-resistant bacterial strains than natural berberine. The alkyl berberine thioamides **8c** and **8e** not only gave good activity against standard *E. coli* DH52 with MIC value of 0.03 μmol/mL, but also displayed 53 times stronger inhibitory effect toward drug-resistant *E. coli* than norfloxacin. Moreover, the clinically drug-resistant *A. baumanii* was sensitive to most of the berberine thioamides such as 2,4-dichlorobenzyl intermediate **17a** (MIC = 3 nmol/mL). These results suggested that the modified berberine backbone was favorable to antibacterial potencies, especially for resistant strains.

Table 1 showed that some target aminothiazolyl berberine derivatives possessed moderate to good efficacies in inhibiting the growth of standard bacterial strains. Hexyl derivative **9c** gave potent activities with MIC values ranging from 0.03 to 0.06 μmol/mL against *S. aureus* ATCC 25923, *M. luteus* ATCC 4698, *E. coli* DH52 and *P. aeruginosa* ATCC 27853, which exerted almost equivalent effects to chloromycin. The replacement of hexyl group with long alkyl chain to yield dodecyl derivative **9f** resulted in decreased activities against standard strains (MIC = 0.03–0.83 μmol/mL), which might be attributed to the excessive

lipophilicity. Comparably, the short alkyl chain modified molecule **9b** gave better inhibitory potencies with MIC values ranging from 0.06 to 0.25 μmol/mL. Aminothiazolyl berberine derivative **9a** incorporating ethyl group exhibited profound efficiency to obviously suppress the growth of *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 and *E. coli* DH52 with MIC value of 0.07 μmol/mL, which was more active than ethoxyl compound **9g** with the same length chain, and this indicated that the hydroxyl group was unfavorable for the antibacterial activity. The unsaturated substituents modified derivatives **12** and **15** displayed no obvious enhancement in inhibitory effect.

Table 1

In comparison with aliphatic modified compounds, most benzyl derivatives **18a**–**i** showed relatively lower activities in inhibiting the growth of standard bacterial strains. Compound **18h** with unsubstituted phenyl moiety did not show apparent inhibition even though the concentration was up to 0.95 μmol/mL. The introduction of halogen atom significantly improved the antibacterial effect. Chlorobenzyl molecules **18b–d** gave good activities against *S. aureus* ATCC 25923, *B. subtilis* ATCC 21216 and *E. coli* DH52 with MIC value of 0.11 μmol/mL. Especially, 4-chlorobenzyl derivative **18d** displayed better inhibitory potencies (MIC = 0.11**–**0.45 μmol/mL) than berberine (MIC = 0.34**–**0.69 μmol/mL), which indicated that the position of chlorine atom on benzyl group would lead to different bioactivities. Compounds **18a** and **18e** with the increased chlorine atom did not remarkably improved effect. The displacement of chlorobenzyl moiety by fluorobenzyl group would afford aminothiazolyl berberine derivatives **18f** and **18g** with similar inhibitory efficiency (MIC = 0.06 μmol/mL) against *S. aureus* ATCC 25923 to chloromycin (MIC = 0.05 μmol/mL) and 12-fold greater anti-*M. luteus* ATCC4698 activity than berberine, respectively. Noticeably, Gram-negative *P. aeruginosa* (ATCC 27853) was sensitive to nitrobenzyl compound **18i** with MIC value of 0.05 μmol/mL, 14- and 2-fold more potent than berberine and chloromycin, respectively, which showed that the electron-withdrawing group was useful for improving antibacterial potentiality.

Fig. 2a and 2b showed the antibacterial data of aminothiazolyl berberine compounds toward drug-resistant strains, it was revealed that the most potent inhibitors were derivatives **9c** and **18a** against the drug-resistant *A. baumanii* with a low nanomolar MIC value of 2 nmol/mL. Moreover, *E. faecalis*, MRSA and *P. aeruginosa* were also sensitive to compound **9c** with MICs of 0.03, 0.03 and 0.06 μmol/mL, respectively. Alkyl derivatives **9a** and **9b** gave MIC values ranging from 0.06 to 0.07 μmol/mL against MRSA and drug-resistant *E. coli*, respectively. Moreover, 3-chlorobenzyl derivative **18c** (MIC = 0.01 μmol/mL) and benzyl one **18h** (MIC = 0.02 μmol/mL) displayed more potent antibacterial efficiencies against *A. baumanii* than their precursors berberine thioamides **17c** and **17h**, which strongly manifested that the aminothiazole moiety could optimize the inhibitory activities against drug-resistant strains, particularly *A. baumanii*.

Fig. 2

The antibacterial evaluation showed that drug-resistant *A. baumanii* was sensitive to most of the aminothiazolyl berberines, especially to derivatives **9c** and **18a**, but almost all the target compounds could not effectively inhibit the growth of drug-resistant *K. pneumonia*. Hydroxyl compound **6** showed slightly weaker activity against the tested strains than derivatives modified by aliphatic chains or substituted benzyl groups, which suggested that the discernible inhibitory benefit of substituents on the antibacterial activity.

2.2.2. Antifungal activity

The antifungal evaluation in Tables S3 and S4 revealed that most of the non-thiazole intermediates exhibited weak inhibition activities against the tested fungi including drug-resistant ones. However, the formation of aminothiazole ring would lead to aminothiazolyl berberine derivatives with good antifungal potencies. Compound **9c** (MIC = 4 nmol/mL) showed comparable inhibition against the growth of *C. parapsilosis* ATCC 22019 to fluconazole (MIC = 7 nmol/mL). It also conferred about 14-fold anti-*A. flavus* ATCC 204304 activity than fluconazole. Particularly, compound **18a** as the most potent bacterial inhibitor also displayed large anti-*A. fumigates* potencies (MIC = 0.03 μmol/mL).

All the antimicrobial results implied that the antimicrobial efficacies should be closely related to aminothiazole ring and the substituents at 9-position of berberine skeleton to some extent. In particular, aminothiazolyl berberine compounds **9c** with a hexyl chain and **18a** with a 2,4-dichlorobenzyl group exhibited unprecedentedly effective inhibition toward drug-resistant *A. baumanii* with the same low MIC value of 2 nmol/mL, and should be deserved to be further investigated as potentially antimicrobial agents. The antibacterial activities of aminothiazolyl berberine derivatives were commonly stronger than those of thioamide intermediates, proper length of the aliphatic chains and halophenyl groups were also important for the activity. In general, halo- or nitro-substituted phenyl derivatives seemed to be less active than those of short aliphatic chain ones, which might be due to a relative decrease in water solubility.

2.3. Aqueous solubility

Aqueous solubility is also a governing property for small molecules interacting with biomolecule in living systems, which plays a critical role in every stage of drug development and discovery.[52] Therefore, the water solubility of all aminothiazolyl berberine derivatives was tested at physiological pH, natural berberine as the control, and the obtained results were depicted in Fig. 3. Generally, most of the aminothiazolyl compounds were more soluble in water than berberine (0.086 mg/mL), except for decyl derivative **9e** (0.061 mg/mL). For aliphatic ethyl molecule **9a**, butyl one **9b**, hydroxyethyl one **9g**, allyl one **12**, and cyano one **15b**, they were more soluble in water (7.02–10.2 mg/mL) than the phenyl ones **18a-i** (0.374–3.19 mg/mL) and hydroxyl one **6** (1.66 mg/mL). In particular, compound **9a** showed the best water solubility of 10.2 mg/mL in the three series, 118 times higher than berberine. Whereas, the highly active compounds **9c** and **18a** possessed the aqueous solubility of 1.42 and 1.90 mg/mL, respectively. These obtained results were almost consistent with the antibacterial activities that the compounds with proper aqueous solubility and lipophilicity showed high inhibitory efficiency against bacterial strains. Moreover,

the insertion of 2-aminothiazolyl and Schiff base moieties might be beneficial for the improvement of aqueous solubility.

Fig. 3

2.4. Cytotoxicity

Despite of the long term clinical use of berberine as a validated antibacterial drug, the genotoxicity or cytotoxicity has been seldom reported. The cytotoxicity is one of the most essential criteria to evaluate the potential of antimicrobial agents. In order to identify the safety profile, the highly active aminothiazolyl berberine derivatives **9c** and **18a** were further evaluated for their toxicities against normal human hepatocyte LO2 cells using the colorimetric cell proliferation MTT assay. The cell viability of compounds **9c** and **18a** against LO2 cells was more than 70.7% and 80.5%, respectively, which suggested that they exhibited low toxicities to the cells even though the concentration up to 100 μg/mL (Fig. 4). Also, it might be concluded that the antibacterial activities exhibited by aminothiazolyl berberine derivatives were not due to the cytotoxic effects.

Fig. 4

Upon the basis of *in vitro* antimicrobial and cytotoxic results, hexyl derivative **9c** and 2,4-dichlorobenzyl one **18a** were selected for detailed evaluation, including bactericidal kinetics, drug resistance development, bacterial membrane permeabilization and molecular docking studies. Furthermore, the preliminary action mechanism of compound **9c** was also investigated.

2.5. Bactericidal kinetics

There is always a need to discover rapidly bactericidal agents to combat antibacterial resistance, and time kill kinetics is an important method to judge the efficacy of investigational antimicrobial agents [53]. To determine whether the aminothiazolyl berberine derivatives have bactericidal efficiency, the viability of exponentially growing drug-resistant *A. baumanii* was checked toward the highly active molecules **9c** and **18a** by time-kill kinetics experiment.

In the time kill assay, the colony forming units (CFUs) of the *A. baumanii* were rapidly decrease after treatment with both compounds **9c** and **18a** at the concentration of 8 nmol/mL, and more than 10^3 CFU/mL reduction in the number of viable bacteria within 2 h. Therefore, it could be interpreted that derivatives **9c** and **18a** gave a similar bactericidal efficacy and had potent killing effects against drug-resistant *A. baumanii* (Fig. 5).

Fig. 5

2.6. Drug resistance development

Resistance to antibiotics has been increasing in recent years and becoming a serious and global challenge

to the drug discovery. The mutagenic properties of bacteria raise the possibility of the resistance even in smaller populations of bacteria exposed to low concentration of antibacterial drugs [54]. Therefore, it is significantly vital to study the resistance of the potent compound against bacterial strains. The highly active compounds **9c** and **18a** were employed to investigate the drug resistance of *A. baumanii*. Fig. 6 showed that the slowly developing resistance of *A. baumanii* to aminothiazolyl berberine derivatives **9c** and **18a** even after 10 passages.

Fig. 6

2.7. Bacterial membrane permeabilization

Bacterial membrane has been considered as a significant and intriguing antibacterial target, and membrane-active nature induces low propensity for bacteria to develop resistance [55,56]. Therefore, it is reasonable to evaluate the membrane permeabilization of compounds **9c** and **18a** against drug-resistant *A. baumanii via* fluorescence spectra using propidium iodide (PI), a common dye that can only pass through the membrane of dead and apoptotic cells and fluoresces upon forming the PI-DNA complex [57,58]. There is an increase of fluorescence intensity within 75 min in Fig. 7, which should be due to the formed PI-DNA complex as the gradual damage of bacterial membrane in the presence of derivatives **9c** and **18a** at 0.024 μmol/mL (12 times MIC). The results manifested that compounds **9c** and **18a** could also effectively disturb the membrane of *A. baumanii*.

Fig. 7

2.8. Molecular docking study

Molecular docking is considered as one of the convenient and effective theoretical methods in binding studies [59]. Thus, a docking investigation was undertaken to explore the inhibitory effects of 2-aminothiazolyl berberine derivatives against DNA gyrase. DNA gyrase catalyzes changes in DNA topology by breaking and rejoining double stranded DNA. In particular, DNA gyrase introduces negative supercoils in DNA in front of the replication fork [60]. Thus, DNA gyrase is involved in very important processes during DNA replication and are essential for cell viability. The crystal of DNA gyrase B was selected as a representative target in the Protein Data Bank, and compounds **9c** and **18a** were used to dock with DNA gyrase B. The docking evaluation gave good binding energy of 6.31 and 5.26 kcal/mol for derivatives **9c** and **18a** against DNA gyrase B, respectively, which should also rationalize the antibacterial mechanism of 2-aminothiazolyl berberine derivatives.

As shown in Fig. 8, the amino group at 2-position of the thiazole ring in molecule **9c** was adjacent to GLY-102 residue, forming a hydrogen bond with a distance of 2.4 Å. The oxygen atom of methoxyl fragment at 10-position of the berberine skeleton and ARG-136 residue could also form a hydrogen bond with a distance of 2.7 Å. For compound **18a**, a similar hydrogen bond was formed between the amino group at 2-position of the thiazole ring and residue ALA-100 with a distance of 2.5 Å. All of these

hydrogen bonds could be favorable to stabilize the 2-aminothiazolyl berberine derivative-enzyme complex, which might be responsible for the good inhibitory efficacy of compounds **9c** and **18a** against the tested strains and the importance of 2-aminothiazolyl pharmacophore.

Fig. 8

2.9. Preliminary antibacterial mechanism study

The good antimicrobial efficiency, no apparent cytotoxicity, rapid killing of *A. baumanii* pathogens, low drug resistance development and bacterial membrane disruptive properties for compounds **9c** and **18a** prompted us to examine the preliminary mechanism of action. It is known that the biological targets of many antibacterial drugs are DNA, cell membrane, enzymes, *etc*. Especially, a growing investigation directs toward the interaction of small drug molecules with DNA for the rational design and construction of new and efficient agents [61]. To explore the preliminary antibacterial action mechanism, the most active hexyl aminothiazolyl berberine derivative **9c** was presumed to exhibit activity due to targeting drug-resistant *A. baumanii* DNA by using UV-vis spectroscopic method.

*2.9.1. Interaction between compound **9c** and drug-resistant A. baumanii DNA*

Berberine and its derivatives could effectively interact with DNA through various binding modes. Therefore, the binding behavior of compound **9c** with *A. baumanii* DNA was studied on molecular level using neutral red (NR) dye as a spectral probe. Genomic DNA was isolated from drug-resistant *A. baumanii* bacteria which were the most sensitive to **9c**, based on the reported procedure and assay evaluated [62]. According to the Bouguer−Lambert−Beer law, the concentration of *A. baumanii* DNA in stock solution was calculated on the basis of UV absorption value at 260 nm and the molar absorption coefficient (ξ_{260} = 6600 L•mol^{-1}•cm^{-1}) expressed as molarity of phosphate groups. The purity of *A. baumanii* DNA was checked by gel electrophoresis (Fig. 10a) and monitoring the ratio of the absorbance at 260 nm to that at 280 nm. A ratio of > 1.8 at A260/A280 was given, which indicated that the DNA was completely free from protein in the solution.

The absorption spectroscopy as one of the most important methods is extensively used in DNA-binding studies [63]. The important spectral characteristics to distinguish the change of DNA double-stranded structure in absorption spectroscopy are hyperchromism and hypochromism. A large hypochromism will be observed because of the strong interaction between the electronic states of DNA bases and that of the intercalating chromophore, which strongly indicates a close proximity of the aromatic chromophore to DNA bases. With a fixed DNA concentration of 8.12 × 10^{-4} mol/L, UV-vis absorption spectra were recorded with the increasing amount of compound **9c** (Fig. 9a). The maximum absorption peak of DNA at 260 nm exhibited proportional increase with the gradually increasing concentration of derivative **9c**. Furthermore, a weak hypochromism existed between DNA and compound **9c** because the absorption value of the **9c**-DNA complex was lower than that of simply sum of free DNA and free molecule **9c**. This

hypochromism effect might be attributed to the formed binary complexes between compound **9c** to DNA bases. More importantly, the intercalation of the aromatic chromophore of **9c** into the DNA double helix and the strong overlap of π-π* states in the π-conjugated system with DNA bases electronic states were agreement with the observed spectral changes [64].

Fig. 9

On the basis of the variations in the absorption spectra of *A. baumanii* DNA upon binding to derivative **9c**, equation (S1) was adopted to calculate the binding constant $K = 2.54 \times 10^3$ L/mol, R = 0.999, SD = 0.01 (R is the correlation coefficient. SD is standard deviation).

2.9.2. Interaction between NR and drug-resistant A. baumanii DNA

It has been demonstrated that the binding of NR, a planar phenazine dye, with DNA is an intercalation mode in recent years [65]. Therefore, NR was employed as the spectral probe to investigate the binding mode of derivative **9c** with *A. baumanii* DNA in the present work.

The absorption peak of the NR around 460 nm was gradual decrease with the continuous addition of *A. baumanii* DNA (Fig. S5). Due to the formation of the DNA-NR complex, a new band emerged at 530 nm.

*2.9.3. Competitive interaction of compound **9c** and NR with drug-resistant A. baumanii DNA*

As shown in Fig. 9b, there was a competitive binding between derivative **9c** and NR with *A. baumanii* DNA. An obvious intensity increase was observed in the developing band around 460 nm with the increasing concentration of **9c**. Moreover, the absorbance at 460 nm exhibited the reverse process in comparison with that of around the same wavelength of free NR in the presence of the increasing concentration of DNA in Fig. S5. The results indicated that compound **9c** could intercalate into *A. baumanii* DNA double helix through displacement of NR in the DNA-NR complex. Furthermore, the increase of absorbance at 276 nm provided another evidence for compound **9c** intercalating into DNA.

2.9.4. Cleavage of drug-resistant A. baumanii DNA

The effective cleavage of biological DNA is a quite important strategy to treat various diseases [66]. An increasing effort is to develop the DNA cleavage agents. In order to investigate the different action modes of small drug molecule with DNA, the cleaving activity of compound **9c** toward *A. baumanii* DNA was studied under physiological conditions (T = 37 ^{o}C and pH = 7.4). As shown in Fig. 10b, there was no DNA cleavage for the control in which without derivative **9c** (Lane 1 and Lane 2). However, the apparent DNA cleaving evidence was observed in the presence of **9c** (Lane 3). It suggested that molecule **9c** might inhibit the growth of *A. baumanii* by dual action modes of both intercalating and cleaving DNA.

Fig. 10

*2.10. Drug combination use of compound **9c** with norfloxacin*

The combination therapy currently is an increasingly prevalent method to treat severely microbial infections, such as the combination use of colistin, auranofin and ceftazidime toward MDR *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* [67]. Along with the rapid emergence of resistance toward clinically antibacterial drugs, more and more effort has been witnessed to develop the drug combination treatment of bacterial infections. A lot of work showed that the combination use of new structural agents with clinically antimicrobial drugs could improve the efficiency, reduce or eliminate side effects and even combat drug resistance *via* different modes of action [68]. In our present work, the drug combination use between the active molecule **9c** and the clinically used norfloxacin was investigated toward the drug-resistant bacteria including MRSA, *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumonia* and *A. baumanii*.

The fraction inhibitory concentration (FIC) index was designated for combination study of **9c** with norfloxacin in inhibiting bacterial growth. The results in Table 2 indicated that **9c** showed significant synergism (FIC < 0.50) with norfloxacin against drug-resistant bacteria, except for against *E. faecalis* (0.50 < FIC < 1.00). Particularly, compound **9c** in combination use resulted in enhancing antibacterial efficacy of norfloxacin by 4-fold (MIC from 0.025 to 0.006 μmol/mL) toward MRSA. At the same time, MIC value of **9c** was lowered by 4-fold in this combination (MIC from 0.030 to 0.007 μmol/mL). It was also worthy to note that the combination could effectively inhibit the growth of drug-resistant *A. baumanii*. These results manifested that the combination use of molecule **9c** with clinical norfloxacin opened up an efficacious approach to combat drug resistance with different action modes. This also represented a dynamic strategy to improve antibacterial potency.

Table 2

3. Conclusion

In this work, a series of aminothiazolyl berberine derivatives as potentially antimicrobial agents were developed *via* convenient procedures from natural berberine. All the structures of the new compounds were characterized by NMR and HRMS spectra. The biological evaluation *in vitro* indicated that some prepared compounds showed moderate to good antibacterial and antifungal activities, and the insertion of 2-aminothiazolyl and Schiff base moieties was beneficial to improve antibacterial efficiency and aqueous solubility. Hexyl aminothiazolyl berberine derivative **9c** and 2,4-dichlorobenzyl one **18a** gave strong inhibitory activities against drug-resistant Gram-negative *A. baumanii* with low MIC values of 2 nmol/mL, low cytotoxicity to human hepatocyte LO2 cells and rapidly bactericidal effects, also displayed quite slow resistance development toward *A. baumanii* and were found to be active toward drug-resistant *A. baumanii* membrane. Molecular docking showed that hydrogen bonds existed in the supramolecular interaction between DNA gyrase and the active molecule **9c** or **18a**. The preliminary exploration showed that compound **9c** could intercalate and/or cleave bacterial DNA from sensitive *A. baumanii* strains and thus might inhibit *A. baumanii* growth. The combination use of the active molecule **9c** with antibacterial norfloxacin could enhance the efficiency, broaden antimicrobial spectrum and effectively combat drug

resistance. It might be concluded that aminothiazole modified berberine derivative **9c** should have great potential in new antibacterial drug development.

4. Experimental

4.1. General methods

All starting material chemicals and solvents were achieved from commercial sources unless otherwise indicated and used without any additional purification. The masses were weighed on a microbalance with a resolution of 0.0001 g. Thin-layer chromatography (TLC) was made using glass plates precoated with silica gel (HG/T2354-92) impregnated with a fluorescent indicator (254 nm). Visualization on TLC was acquired using UV light (254 nm) and also staining with iodine. Column chromatography was undertaken on silica gel (500-600 mesh size). Melting point (mp) was measured on a melting point apparatus (X-6 type). High resolution mass spectra (HRMS) were obtained with IonSpec FT-ICR mass spectrometer with ESI-TOF method. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE III 600 MHz spectrometer using DMSO-d_6 or $CDCl_3$ as solvent, or TMS as internal standard. Proton and carbon chemical shifts were expressed in parts per million (δ, ppm) and were referenced to NMR solvent DMSO-d_6, δ 2.50, 39.5 ppm; $CDCl_3$, δ 7.26, 77.0 ppm. The following abbreviations were used to describe peak patterns: s = singlet, d= doublet, t = triplet, q = quadruplet, m = multiplet. Coupling constants (*J*) were expressed in hertz unit (Hz). The abbreviations of THPB and Ph were adopted to assign the berberine skeleton and aryl group, respectively.

*4.1.1. Synthesis of 9-hydroxy-10-methoxy-5,6-dihydro-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-7-ium chloride (**2**), 10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-ol (**3**) and 9-hydroxy-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**4**)*

The intermediates **2–4** were prepared according to the previously reported procedures [69].

*4.1.2. Synthesis of 2-((9-Hydroxy-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**5**)*

A mixture of compound **4** (1.0 g, 2.8 mmol), hydrazinecarbothioamide (0.28 g, 3.1 mmol) and glacial acetic acid (0.15 eq) as the catalyst in anhydrous alcohol (20 mL) was stirred at 80 ^{o}C under N_2 protection for 6 h. Upon completion of the reaction, the solvent was evaporated. The residue was washed with methanol, and further purified by silica gel column chromatography (eluent, chloroform/acetone (3/1, V/V)) to afford the desired compound **5** (0.94 g, 78.8%) as yellow solid. Mp: 196–197 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.16 (s, 1H, N*H*), 9.81 (s, 1H, O*H*), 8.33 (s, 1H, N=C*H*), 8.17 (s, 1H, N*H*), 8.03 (s, 1H, N*H*), 7.64 (s, 1H, THPB-11-*H*), 6.92 (s, 1H, THPB-1-*H*), 6.67 (s, 1H, THPB-4-*H*), 6.01 (d, *J* = 3.0 Hz, 2H, OCH_2O), 4.67 (s, 1H, THPB-8-*H*), 4.43 (d, *J* = 14.5 Hz, 1H, C*H*), 4.30–4.26 m, 1H, THPB-8-*H*),

4.11–4.07 (m, 1H, THPB-13-*H*), 3.91 (s, 3H, OCH_3), 3.86 (s, 1H, THPB-13-*H*), 3.42 (s, 1H, THPB-5-*H*), 3.34–3.29 (m, 1H, THPB-5-*H*), 3.06 (s, 1H, THPB-6-*H*), 2.90 (d, *J* = 11.4 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.1, 150.4, 147.3, 146.1, 145.7, 144.8, 141.1, 131.3, 128.5, 127.7, 125.8, 124.0, 118.5, 109.1, 108.3, 106.3, 101.8, 68.2, 59.1, 56.5, 49.1, 33.8, 29.5 ppm.

*4.1.3. Synthesis of 10-Methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3] dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-ol (**6**)*

A mixture of compound **5** (0.31 g, 0.72 mmol) and 2-chloroacetaldehyde (0.08 g, 1.1 mmol) in anhydrous alcohol (20 mL) was stirred at 80 ^{o}C under N_2 protection for 3 h. Upon completion of the reaction, the solvent was evaporated. The residue was washed with methanol, and further purified by silica gel column chromatography (eluent, chloroform/acetone (2/1, V/V)) to afford the desired compound **6** (0.15 g, 45.0%) as light yellow solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.40 (s, 1H, N*H*), 9.83 (s, 1H, O*H*), 8.29 (s, 1H, N=C*H*), 7.32 (s, 1H, THPB-11-*H*), 7.26 (d, *J* = 3.1 Hz, 1H, thiazolyl-4-*H*), 7.19 (s, 1H, thiazolyl-5-*H*), 6.84 (s, 2H, THPB-1-*H*, THPB-4-*H*), 6.05 (d, *J* = 3.0 Hz, 2H, OCH_2O), 4.68 (s, 1H, THPB-8-*H*), 4.58 (d, *J* = 15.6 Hz, 1H, C*H*), 4.30 (dd, *J* = 15.3, 7.7 Hz, 1H, THPB-8-*H*), 4.09–4.05 (m, 1H, THPB-13-*H*), 3.89 (s, 3H, OCH_3), 3.85 (s, 1H, THPB-13-*H*), 3.43 (d, *J* = 6.8 Hz, 1H, THPB-5-*H*), 3.37–3.31 (m, 1H, THPB-5-*H*), 3.06 (dd, *J* = 16.9, 12.4 Hz, 1H, THPB-6-*H*), 2.90 (d, *J* = 15.8 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.4, 150.3, 147.4, 146.2, 145.9, 144.9, 141.2, 131.4, 128.1, 127.9, 125.6, 124.2, 117.5, 109.0, 108.6, 106.4, 101.8, 68.2, 59.1, 56.5, 49.1, 33.7, 29.4 ppm; HRMS (ESI) calcd. for $C_{23}H_{22}N_4O_4S$ $[M + H]^+$, 451.1440; found, 451.1441.

*4.1.4. Synthesis of 9-Ethoxy-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a] isoquinoline-12-carbaldehyde (**7a**)*

To a solution of compound **4** (2.0 g, 5.7 mmol) in DMF (50 mL) was added potassium carbonate (1.2 g, 8.5 mmol), the mixture was stirred at 80 ^{o}C for 1 h and bromoethane (0.93 g, 8.5 mmol) was then added and again the temperature maintained at 80 °C for 6 h, then diluted with chloroform (3 × 20 mL). The organic phase was washed with saturated sodium chloride, dried over anhydrous sodium sulphate and further purified by silica gel column chromatography (eluent, petroleum ether/chloroform (20/1, V/V)) to afford the desired compound **7a** (1.75 g, 80.4%) as yellow solid. Mp: 190–191 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.21 (s, 1H, C*H*O), 7.32 (s, 1H, THPB-11-*H*), 6.79 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.93 (s, 2H, OCH_2O), 4.28–4.21 (m, 2H, CH_2CH$_3$), 4.17 (dd, *J* = 9.5, 7.1 Hz, 1H, C*H*), 3.90 (s, 3H, OCH_3), 3.85 (s, 1H, THPB-8-*H*), 3.51 (d, *J* = 15.6 Hz, 2H, THPB-8-*H*, THPB-13-*H*), 3.22–3.16 (m, 1H, THPB-13-*H*), 3.12 (s, 1H, THPB-5-*H*), 3.07–2.99 (m, 1H, THPB-5-*H*), 2.70–2.59 (m, 2H, THPB-6-*H*), 1.40 (t, *J* = 7.0 Hz, 3H, CH$_2$CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.2, 149.5, 146.3, 146.1, 131.7, 130.4, 129.9, 129.0, 127.7, 113.7, 108.4, 105.6, 100.8, 68.7, 59.2, 55.9, 54.2, 51.3, 50.7, 33.8, 29.5, 15.9 ppm.

4.1.5. Synthesis of 9-Butoxy-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]

*isoquinoline-12-carbaldehyde (**7b**)*

Compound **7b** was prepared according to the procedure described for compound **7a**, starting from compound **4** (2.0 g, 5.7 mmol), 1-bromobutane (1.2 g, 8.5 mmol) and potassium carbonate (1.2 g, 8.5 mmol). The pure product **7b** (1.91 g, 81.7%) was obtained as light yellow solid. Mp: 168–169 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.20 (s, 1H, C*H*O), 7.31 (s, 1H, THPB-11-*H*), 6.78 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.92 (s, 2H, OCH_2O), 4.23 (d, *J* = 15.9 Hz, 1H, C*H*), 4.20–4.15 (m, 1H, C*H*$CH_2CH_2CH_3$), 4.09 (dt, *J* = 9.3, 6.7 Hz, 1H, C*H*$CH_2CH_2CH_3$), 3.89 (s, 3H, OCH_3), 3.85 (dd, *J* = 16.9, 3.4 Hz, 1H, THPB-8-*H*), 3.50 (d, *J* = 15.7 Hz, 2H, THPB-8-*H*, THPB-13-*H*), 3.18 (dd, *J* = 10.3, 4.5 Hz, 1H, THPB-13-*H*), 3.15–3.07 (m, 1H, THPB-5-*H*), 3.03 (dd, *J* = 16.7, 11.3 Hz, 1H, THPB-5-*H*), 2.70–2.59 (m, 2H, THPB-6-*H*), 1.80–1.73 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.54–1.47 (m, 2H, $CH_2CH_2CH_2CH_3$), 0.99 (t, *J* = 7.4 Hz, 3H, $CH_2CH_2CH_2CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.6 (*C*=O), 150.1, 149.7, 146.3, 146.1, 131.8, 130.5, 129.9, 129.0, 127.7, 113.8, 108.4, 105.7, 100.8, 72.7, 59.2, 56.0, 54.2, 51.4, 33.9, 32.5, 29.5, 19.1, 13.8 ppm.

*4.1.6. Synthesis of 9-(Hexyloxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino [3,2-a]isoquinoline-12-carbaldehyde (**7c**)*

Compound **7c** was prepared according to the procedure described for compound **7a**, starting from compound **4** (2.0 g, 5.7 mmol), 1-bromohexane (1.4 g, 8.5 mmol) and potassium carbonate (1.2 g, 8.5 mmol). The pure product **7c** (1.96 g, 78.7%) was obtained as yellow solid. Mp: 64–65 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.20 (s, 1H, C*H*O), 7.31 (s, 1H, THPB-11-*H*), 6.79 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.92 (s, 2H, OCH_2O), 4.23 (d, *J* = 15.9 Hz, 1H, C*H*), 4.16 (dd, *J* = 11.5, 4.6 Hz, 1H, C*H*$(CH_2)_4CH_3$), 4.08 (dd, *J* = 11.4, 4.6 Hz, 1H, C*H*$(CH_2)_4CH_3$), 3.89 (s, 3H, OCH_3), 3.84 (d, *J* = 4.2 Hz, 1H, THPB-8-*H*), 3.50 (d, *J* = 15.6 Hz, 2H, THPB-8-*H*, THPB-13-*H*), 3.17 (dd, *J* = 10.2, 4.9 Hz, 1H, THPB-13-*H*), 3.11 (s, 1H, THPB-5-*H*), 3.02 (dd, *J* = 16.8, 11.3 Hz, 1H, THPB-5-*H*), 2.69–2.60 (m, 2H, THPB-6-*H*), 1.77 (dd, *J* = 14.5, 7.2 Hz, 2H, $CH_2CH_2(CH_2)_3CH_3$), 1.58–1.51 (m, 1H, $(CH_2)_2CH_2(CH_2)_2CH_3$), 1.50–1.43 (m, 2H, $(CH_2)_3CH_2CH_2CH_3$), 1.29 (d, *J* = 10.6 Hz, 2H, $(CH_2)_4CH_2CH_3$), 0.92 (t, *J* = 6.6 Hz, 3H, $(CH_2)_5CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.1, 149.7, 146.3, 146.1, 131.7, 130.4, 129.8, 128.9, 127.6, 113.8, 108.4, 105.6, 100.8, 73.1, 62.9, 59.1, 55.9, 54.2, 51.3, 33.8, 31.6, 29.4, 25.6, 22.6, 14.0 ppm.

*4.1.7. Synthesis of 10-Methoxy-9-(octyloxy)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino [3,2-a]isoquinoline-12-carbaldehyde (**7d**)*

Compound **7d** was prepared according to the procedure described for compound **7a**, starting from compound **4** (2.0 g, 5.7 mmol), 1-bromooctane (1.6 g, 8.5 mmol) and potassium carbonate (1.2 g, 8.5 mmol). The pure product **7d** (2.12 g, 79.9%) was obtained as yellow solid. Mp: 97–98 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.20 (s, 1H, C*H*O), 7.32 (s, 1H, THPB-11-*H*), 6.79 (s, 1H, THPB-1-*H*), 6.60 (s, 1H, THPB-4-*H*), 5.93 (s, 2H, OCH_2O), 4.23 (d, *J* = 15.9 Hz, 1H, C*H*), 4.19–4.14 (m, 1H, C*H*$(CH_2)_6CH_3$), 4.08

(d, *J* = 9.3 Hz, 1H, C*H*$(CH_2)_6CH_3$), 3.90 (s, 3H, OCH_3), 3.85 (s, 1H, THPB-8-*H*), 3.51 (d, *J* = 15.7 Hz, 2H, THPB-8-*H*, THPB-13-*H*), 3.18 (dd, *J* = 10.3, 4.7 Hz, 1H, THPB-13-*H*), 3.15–3.09 (m, 1H, THPB-5-*H*), 3.01 (dd, *J* = 15.9, 4.8 Hz, 1H, THPB-5-*H*), 2.67 (d, *J* = 16.9 Hz, 1H, THPB-6-*H*), 2.65–2.60 (m, 1H, THPB-6-*H*), 1.81–1.74 (m, 2H, $CH_2CH_2(CH_2)_5CH_3$), 1.46 (dd, *J* = 14.7, 7.2 Hz, 2H, $(CH_2)_2CH_2(CH_2)_4CH_3$), 1.29 (d, *J* = 5.9 Hz, 8H, $(CH_2)_3(CH_2)_4CH_3$), 0.89 (s, 3H, $(CH_2)_7CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.1, 149.7, 146.3, 146.1, 131.6, 130.4, 129.8, 128.9, 127.6, 113.9, 108.4, 105.6, 100.8, 73.0, 62.9, 59.1, 55.9, 54.2, 51.3, 36.4, 32.8, 31.4, 30.4, 29.3, 25.7, 22.6, 14.0 ppm.

*4.1.8. Synthesis of 9-(Decyloxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino [3,2-a]isoquinoline-12-carbaldehyde (**7e**)*

Compound **7e** was prepared according to the procedure described for compound **7a**, starting from compound **4** (2.0 g, 5.7 mmol), 1-bromodecane (1.9 g, 8.5 mmol) and potassium carbonate (1.2 g, 8.5 mmol). The pure product **7e** (2.28 g, 81.1%) was obtained as yellow solid. Mp: 50–51 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.20 (s, 1H, C*H*O), 7.31 (s, 1H, THPB-11-*H*), 6.79 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.93 (s, 2H, OCH_2O), 4.23 (d, *J* = 15.8 Hz, 1H, C*H*), 4.19–4.14 (m, 1H, C*H*$(CH_2)_8CH_3$), 4.10–4.05 (m, 1H, C*H*$(CH_2)_8CH_3$), 3.89 (s, 3H, OCH_3), 3.85 (s, 1H, THPB-8-*H*), 3.51 (d, *J* = 15.2 Hz, 2H, THPB-8-*H*, THPB-13-*H*), 3.21–3.16 (m, 1H, THPB-13-*H*), 3.12 (s, 1H, THPB-5-*H*), 3.03 (d, *J* = 4.5 Hz, 1H, THPB-5-*H*), 2.70–2.60 (m, 2H, THPB-6-*H*), 1.80–1.74 (m, 2H, $CH_2CH_2(CH_2)_7CH_3$), 1.46 (dd, *J* = 14.5, 7.2 Hz, 2H, $(CH_2)_2CH_2(CH_2)_6CH_3$), 1.31 (d, *J* = 37.5 Hz, 12H, $(CH_2)_3(CH_2)_6CH_3$), 0.89 (d, *J* = 5.3 Hz, 3H, $(CH_2)_9CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.1, 149.7, 146.3, 146.1, 131.7, 130.4, 129.7, 128.9, 127.6, 113.8, 108.4, 105.6, 100.8, 73.1, 63.0, 59.1, 55.9, 54.2, 51.3, 50.6, 33.7, 32.8, 31.9, 30.4, 29.3, 25.9, 25.7, 22.6, 14.0 ppm.

*4.1.9. Synthesis of 9-(Dodecyloxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino [3,2-a]isoquinoline-12-carbaldehyde (**7f**)*

Compound **7f** was prepared according to the procedure described for compound **7a**, starting from compound **4** (2.0 g, 5.7 mmol), 1-bromododecane (2.1 g, 8.5 mmol) and potassium carbonate (1.2 g, 8.5 mmol). The pure product **7f** (2.26 g, 76.0%) was obtained as yellow solid. Mp: 75–76 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.20 (s, 1H, C*H*O), 7.31 (s, 1H, THPB-11-*H*), 6.79 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.92 (d, *J* = 8.6 Hz, 2H, OCH_2O), 4.23 (d, *J* = 15.9 Hz, 1H, C*H*), 4.16 (d, *J* = 9.2 Hz, 1H, C*H*$(CH_2)_{10}CH_3$), 4.11–4.04 (m, 1H, C*H*$(CH_2)_{10}CH_3$), 3.89 (s, 3H, OCH_3), 3.84 (s, 1H, THPB-8-*H*), 3.50 (d, *J* = 15.7 Hz, 2H, THPB-8-*H*, THPB-13-*H*), 3.21–3.15 (m, 1H, THPB-13-*H*), 3.12 (s, 1H, THPB-5-*H*), 3.03 (dd, *J* = 16.6, 11.4 Hz, 1H, THPB-5-*H*), 2.69–2.60 (m, 2H, THPB-6-*H*), 1.82–1.72 (m, 2H, $CH_2CH_2(CH_2)_9CH_3$), 1.50–1.42 (m, 2H, $(CH_2)_2CH_2(CH_2)_8CH_3$), 1.32 (d, *J* = 28.7 Hz, 16H, $(CH_2)_3(CH_2)_8CH_3$), 0.88 (t, *J* = 6.9 Hz, 3H, $(CH_2)_{11}CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.1, 149.7, 146.3, 146.1, 131.7, 130.4, 129.8, 128.9, 127.7, 113.8, 108.4, 105.6, 100.8, 73.1, 63.0,

59.2, 55.9, 54.2, 51.3, 50.6, 33.8, 32.8, 31.9, 30.4, 29.7, 29.5, 29.3, 25.9, 25.7, 22.6, 14.0 ppm.

*4.1.10. Synthesis of 9-(2-Hydroxyethoxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**7g**)*

Compound **7g** was prepared according to the procedure described for compound **7a**, starting from compound **4** (1.3 g, 3.7 mmol), 2-bromoethan-1-ol (0.69 g, 5.5 mmol) and potassium carbonate (0.76 g, 5.5 mmol). The pure product **7g** (1.07 g, 73.1%) was obtained as yellow solid. Mp: 116–117 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.22 (s, 1H, C*H*O), 7.33 (s, 1H, THPB-11-*H*), 6.78 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.93 (s, 2H, OCH_2O), 4.30–4.25 (m, 2H, OCH_2C*H*OH, C*H*), 4.16 (ddd, *J* = 10.6, 5.7, 3.2 Hz, 1H, OCH_2C*H*OH), 3.91 (s, 3H, OCH_3), 3.89 (ddd, *J* = 9.3, 6.7, 3.8 Hz, 3H, THPB-8-*H*, OCH_2CH_2OH), 3.53 (d, *J* = 15.5 Hz, 2H, THPB-8-*H*, THPB-13-*H*), 3.18 (ddd, *J* = 10.8, 5.2, 1.7 Hz, 1H, THPB-13-*H*), 3.14–3.08 (m, 1H, THPB-5-*H*), 3.06–3.01 (m, 1H, THPB-5-*H*), 2.69–2.62 (m, 2H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.2, 149.5, 146.3, 146.1, 131.7, 130.4, 129.9, 129.0, 127.7, 113.7, 108.4, 105.6, 100.8, 68.7, 59.2, 55.9, 54.2, 51.3, 50.7, 33.8, 29.5 ppm.

*4.1.11. Synthesis of 9-(Allyloxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino [3,2-a]isoquinoline-12-carbaldehyde (**10**)*

Compound **10** was prepared according to the procedure described for compound **7a**, starting from compound **4** (1.3 g, 3.7 mmol), 3-bromoprop-1-ene (0.67 g, 5.5 mmol) and potassium carbonate (0.76 g, 5.5 mmol). The pure product **10** (1.05 g, 72.5%) was obtained as yellow solid. Mp: 88–89 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.21 (s, 1H, C*H*O), 7.33 (s, 1H, THPB-11-*H*), 6.78 (s, 1H, THPB-1-*H*), 6.60 (s, 1H, THPB-4-*H*), 6.07 (ddd, *J* = 17.0, 5.8, 4.6 Hz, 1H, CH_2C*H*=CH_2), 5.93 (s, 2H, OCH_2O), 5.38 (dd, *J* = 17.1, 1.4 Hz, 1H, CH_2CH=C*H*), 5.25 (d, *J* = 10.3 Hz, 1H, CH_2CH=C*H*), 4.72 (dd, *J* = 12.3, 5.9 Hz, 1H, C*H*CH=CH), 4.63 (dd, *J* = 12.4, 5.9 Hz, 1H, C*H*CH=CH), 4.27 (d, *J* = 15.5 Hz, 1H, C*H*), 3.91 (s, 3H, OCH_3), 3.87 (d, *J* = 15.0 Hz, 1H, THPB-8-*H*), 3.56 (s, 2H, THPB-8-*H*, THPB-13-*H*), 3.20 (s, 1H, THPB-13-*H*), 3.13 (s, 1H, THPB-5-*H*), 3.06 (s, 1H, THPB-5-*H*), 2.69 (d, *J* = 15.5 Hz, 2H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.1, 149.0, 146.3, 146.1, 131.7, 130.4, 129.8, 128.9, 127.7, 118.1, 113.8, 108.4, 105.6, 100.9, 73.6, 73.1, 63.0, 58.4, 55.9, 33.8, 31.6, 29.3 ppm.

*4.1.12. Synthesis of 10-Methoxy-9-(prop-2-yn-1-yloxy)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**13a**)*

Compound **13a** was prepared according to the procedure described for compound **7a**, starting from compound **4** (1.3 g, 3.7 mmol), 3-bromoprop-1-yne (0.66 g, 5.5 mmol) and potassium carbonate (0.76 g, 5.5 mmol). The pure product **13a** (1.02 g, 70.6%) was obtained as yellow solid. Mp: 89–90 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.21 (s, 1H, C*H*O), 7.37 (s, 1H, THPB-11-*H*), 6.74 (s, 1H, THPB-1-*H*), 6.61 (s, 1H, THPB-4-*H*), 5.94 (s, 2H, OCH_2O), 4.76 (d, *J* = 2.0 Hz, 2H, CH_2C≡CH), 4.23 (d, *J* = 15.9 Hz, 1H, C*H*), 3.89 (s, 3H, OCH_3), 3.50 (s, 1H, CH_2C≡C*H*), 3.45–3.41 (m, 2H, THPB-8-*H*), 3.23–3.19 (m, 2H, THPB-13-*H*), 3.07 (d, *J* = 6.6 Hz, 1H, THPB-5-*H*), 2.95 (d, *J* = 19.9Hz, 1H, THPB-5-*H*), 2.60 (d, *J* = 15.5

Hz, 1H, THPB-6-*H*), 2.46 (d, *J* = 11.0 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.6 (*C*=O), 150.1, 149.7, 146.3, 146.1, 131.6, 130.2, 129.8, 128.9, 127.6, 118.0, 113.8, 108.4, 105.6, 100.9, 79.5, 73.6, 63.0, 58.4, 57.8, 56.1, 33.7, 29.6 ppm.

*4.1.13. Synthesis of 2-((12-Formyl-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino [3,2-a]isoquinolin-9-yl)oxy)acetonitrile (**13b**)*

Compound **13b** was prepared according to the procedure described for compound **7a**, starting from compound **4** (1.3 g, 3.7 mmol), 2-bromoacetonitrile (0.42 g, 5.5 mmol) and potassium carbonate (0.76 g, 5.5 mmol). The pure product **13b** (1.09 g, 75.0%) was obtained as yellow solid. Mp: 164–165 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.24 (s, 1H, C*H*O), 7.36 (s, 1H, THPB-11-*H*), 6.76 (s, 1H, THPB-1-*H*), 6.60 (s, 1H, THPB-4-*H*), 5.93 (s, 2H, OCH_2O), 5.01 (d, *J* = 16.0 Hz, 1H, C*H*C≡N), 4.92 (d, *J* = 16.0 Hz, 1H, C*H*C≡N), 4.26 (d, *J* = 16.0 Hz, 1H, C*H*), 3.94 (d, *J* = 6.1 Hz, 3H, OCH_3), 3.86–3.82 (m, 1H, THPB-8-*H*), 3.59 (d, *J* = 16.0 Hz, 1H, THPB-8-*H*), 3.55 (dd, *J* = 10.9, 3.1 Hz, 1H, THPB-13-*H*), 3.20–3.16 (m, 1H, THPB-13-*H*), 3.13–3.08 (m, 1H, THPB-5-*H*), 3.03 (dd, *J* = 16.9, 11.2 Hz, 1H, THPB-5-*H*), 2.66 (t, *J* = 12.5 Hz, 2H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.1, 149.7, 146.3, 146.1, 131.7, 130.4, 129.8, 128.9, 127.7, 118.1, 113.8, 108.4, 105.6, 100.9, 73.6, 73.1, 63.0, 58.4, 57.6, 55.9, 33.5, 29.4 ppm.

*4.1.14. Synthesis of 2-((9-Ethoxy-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino [3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**8a**)*

Compound **8a** was prepared according to the procedure described for compound **5**, starting from compound **7a** (2.0 g, 5.2 mmol), hydrazinecarbothioamide (0.52 g, 5.7 mmol) and glacial acetic acid (0.15 eq). The pure product **8a** (1.20 g, 50.8%) was obtained as brown solid. Mp: 201–202 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.16 (s, 1H, N*H*), 8.32 (s, 1H, N=C*H*), 7.20 (s, 1H, THPB-11-*H*), 7.12 (s, 1H, N*H*), 6.90 (s, 1H, THPB-1-*H*), 6.60 (s, 1H, THPB-4-*H*), 6.51 (s, 1H, N*H*), 5.95 (s, 1H, OC*H*O), 5.92 (s, 1H, OC*H*O), 4.23 (d, *J* = 15.7 Hz, 1H, C*H*), 4.15 (d, *J* = 7.1 Hz, 1H, C*H*CH_3), 4.09 (d, *J* = 7.1 Hz, 1H, C*H*CH_3), 3.88 (s, 3H, OCH_3), 3.48 (s, 3H, THPB-8-*H*, THPB-13-*H*), 3.20 (s, 1H, THPB-13-*H*), 3.12 (s, 1H, THPB-5-*H*), 2.79 (t, *J* = 13.8 Hz, 1H, THPB-5-*H*), 2.67 (d, *J* = 16.0 Hz, 1H, THPB-6-*H*), 2.61 (s, 1H, THPB-6-*H*), 1.39 (t, *J* = 7.0 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 150.4, 146.2, 146.0, 145.9, 141.2, 131.4, 129.4, 128.1, 128.0, 127.3, 108.6, 108.3, 106.1, 101.1, 68.2, 59.2, 56.5, 53.9, 51.1, 33.7, 29.4, 16.1 ppm.

*4.1.15. Synthesis of 2-((9-Butoxy-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino [3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**8b**).*

Compound **8b** was prepared according to the procedure described for compound **5**, starting from compound **7b** (0.33 g, 0.81 mmol), hydrazinecarbothioamide (0.08 g, 0.90 mmol) and glacial acetic acid (0.15 eq). The pure product **8b** (0.30 g, 77.4%) was obtained as brown solid. Mp: 148–149 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.14 (s, 1H, N*H*), 8.43 (s, 1H, N=C*H*), 8.18 (s, 1H, N*H*), 8.01 (s, 1H, N*H*), 7.61 (s, 1H, THPB-11-*H*), 6.93 (s, 1H, THPB-1-*H*), 6.68 (s, 1H, THPB-4-*H*), 5.96 (d, *J* = 18.6 Hz, 2H, OCH_2O),

4.05 (d, *J* = 15.7 Hz, 1H, C*H*), 3.98 (dt, *J* = 13.3, 6.7 Hz, 1H, C*H*$CH_2CH_2CH_3$), 3.95–3.91 (m, 1H, C*H*$CH_2CH_2CH_3$), 3.85 (d, *J* = 9.8 Hz, 3H, OCH_3), 3.46 (d, *J* = 15.6 Hz, 1H, THPB-8-*H*), 3.42–3.34 (m, 2H, THPB-8-*H*, THPB-13-*H*), 3.08 (d, *J* = 6.5 Hz, 1H, THPB-13-*H*), 2.91 (t, *J* = 11.1 Hz, 1H, THPB-5-*H*), 2.61 (d, *J* = 15.5 Hz, 1H, THPB-5-*H*), 2.49–2.41 (m, 2H, THPB-6-*H*), 1.70–1.63 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.49–1.41 (m, 2H, $CH_2CH_2CH_2CH_3$), 0.94 (t, *J* = 7.3 Hz, 3H, $CH_2CH_2CH_2CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 150.6, 146.2, 146.0, 145.9, 141.2, 131.4, 129.2, 128.1, 128.0, 127.4, 108.6, 108.4, 106.1, 101.1, 72.2, 59.1, 56.5, 54.1, 51.1, 33.7, 32.4, 29.4, 19.2, 14.2 ppm.

*4.1.16. Synthesis of 2-((9-(Hexyloxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**8c**).*

Compound **8c** was prepared according to the procedure described for compound **5**, starting from compound **7c** (1.0 g, 2.3 mmol), hydrazinecarbothioamide (0.23 g, 2.5 mmol) and glacial acetic acid (0.15 eq). The pure product **8c** (0.64 g, 54.1%) was obtained as brown solid. Mp: 138–139 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.31 (s, 1H, N*H*), 8.36 (s, 1H, N=C*H*), 7.20 (s, 1H, THPB-11-*H*), 7.12 (s, 1H, N*H*), 6.92 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 6.54 (s, 1H, N*H*), 5.95 (s, 1H, OC*H*O), 5.91 (s, 1H, OC*H*O), 4.23 (d, *J* = 15.7 Hz, 1H, C*H*), 4.07 (dd, *J* = 11.4, 4.5 Hz, 1H, C*H*$(CH_2)_4CH_3$), 4.01–3.96 (m, 1H, C*H*$(CH_2)_4CH_3$), 3.87 (s, 3H, OCH_3), 3.47 (t, *J* = 13.6 Hz, 3H, THPB-8-*H*, THPB-13-*H*), 3.21–3.15 (m, 1H, THPB-13-*H*), 3.11 (s, 1H, THPB-5-*H*), 2.78 (t, *J* = 14.2 Hz, 1H, THPB-5-*H*), 2.67 (d, *J* = 16.1 Hz, 1H, THPB-6-*H*), 2.61 (s, 1H, THPB-6-*H*), 1.76 (dd, *J* = 14.6, 7.3 Hz, 2H, $CH_2CH_2(CH_2)_3CH_3$), 1.50–1.43 (m, 2H, $(CH_2)_2CH_2(CH_2)_2CH_3$), 1.38–1.32 (m, 4H, $(CH_2)_3(CH_2)_2CH_3$), 0.92 (t, *J* = 6.6 Hz, 3H, $(CH_2)_5CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.1 (*C*=S), 150.4, 147.0, 146.2, 146.1, 143.2, 131.4, 128.4, 127.8, 125.8, 109.2, 108.4, 105.7, 100.8, 73.0, 62.9, 59.1, 55.9, 54.2, 51.3, 36.4, 32.8, 31.4, 30.4, 29.3, 25.7, 22.6, 14.0 ppm.

*4.1.17. Synthesis of 2-((10-Methoxy-9-(octyloxy)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**8d**)*

Compound **8d** was prepared according to the procedure described for compound **5**, starting from compound **7d** (1.9 g, 4.0 mmol), hydrazinecarbothioamide (0.40 g, 4.4 mmol) and glacial acetic acid (0.15 eq). The pure product **8d** (1.13 g, 52.6%) was obtained as brown solid. Mp: 157–158 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.15 (s, 1H, N*H*), 8.43 (s, 1H, N=C*H*), 8.11 (s, 1H, N*H*), 8.03 (s, 1H, N*H*), 7.36 (s, 1H, THPB-11-*H*), 6.78 (s, 1H, THPB-1-*H*), 6.62 (s, 1H, THPB-4-*H*), 5.98 (s, 2H, OCH_2O), 4.23 (d, *J* = 15.9 Hz, 1H, C*H*), 4.17–4.12 (m, 1H, C*H*$(CH_2)_6CH_3$), 4.06 (d, *J* = 9.2 Hz, 1H, C*H*$(CH_2)_6CH_3$), 3.89 (s, 3H, OCH_3), 3.86 (s, 1H, THPB-8-*H*), 3.52 (d, *J* = 15.7 Hz, 2H, THPB-8-*H*, THPB-13-*H*), 3.19 (d *J* = 10.3 Hz, 1H, THPB-13-*H*), 3.14–3.06 (m, 1H, THPB-5-*H*), 3.03 (dd, *J* = 15.9, 4.8 Hz, 1H, THPB-5-*H*), 2.79–2.75 (m, 1H, THPB-6-*H*), 2.65–2.60 (m, 1H, THPB-6-*H*), 1.81–1.75 (m, 2H, $CH_2CH_2(CH_2)_5CH_3$), 1.48–1.44 (m, 2H, $(CH_2)_2CH_2(CH_2)_4CH_3$), 1.29–1.25 (m, 8H, $(CH_2)_3(CH_2)_4CH_3$), 0.89 (s, 3H, $(CH_2)_7CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.2 (*C*=S), 150.6, 149.7, 147.0, 146.2, 146.0, 143.2, 141.2, 131.4, 130.4,

129.2, 128.1, 128.0, 127.4, 125.8, 108.6, 108.4, 106.1, 101.1, 72.2, 59.1, 56.5, 54.1, 51.1, 33.7, 32.4, 29.4, 19.2, 14.2 ppm.

*4.1.18. Synthesis of 2-((9-(Decyloxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**8e**)*

Compound **8e** was prepared according to the procedure described for compound **5**, starting from compound **7e** (1.9 g, 3.9 mmol), hydrazinecarbothioamide (0.39 g, 4.2 mmol) and glacial acetic acid (0.15 eq). The pure product **8e** (1.12 g, 50.8%) was obtained as brown solid. Mp: 118–119 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.20 (s, 1H, N*H*), 8.33 (s, 1H, N=C*H*), 7.20 (s, 1H, THPB-11-*H*), 7.12 (s, 1H, N*H*), 6.91 (s, 1H, THPB-1-*H*), 6.60 (s, 1H, THPB-4-*H*), 6.51 (s, 1H, N*H*), 5.95 (s, 1H, OC*H*O), 5.91 (s, 1H, OC*H*O), 4.23 (d, *J* = 15.6 Hz, 1H, C*H*), 4.07 (dt, *J* = 13.8, 6.8 Hz, 1H, C*H*$(CH_2)_8CH_3$), 3.98 (dd, *J* = 15.8, 6.8 Hz, 1H, C*H*$(CH_2)_8CH_3$), 3.87 (s, 3H, OCH_3), 3.48 (d, *J* = 8.1 Hz, 2H, THPB-8-*H*), 3.47–3.43 (m, 1H, THPB-13-*H*), 3.18 (s, 1H, THPB-13-*H*), 3.11 (s, 1H, THPB-5-*H*), 2.78 (t, *J* = 13.8 Hz, 1H, THPB-5-*H*), 2.67 (d, *J* = 16.0 Hz, 1H, THPB-6-*H*), 2.62 (d, *J* = 10.9 Hz, 1H, THPB-6-*H*), 1.76 (dt, *J* = 13.9, 6.8 Hz, 2H, $CH_2CH_2(CH_2)_7CH_3$), 1.45 (dd, *J* = 14.5, 7.0 Hz, 2H, $(CH_2)_2CH_2(CH_2)_6CH_3$), 1.39–1.26 (m, 12H, $(CH_2)_3(CH_2)_6CH_3$), 0.88 (t, *J* = 6.8 Hz, 3H, $(CH_2)_9CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.1 (*C*=S), 150.4, 147.0, 146.2, 146.1, 143.3, 131.4, 130.4, 128.4, 127.8, 125.8, 109.2, 108.4, 105.8, 100.8, 73.0, 59.2, 56.0, 54.2, 51.5, 50.6, 34.6, 32.8, 31.9, 30.4, 29.6, 29.4, 26.0, 25.7, 22.7, 14.1 ppm.

*4.1.19. Synthesis of 2-((9-(Dodecyloxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**8f**)*

Compound **8f** was prepared according to the procedure described for compound **5**, starting from compound **7f** (1.9 g, 3.6 mmol), hydrazinecarbothioamide (0.36 g, 4.0 mmol) and glacial acetic acid (0.15 eq). The pure product **8f** (1.16 g, 54.0%) was obtained as brown solid. Mp: 119–120 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.22 (s, 1H, N*H*), 8.34 (s, 1H, N=C*H*), 7.20 (s, 1H, THPB-11-*H*), 7.12 (s, 1H, N*H*), 6.91 (s, 1H, THPB-1-*H*), 6.60 (s, 1H, THPB-4-*H*), 6.52 (s, 1H, N*H*), 5.95 (s, 1H, OC*H*O), 5.91 (s, 1H, OC*H*O), 4.23 (d, *J* = 15.7 Hz, 1H, C*H*), 4.07 (dd, *J* = 15.1, 7.2 Hz, 1H, C*H*$(CH_2)_{10}CH_3$), 3.98 (dd, *J* = 15.3, 6.9 Hz, 1H, C*H*$(CH_2)_{10}CH_3$), 3.87 (s, 3H, OCH_3), 3.49 (s, 2H, THPB-8-*H*), 3.45 (s, 1H, THPB-13-*H*), 3.18 (d, *J* = 5.3 Hz, 1H, THPB-13-*H*), 3.11 (s, 1H, THPB-5-*H*), 2.78 (t, *J* = 13.9 Hz, 1H, THPB-5-*H*), 2.67 (d, *J* = 16.0 Hz, 1H, THPB-6-*H*), 2.61 (t, *J* = 10.3 Hz, 1H, THPB-6-*H*), 1.79–1.73 (m, 2H, $CH_2CH_2(CH_2)_9CH_3$), 1.45 (dd, *J* = 14.2, 7.0 Hz, 2H, $(CH_2)_2CH_2(CH_2)_8CH_3$), 1.38–1.26 (m, 16H, $(CH_2)_3(CH_2)_8CH_3$), 0.88 (t, *J* = 6.6 Hz, 3H, $(CH_2)_{11}CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.1 (*C*=S), 150.6, 149.7, 146.2, 146.0, 141.1, 131.3, 129.2, 128.0, 127.9, 127.4, 113.8, 108.6, 108.3, 106.1, 101.1, 72.4, 59.2, 56.5, 54.1, 51.2, 50.6, 33.7, 32.8, 31.8, 30.3, 29.7, 29.5, 29.2, 25.9, 25.7, 22.6, 14.4 ppm.

*4.1.20. Synthesis of 2-((9-(2-Hydroxyethoxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**8g**)*

Compound **8g** was prepared according to the procedure described for compound **5**, starting from

compound **7g** (0.20 g, 0.50 mmol), hydrazinecarbothioamide (0.050 g, 0.55 mmol) and glacial acetic acid (0.15 eq). The pure product **8g** (0.079 g, 33.4%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.15 (s, 1H, N*H*), 8.44 (s, 1H, N=C*H*), 8.19 (s, 1H, N*H*), 8.01 (s, 1H, N*H*), 7.61 (s, 1H, THPB-11-*H*), 6.93 (s, 1H, THPB-1-*H*), 6.68 (s, 1H, THPB-4-*H*), 5.96 (d, *J* = 18.0 Hz, 2H, OCH_2O), 4.76 (t, *J* = 5.3 Hz, 1H, OC*H*CH$_2$OH), 4.34 (t, *J* = 5.0 Hz, 1H, OC*H*CH$_2$OH), 4.19 (d, *J* = 15.9 Hz, 1H, C*H*), 4.07 (dt, *J* = 10.1, 5.4 Hz, 1H, OCH$_2$C*H*OH), 3.96 (dt, *J* = 10.3, 5.1 Hz, 1H, OCH$_2$C*H*OH), 3.85 (s, 3H, OCH_3), 3.49–3.44 (m, 2H, THPB-8-*H*), 3.38 (dd, *J* = 23.7, 13.5 Hz, 2H, THPB-13-*H*), 3.10–3.05 (m, 1H, THPB-5-*H*), 2.95–2.87 (m, 1H, THPB-5-*H*), 2.62 (d, *J* = 15.7 Hz, 1H, THPB-6-*H*), 2.48–2.43 (m, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 150.4, 146.2, 145.9, 141.2, 131.4, 129.4, 128.0, 127.3, 108.6, 108.3, 106.1, 101.1, 79.6, 74.6, 60.9, 59.2, 56.5, 55.3, 53.9, 51.1, 49.1, 33.7, 29.4 ppm.

*4.1.21. Synthesis of 2-((9-(Allyloxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**11**)*

Compound **11** was prepared according to the procedure described for compound **5**, starting from compound **10** (0.31 g, 0.79 mmol), hydrazinecarbothioamide (0.079 g, 0.87 mmol) and glacial acetic acid (0.15 eq). The pure product **11** (0.12 g, 32.1%) was obtained as brown solid. Mp: 151–152 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.16 (s, 1H, N*H*), 8.43 (s, 1H, N=C*H*), 8.20 (s, 1H, N*H*), 8.03 (s, 1H, N*H*), 7.62 (s, 1H, THPB-11-*H*), 6.93 (s, 1H, THPB-1-*H*), 6.68 (s, 1H, THPB-4-*H*), 6.04 (dd, *J* = 11.0, 5.4 Hz, 1H, CH$_2$C*H*=CH$_2$), 5.96 (d, *J* = 18.7 Hz, 2H, OCH_2O), 5.35 (d, *J* = 17.2 Hz, 1H, CH$_2$CH=C*H*), 5.21 (d, *J* = 10.3 Hz, 1H, CH$_2$CH=C*H*), 4.57–4.47 (m, 2H, CH_2CH=CH$_2$), 4.06 (d, *J* = 15.9 Hz, 1H, C*H*), 3.86 (s, 3H, OCH_3), 3.45 (dd, *J* = 17.1, 10.9 Hz, 2H, THPB-8-*H*), 3.42–3.34 (m, 2H, THPB-13-*H*), 3.08 (d, *J* = 6.8 Hz, 1H, THPB-5-*H*), 2.90 (d, *J* = 11.2 Hz, 1H, THPB-5-*H*), 2.61 (d, *J* = 15.5 Hz, 1H, THPB-6-*H*), 2.47–2.42 (m, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 150.5, 146.2, 146.0, 145.5, 141.1, 135.0, 131.3, 129.3, 128.0, 127.6, 118.0, 108.6, 108.2, 106.1, 101.1, 79.6, 73.2, 59.1, 56.5, 54.2, 51.1, 33.7, 29.4 ppm.

*4.1.22. Synthesis of 2-((10-Methoxy-9-(prop-2-yn-1-yloxy)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**14a**)*

Compound **14a** was prepared according to the procedure described for compound **5**, starting from compound **13a** (0.55 g, 1.4 mmol), hydrazinecarbothioamide (0.14 g, 1.6 mmol) and glacial acetic acid (0.15 eq). The pure product **14a** (0.33 g, 50.1%) was obtained as brown solid. Mp: 174–175 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.18 (s, 1H, N*H*), 8.43 (s, 1H, N=C*H*), 8.22 (s, 1H, N*H*), 8.06 (s, 1H, N*H*), 7.64 (s, 1H, THPB-11-*H*), 6.94 (s, 1H, THPB-1-*H*), 6.69 (s, 1H, THPB-4-*H*), 5.98 (s, 1H, OC*H*O), 5.95 (s, 1H, OC*H*O), 4.78 (d, *J* = 1.9 Hz, 2H, CH_2C≡CH), 4.14 (d, *J* = 15.8 Hz, 1H, C*H*), 3.87 (s, 3H, OCH_3), 3.51 (s, 1H, CH$_2$C≡C*H*), 3.45 (dd, *J* = 11.9, 6.7 Hz, 4H, THPB-8-*H*, THPB-13-*H*), 3.08 (d, *J* = 6.7 Hz, 1H, THPB-5-*H*), 2.92 (dd, *J* = 19.9, 8.8 Hz, 1H, THPB-5-*H*), 2.62 (d, *J* = 15.5 Hz, 1H, THPB-6-*H*), 2.45 (d, *J*

= 11.1 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 150.4, 146.2, 146.0, 145.5, 141.1, 134.8, 131.3, 128.9, 128.1, 119.6, 108.6, 106.4, 101.8, 100.0, 79.7, 76.3, 60.0, 59.0, 56.5, 55.3, 51.7, 33.5, 29.8 ppm.

*4.1.23. Synthesis of 2-((9-(Cyanomethoxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**14b**)*

Compound **14b** was prepared according to the procedure described for compound **5**, starting from compound **13b** (0.66 g, 1.7 mmol), hydrazinecarbothioamide (0.17 g, 1.9 mmol) and glacial acetic acid (0.15 eq). The pure product **14b** (0.55 g, 69.2%) was obtained as brown solid. Mp: 219–220 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.21 (s, 1H, N*H*), 8.44 (s, 1H, N=C*H*), 8.25 (s, 1H, N*H*), 8.10 (s, 1H, N*H*), 7.68 (s, 1H, THPB-11-*H*), 6.95 (s, 1H, THPB-1-*H*), 6.69 (s, 1H, THPB-4-*H*), 5.98 (d, *J* = 0.8 Hz, 1H, OC*H*O), 5.95 (d, *J* = 0.7 Hz, 1H, OC*H*O), 5.05 (d, *J* = 3.3 Hz, 2H, CH_2C≡N), 4.11 (d, *J* = 15.7 Hz, 1H, C*H*), 3.90 (s, 3H, OCH_3), 3.47–3.43 (m, 4H, THPB-8-*H*, THPB-13-*H*), 3.10 (d, *J* = 7.3 Hz, 1H, THPB-5-*H*), 2.95–2.88 (m, 1H, THPB-5-*H*), 2.63 (d, *J* = 15.5 Hz, 1H, THPB-6-*H*), 2.47 (d, *J* = 12.8 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 150.5, 146.3, 146.1, 131.7, 130.4, 129.8, 128.9, 127.7, 118.1, 113.8, 108.4, 105.6, 101.1, 100.8, 73.6, 73.1, 63.0, 58.4, 57.6, 55.9, 33.8, 29.4 ppm.

*4.1.24. Synthesis of 9-Ethoxy-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro -6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**9a**)*

Compound **9a** was prepared according to the procedure described for compound **6**, starting from compound **8a** (0.31 g, 0.69 mmol) and 2-chloroacetaldehyde (0.08 g, 1.1 mmol). The pure product **9a** (0.15 g, 45.8%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.10 (s, 1H, N=C*H*), 7.30 (s, 1H, THPB-11-*H*), 7.26–7.24 (m, 1H, thiazolyl-4-*H*), 6.83 (s, 1H, THPB-1-*H*), 6.70 (s, 1H, thiazolyl-5-*H*), 6.61 (s, 1H, THPB-1-*H*), 5.95 (d, *J* = 15.5 Hz, 2H, OCH_2O), 4.26 (d, *J* = 14.6 Hz, 1H, C*H*), 4.18–4.12 (m, 1H, C*H*CH_3), 4.10–4.04 (m, 1H, C*H*CH_3), 3.90 (s, 3H, OCH_3), 3.52 (d, *J* = 15.2 Hz, 3H, THPB-8-*H*, THPB-13-*H*), 3.22 (s, 1H, THPB-13-*H*), 3.14 (d, *J* = 11.6 Hz, 1H, THPB-5-*H*), 2.86 (t, *J* = 12.5 Hz, 1H, THPB-5-*H*), 2.67 (dd, *J* = 24.0, 13.9 Hz, 2H, THPB-6-*H*), 1.39 (t, *J* = 6.9 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.4, 150.3, 147.4, 146.2, 145.9, 144.9, 141.2, 131.4, 128.1, 127.9, 126.0, 124.2, 112.0, 109.0, 108.5, 106.5, 101.1, 68.2, 59.3, 56.1, 53.9, 51.1, 33.7, 29.4, 16.2 ppm; HRMS (ESI) calcd. for $C_{25}H_{26}N_4O_4S$ $[M + H]^+$, 479.1753; found, 479.1752.

*4.1.25. Synthesis of 9-Butoxy-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro -6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**9b**)*

Compound **9b** was prepared according to the procedure described for compound **6**, starting from compound **8b** (0.14 g, 0.29 mmol) and 2-chloroacetaldehyde (0.034 g, 0.44 mmol). The pure product **9b** (0.061 g, 41.6%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.76 (s, 1H, N*H*), 8.28 (s, 1H, N=C*H*), 7.36 (s, 1H, THPB-11-*H*), 7.24 (s, 1H, thiazolyl-4-*H*), 7.19 (s, 1H, thiazolyl-5-*H*), 6.84 (s, 2H, THPB-1-*H*, THPB-4-*H*), 6.05 (s, 2H, OCH_2O), 4.66 (s, 1H, C*H*), 4.59 (d, *J* =

15.5 Hz, 1H, C*H*$CH_2CH_2CH_3$), 4.41 (d, *J* = 13.6 Hz, 1H, C*H*$CH_2CH_2CH_3$), 4.05 (dd, *J* = 16.7, 10.7 Hz, 3H, THPB-8-*H*, THPB-13-*H*), 3.87 (s, 3H, OC*H*$_3$), 3.40 (s, 3H, THPB-5-*H*, THPB-13-*H*), 3.13–3.05 (m, 1H, THPB-6-*H*), 2.88 (d, *J* = 12.7 Hz, 1H, THPB-6-*H*), 1.75–1.67 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.46 (dd, *J* = 13.7, 6.9 Hz, 2H, $CH_2CH_2CH_2CH_3$), 0.96 (t, *J* = 7.3 Hz, 3H, $CH_2CH_2CH_2CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.3, 150.7, 147.4, 147.1, 145.9, 144.8, 141.2, 131.4, 128.1, 127.4, 126.0, 124.2, 112.0, 109.0, 108.8, 106.4, 101.8, 72.6, 58.9, 56.4, 51.5, 50.4, 49.1, 32.4, 32.2, 19.1, 14.2 ppm; HRMS (ESI) calcd. for $C_{27}H_{30}N_4O_4S$ $[M + H]^+$, 507.2066; found, 507.2064.

*4.1.26. Synthesis of 9-(Hexyloxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**9c**)*

Compound **9c** was prepared according to the procedure described for compound **6**, starting from compound **8c** (0.20 g, 0.39 mmol) and 2-chloroacetaldehyde (0.046 g, 0.59 mmol). The pure product **9c** (0.090 g, 43.2%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.71 (s, 1H, N*H*), 8.24 (s, 1H, N=C*H*), 7.25 (s, 1H, THPB-11-*H*), 7.21 (d, *J* = 3.4 Hz, 1H, thiazolyl-4-*H*), 7.00 (s, 1H, THPB-1-*H*), 6.81 (s, 1H, thiazolyl-5-*H*), 6.69 (s, 1H, THPB-4-*H*), 5.97 (d, *J* = 7.0 Hz, 2H, OC*H*$_2$O), 4.09 (d, *J* = 15.9 Hz, 1H, C*H*), 3.98 (dd, *J* = 11.1, 4.6 Hz, 1H, C*H*$(CH_2)_4CH_3$), 3.94 (dd, *J* = 11.1, 4.6 Hz, 1H, C*H*$(CH_2)_4CH_3$), 3.82 (s, 3H, OC*H*$_3$), 3.59 (d, *J* = 14.1 Hz, 2H, THPB-8-*H*), 3.30 (d, *J* = 7.6 Hz, 2H, THPB-13-*H*), 3.10 (s, 1H, THPB-5-*H*), 2.97–2.88 (m, 1H, THPB-5-*H*), 2.63 (d, *J* = 16.1 Hz, 1H, THPB-6-*H*), 2.56 (d, *J* = 16.0 Hz, 1H, THPB-6-*H*), 1.68 (dd, *J* = 14.3, 6.9 Hz, 2H, $CH_2CH_2(CH_2)_3CH_3$), 1.43 (d, *J* = 6.6 Hz, 2H, $(CH_2)_2CH_2(CH_2)_2CH_3$), 1.34–1.31 (m, 4H, $(CH_2)_3(CH_2)_2CH_3$), 0.89 (d, *J* = 6.6 Hz, 3H, $(CH_2)_5CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.4, 150.7, 147.4, 145.9, 144.8, 140.0, 131.4, 129.3, 128.1, 127.4, 124.2, 112.1, 108.8, 106.4, 101.8, 73.0, 62.9, 59.1, 56.4, 54.2, 49.1, 45.2, 36.4, 31.5, 30.1, 29.3, 25.5, 22.5, 14.4 ppm; HRMS (ESI) calcd. for $C_{29}H_{34}N_4O_4S$ $[M + H]^+$, 535.2379; found, 535.2378.

*4.1.27. Synthesis of 10-Methoxy-9-(octyloxy)-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**9d**)*

Compound **9d** was prepared according to the procedure described for compound **6**, starting from compound **8d** (0.20 g, 0.37 mmol) and 2-chloroacetaldehyde (0.043 g, 0.56 mmol). The pure product **9d** (0.084 g, 40.2%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.83 (s, 1H, N*H*), 8.48 (s, 1H, N=C*H*), 7.49 (s, 1H, THPB-11-*H*), 7.38 (s, 1H, thiazolyl-4-*H*), 7.19 (s, 1H, THPB-1-*H*), 7.00 (s, 1H, thiazolyl-5-*H*), 6.84 (s, 1H, THPB-4-*H*), 6.05 (d, *J* = 6.2 Hz, 2H, OC*H*$_2$O), 4.68 (s, 1H, C*H*), 4.59 (d, *J* = 15.6 Hz, 1H, C*H*$(CH_2)_6CH_3$), 4.42 (d, *J* = 14.1 Hz, 1H, C*H*$(CH_2)_4CH_3$), 4.04 (s, 2H, THPB-8-*H*), 3.89 (s, 3H, OC*H*$_3$), 3.41 (s, 2H, THPB-13-*H*), 3.14–3.07 (m, 1H, THPB-5-*H*), 2.89 (s, 2H, THPB-5-*H*, THPB-6-*H*), 2.73 (s, 1H, THPB-6-*H*), 1.73 (s, 2H, $CH_2CH_2(CH_2)_5CH_3$), 1.43 (s, 2H, $(CH_2)_2CH_2(CH_2)_4CH_3$), 1.30 (d, *J* = 20.0 Hz, 8H, $(CH_2)_3(CH_2)_4CH_3$), 0.88 (s, 3H, $(CH_2)_7CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.3, 150.7, 147.4, 147.1, 145.9, 144.8, 141.2, 131.4, 128.1, 127.4, 125.9,

125.5, 124.2, 112.1, 108.6, 108.4, 106.3, 101.8, 73.0, 58.9, 56.5, 54.1, 49.1, 36.3, 31.7, 31.3, 30.2, 29.1, 25.8, 22.6, 14.4 ppm; HRMS (ESI) calcd. for $C_{31}H_{38}N_4O_4S$ $[M + H]^+$, 563.2692; found, 563.2693.

*4.1.28. Synthesis of 9-(Decyloxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**9e**)*

Compound **9e** was prepared according to the procedure described for compound **6**, starting from compound **8e** (0.50 g, 0.88 mmol) and 2-chloroacetaldehyde (0.10 g, 1.3 mmol). The pure product **9e** (0.21 g, 41.3%) was obtained as brown solid. Mp: > 250 ^oC; ^1H NMR (600 MHz, DMSO-d_6) δ 11.91 (s, 1H, N*H*), 8.26 (s, 1H, N=C*H*), 7.34 (s, 1H, THPB-11-*H*), 7.23 (d, *J* = 3.0 Hz, 1H, thiazolyl-4-*H*), 7.13 (d, *J* = 39.4 Hz, 1H, thiazolyl-5-*H*), 6.82 (s, 2H, THPB-1-*H*, THPB-4-*H*), 6.04 (s, 2H, OCH_2O), 4.57 (s, 2H, CH_2(CH_2)$_8$$CH_3$), 4.39 (s, 1H, C*H*), 4.04–3.97 (m, 3H, THPB-8-*H*, THPB-13-*H*), 3.86 (s, 3H, OCH_3), 3.33 (s, 3H, THPB-5-*H*, THPB-13-*H*), 3.05 (s, 1H, THPB-6-*H*), 2.88 (d, *J* = 12.7 Hz, 1H, THPB-6-*H*), 1.74–1.69 (m, 2H, CH_2CH_2(CH_2)$_7$$CH_3$), 1.42 (d, *J* = 6.7 Hz, 2H, (CH_2)$_2$CH_2(CH_2)$_6$$CH_3$), 1.27 (s, 12H, ($CH_2$)$_3$(C$H_2$)$_6$$CH_3$), 0.86 (t, *J* = 6.6 Hz, 3H, (CH_2)$_9$CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.4, 150.6, 147.4, 147.1, 145.9, 144.8, 141.2, 131.4, 130.4, 128.1, 127.4, 126.0, 124.2, 112.0, 109.0, 108.7, 106.5, 101.7, 72.9, 59.2, 56.7, 56.4, 50.6, 49.1, 34.6, 32.8, 31.8, 30.2, 29.5, 29.2, 25.8, 22.5, 14.4 ppm; HRMS (ESI) calcd. for $C_{33}H_{42}N_4O_4S$ $[M + H]^+$, 591.3005; found, 591.3006.

*4.1.29. Synthesis of 9-(Dodecyloxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**9f**)*

Compound **9f** was prepared according to the procedure described for compound **6**, starting from compound **8f** (0.30 g, 0.50 mmol) and 2-chloroacetaldehyde (0.059 g, 0.76 mmol). The pure product **9f** (0.13 g, 40.5%) was obtained as brown solid. Mp: > 250 ^oC; ^1H NMR (600 MHz, DMSO-d_6) δ 11.84 (s, 1H, N*H*), 8.47 (s, 1H, N=C*H*), 7.48 (s, 1H, THPB-11-*H*), 7.37 (s, 1H, thiazolyl-4-*H*), 7.19 (s, 1H, THPB-1-*H*), 6.99 (s, 1H, thiazolyl-5-*H*), 6.84 (s, 1H, THPB-4-*H*), 6.05 (d, *J* = 6.4 Hz, 2H, OCH_2O), 4.68 (s, 1H, C*H*(CH_2)$_{10}$$CH_3$), 4.59 (d, *J* = 15.6 Hz, 1H, C*H*), 4.41 (d, *J* = 14.7 Hz, 1H, C*H*(CH_2)$_{10}$$CH_3$), 4.05–4.01 (m, 2H. THPB-8-*H*), 3.89 (s, 3H, OCH_3), 3.42 (d, *J* = 12.5 Hz, 2H, THPB-13-*H*), 3.14–3.07 (m, 1H, THPB-5-*H*), 2.88 (d, *J* = 10.7 Hz, 2H, THPB-6-*H*), 2.73 (s, 1H, THPB-5-*H*), 1.72 (d, *J* = 6.5 Hz, 2H, CH_2CH_2(CH_2)$_9$$CH_3$), 1.42 (s, 2H, ($CH_2$)$_2CH_2$($CH_2$)$_8$$CH_3$), 1.26 (s, 16H, ($CH_2$)$_3$(C$H_2$)$_8$$CH_3$), 0.86 (t, *J* = 6.4 Hz, 3H, (CH_2)$_{11}$CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.3, 150.6, 147.4, 147.1, 145.9, 144.8, 141.2, 131.4, 128.1, 127.4, 126.0, 125.5, 124.0, 113.0, 108.6, 108.3, 106.4, 101.8, 73.0, 58.9, 56.5, 54.1, 51.5, 49.1, 36.2, 33.7, 31.8, 30.2, 29.7, 29.5, 29.2, 25.8, 25.7, 22.5, 14.4 ppm; HRMS (ESI) calcd. for $C_{35}H_{46}N_4O_4S$ $[M + H]^+$, 619.3318; found, 619.3317.

*4.1.30. Synthesis of 2-((10-Methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl)oxy)ethan-1-ol (**9g**).*

Compound **9g** was prepared according to the procedure described for compound **6**, starting from compound **8g** (0.10 g, 0.21 mmol) and 2-chloroacetaldehyde (0.025 g, 0.32 mmol). The pure product **9g**

(0.041 g, 39.4%) was obtained as brown solid. Mp: 236–237 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.85 (s, 1H, N*H*), 8.33 (s, 1H, N=C*H*), 7.43 (s, 1H, THPB-11-*H*), 7.25 (s, 1H, thiazolyl-4-*H*), 7.06 (s, 1H, THPB-1-*H*), 6.95 (s, 1H, thiazolyl-5-*H*), 6.84 (s, 1H, THPB-4-*H*), 6.02 (d, *J* = 18.6 Hz, 2H, OCH_2O), 4.77 (s, 1H, OC*H*CH$_2$OH), 4.71 (s, 1H, OC*H*CH$_2$OH), 4.53 (d, *J* = 15.9 Hz, 1H, C*H*), 4.23–4.19 (m, 1H, OCH$_2$C*H*OH), 4.17–4.13 (m, 1H, OCH$_2$C*H*OH), 3.89 (s, 3H, OCH_3), 3.45 (d, *J* = 15.8 Hz, 2H, THPB-8-*H*), 3.38 (dd, *J* = 23.3, 13.5 Hz, 2H, THPB-13-*H*), 3.05 (d, *J* = 6.1 Hz, 1H, THPB-5-*H*), 2.92 (t, *J* = 11.1 Hz, 1H, THPB-5-*H*), 2.62 (d, *J* = 15.5 Hz, 1H, THPB-6-*H*), 2.49–2.45 (m, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.4, 150.4, 146.2, 146.0, 145.9, 141.2, 131.4, 129.4, 128.0, 127.3, 112.1, 108.6, 108.2, 106.1, 101.1, 79.6, 74.6, 60.9, 59.2, 56.5, 54.0, 51.1, 49.1, 33.7, 29.5 ppm; HRMS (ESI) calcd. for $C_{25}H_{26}N_4O_5S$ $[M + H]^+$, 495.1702; found, 495.1704.

*4.1.31. Synthesis of 9-(Allyloxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**12**)*

Compound **12** was prepared according to the procedure described for compound **6**, starting from compound **11** (0.10 g, 0.22 mmol) and 2-chloroacetaldehyde (0.026 g, 0.33 mmol). The pure product **12** (0.032 g, 30.1%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.85 (s, 1H, N*H*), 8.32 (s, 1H, N=C*H*), 8.25 (s, 1H, THPB-11-*H*), 7.32 (s, 1H, THPB-1-*H*), 7.23 (d, *J* = 3.6 Hz, 1H, thiazolyl-4-*H*), 6.82 (d, *J* = 3.3 Hz, 1H, thiazolyl-5-*H*), 6.78 (s, 1H, THPB-4-*H*), 6.07 (dd, *J* = 11.1, 5.8 Hz, 1H, CH$_2$C*H*=CH$_2$), 6.02 (s, 2H, OCH_2O), 5.38 (d, *J* = 17.2 Hz, 1H, CH$_2$CH=C*H*), 5.24 (d, *J* = 9.5 Hz, 1H, CH$_2$CH=C*H*), 4.58 (d, *J* = 5.4 Hz, 3H, CH_2CH=CH$_2$, C*H*), 4.34 (s, 1H, THPB-8-*H*), 3.89 (s, 1H, THPB-8-*H*), 3.87 (s, 3H, OCH_3), 3.44 (dd, *J* = 13.9, 6.9 Hz, 2H, THPB-13-*H*), 3.32 (s, 3H, THPB-5-*H*, THPB-6-*H*), 3.17 (s, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.3, 150.5, 146.2, 146.0, 145.5, 141.1, 134.8, 131.3, 129.3, 128.3, 127.6, 118.5, 112.0, 108.6, 106.5, 101.5, 79.7, 73.4, 59.1, 56.6, 56.5, 55.4, 54.2, 49.1, 33.7, 29.4 ppm; HRMS (ESI) calcd. for $C_{26}H_{26}N_4O_4S$ $[M + H]^+$, 491.1753; found, 491.1756.

*4.1.32. Synthesis of 10-Methoxy-9-(prop-2-yn-1-yloxy)-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**15a**)*

Compound **15a** was prepared according to the procedure described for compound **6**, starting from compound **14a** (0.18 g, 0.39 mmol) and 2-chloroacetaldehyde (0.045 g, 0.58 mmol). The pure product **15a** (0.067 g, 35.2%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.73 (s, 1H, N*H*), 8.33 (s, 1H, N=C*H*), 7.42 (s, 1H, THPB-11-*H*), 7.27 (s, 1H, thiazolyl-4-*H*), 7.19 (s, 1H, THPB-1-*H*), 6.87 (s, 1H, thiazolyl-5-*H*), 6.84 (s, 1H, THPB-4-*H*), 6.05 (s, 2H, OCH_2O), 4.67 (d, *J* = 14.2 Hz, 2H, CH_2C≡CH), 4.45 (d, *J* = 15.2 Hz, 1H, C*H*), 4.08 (d, *J* = 16.0 Hz, 1H, THPB-8-*H*), 3.90 (s, 3H, OCH_3), 3.85 (s, 1H, THPB-8-*H*), 3.55 (s, 1H, THPB-13-*H*), 3.44 (dd, *J* = 13.6, 6.6 Hz, 2H, CH$_2$C≡C*H*, THPB-13-*H*), 3.40 (d, *J* = 16.0 Hz, 2H, THPB-5-*H*), 3.14–3.08 (m, 1H, THPB-6-*H*), 2.89 (d, *J* = 15.6 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.3, 150.5, 147.4, 146.2, 146.0, 145.5, 141.1,

134.8, 131.3, 128.9, 127.6, 118.5, 112.0, 108.6, 106.4, 101.8, 100.0, 79.7, 60.0, 59.0, 56.5, 55.3, 51.7, 49.1, 32.1, 26.8 ppm; HRMS (ESI) calcd. for $C_{26}H_{24}N_4O_4S$ $[M + H]^+$, 489.1597; found, 489.1598.

*4.1.33. Synthesis of 2-((10-Methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3] dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl)oxy)acetonitrile (**15b**)*

Compound **15b** was prepared according to the procedure described for compound **6**, starting from compound **14b** (0.18 g, 0.39 mmol) and 2-chloroacetaldehyde (0.046 g, 0.58 mmol). The pure product **15b** (0.063 g, 33.1%) was obtained as brown solid. Mp: > 250 ^oC; ^1H NMR (600 MHz, DMSO-d_6) δ 11.85 (s, 1H, N*H*), 8.46 (s, 1H, N=C*H*), 7.55 (s, 1H, THPB-11-*H*), 7.37 (s, 1H, thiazolyl-4-*H*), 7.21 (s, 1H, THPB-1-*H*), 6.99 (s, 1H, thiazolyl-5-*H*), 6.85 (s, 1H, THPB-4-*H*), 6.06 (d, *J* = 5.8 Hz, 2H, OCH_2O), 5.15 (d, *J* = 7.2 Hz, 2H, CH_2C≡CN), 4.71 (d, *J* = 14.9 Hz, 2H, THPB-8-*H*), 4.47 (d, *J* = 15.2 Hz, 1H, C*H*), 4.11 (d, *J* = 15.7 Hz, 1H, THPB-13-*H*), 3.95 (s, 3H, OCH_3), 3.86 (d, *J* = 7.1 Hz, 1H, THPB-13-*H*), 3.40 (d, *J* = 16.4 Hz, 2H, THPB-5-*H*), 3.12 (d, *J* = 15.9 Hz, 1H, THPB-6-*H*), 2.91 (d, *J* = 15.3 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.4, 150.1, 147.4, 146.2, 146.0, 142.8, 131.7, 130.4, 129.8, 128.9, 127.7, 117.2, 112.1, 109.6, 108.67, 105.6, 101.8, 79.7, 73.6, 63.0, 58.9, 58.0, 56.7, 33.5, 29.4 ppm; HRMS (ESI) calcd. for $C_{25}H_{23}N_5O_4S$ $[M + H]^+$, 490.1549; found, 490.1548.

*4.1.34. Synthesis of 9-((2,4-Dichlorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**16a**)*

Compound **16a** was prepared according to the procedure described for compound **7a**, starting from compound **4** (2.0 g, 5.7 mmol), 2,4-dichloro-1-(chloromethyl)benzene (1.7 g, 8.5 mmol) and potassium carbonate (1.2 g, 8.5 mmol). The pure product **16a** (2.34 g, 80.1%) was obtained as yellow solid. Mp: 100–101 ^oC; ^1H NMR (600 MHz, $CDCl_3$) δ 10.21 (s, 1H, C*H*O), 7.55 (d, *J* = 8.2 Hz, 1H, Ph-3-*H*), 7.35 (s, 1H, THPB-11-*H*), 7.30 (dd, *J* = 8.3, 1.7 Hz, 1H, Ph-5-*H*), 7.25 (d, *J* = 1.8 Hz, 1H, Ph-6-*H*), 6.77 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.93 (s, 2H, OCH_2O), 5.27 (d, *J* = 12.5 Hz, 1H, Ph-1-C*H*), 5.19 (d, *J* = 12.5 Hz, 1H, Ph-1-C*H*), 4.19 (d, *J* = 15.8 Hz, 1H, C*H*), 3.92 (s, 3H, OCH_3), 3.84 (d, *J* = 17.0 Hz, 1H, THPB-8-*H*), 3.48 (s, 1H, THPB-8-*H*), 3.44 (s, H, THPB-13-*H*), 3.10 (s, 2H, THPB-5-*H*), 3.06–2.99 (m, 1H, THPB-13-*H*), 2.65 (d, *J* = 15.7 Hz, 1H, THPB-6-*H*), 2.58 (s, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.0, 148.5, 146.3, 137.0, 134.5, 133.9, 133.7, 133.0, 130.4, 129.5, 129.3, 129.0, 127.5, 127.3, 113.8, 108.4, 105.6, 100.9, 70.7, 61.9, 59.1, 56.0, 54.0, 51.3, 33.5, 29.2 ppm.

*4.1.35. Synthesis of 9-((2-Chlorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**16b**)*

Compound **16b** was prepared according to the procedure described for compound **7a**, starting from compound **4** (2.0 g, 5.7 mmol), 1-chloro-2-(chloromethyl)benzene (1.4 g, 8.5 mmol) and potassium carbonate (1.2 g, 8.5 mmol). The pure product **16b** (2.13 g, 78.2%) was obtained as yellow solid. Mp: 48–49 ^oC; ^1H NMR (600 MHz, $CDCl_3$) δ 10.20 (s, 1H, C*H*O), 7.60 (dd, *J* = 7.4, 1.7 Hz, 1H, Ph-3-*H*), 7.34 (d, *J* = 1.2 Hz, 1H, THPB-11-*H*), 7.31–7.29 (m, 1H, Ph-4-*H*), 7.26 (s, 1H, Ph-6-*H*), 7.22 (d, *J* = 1.7 Hz, 1H,

Ph-5-*H*), 6.77 (s, 1H, THPB-1-*H*), 6.58 (s, 1H, THPB-4-*H*), 5.92 (s, 2H, OCH_2O), 5.31 (t, *J* = 12.7 Hz, 1H, Ph-1-C*H*), 5.24 (d, *J* = 12.2 Hz, 1H, Ph-1-C*H*), 4.23 (d, *J* = 15.9 Hz, 1H, C*H*), 3.93 (s, 3H, OCH_3), 3.84 (dd, *J* = 16.9, 3.2 Hz, 1H, THPB-8-*H*), 3.50 (s, 1H, THPB-8-*H*), 3.46 (s, 1H, THPB-13-*H*), 3.11 (s, 2H, THPB-5-*H*), 3.05 (d, *J* = 12.4 Hz, 1H, THPB-13-*H*), 2.65 (d, *J* = 15.8 Hz, 1H, THPB-6-*H*), 2.58 (d, *J* = 11.0 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.2, 148.9, 146.3, 146.2, 138.4, 135.3, 133.2, 132.7, 129.7, 129.4, 129.3, 128.7, 127.0, 113.8, 108.4, 105.6, 100.8, 71.3, 62.7, 59.0, 58.4, 56.1, 54.0, 51.1, 31.6, 26.9 ppm.

*4.1.36. Synthesis of 9-((3-Chlorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**16c**)*

Compound **16c** was prepared according to the procedure described for compound **7a**, starting from compound **4** (1.5 g, 4.2 mmol), 1-chloro-3-(chloromethyl)benzene (1.0 g, 6.4 mmol) and potassium carbonate (0.88 g, 6.4 mmol). The pure product **16c** (1.56 g, 77.5%) was obtained as yellow solid. Mp: 45–46 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.22 (s, 1H, C*H*O), 7.47 (s, 1H, Ph-2-*H*), 7.36 (s, 1H, THPB-11-*H*), 7.32 (s, 2H, Ph-5,6-*H*), 7.22 (d, *J* = 7.2 Hz, 1H, Ph-4-*H*), 6.77 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.93 (s, 2H, OCH_2O), 5.20 (d, *J* = 11.6 Hz, 1H, Ph-1-C*H*), 5.08 (d, *J* = 11.6 Hz, 1H, Ph-1-C*H*), 4.20 (d, *J* = 15.7 Hz, 1H, C*H*), 3.94 (s, 3H, OCH_3), 3.85 (d, *J* = 5.2 Hz, 1H, THPB-8-*H*), 3.53 (s, 1H, THPB-8-*H*), 3.45 (s, 1H, THPB-13-*H*), 3.13 (s, 2H, THPB-5-*H*), 3.08–3.01 (m, 1H, THPB-13-*H*), 2.67 (d, *J* = 16.0 Hz, 1H, THPB-6-*H*), 2.62 (d, *J* = 11.4 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.6 (*C*=O), 150.1, 148.7, 146.4, 139.4, 134.4, 129.8, 129.5, 128.3, 128.1, 127.6, 126.9, 126.0, 124.8, 113.7, 108.4, 105.6, 100.9, 73.6, 64.4, 59.0, 58.4, 56.0, 54.1, 51.2, 31.6, 26.9 ppm.

*4.1.37. Synthesis of 9-((4-Chlorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**16d**)*

Compound **16d** was prepared according to the procedure described for compound **7a**, starting from compound **4** (2.0 g, 5.7 mmol), 1-chloro-4-(chloromethyl)benzene (1.4 g, 8.5 mmol) and potassium carbonate (1.2 g, 8.5 mmol). The pure product **16d** (2.07 g, 76.0%) was obtained as yellow solid. Mp: 66–67 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.19 (s, 1H, C*H*O), 7.34 (d, *J* = 3.9 Hz, 2H, Ph-3,5-*H*), 7.32 (s, 2H, Ph-2,6-*H*), 7.26 (s, 1H, THPB-11-*H*), 6.76 (s, 1H, THPB-1-*H*), 6.58 (s, 1H, THPB-4-*H*), 5.92 (s, 2H, OCH_2O), 5.19 (d, *J* = 11.5 Hz, 1H, Ph-1-C*H*), 5.07 (d, *J* = 11.5 Hz, 1H, Ph-1-C*H*), 4.14 (d, *J* = 15.7 Hz, 1H, C*H*), 3.93 (s, 3H, OCH_3), 3.86–3.81 (m, 1H, THPB-8-*H*), 3.52–3.47 (m, 1H, THPB-8-*H*), 3.40 (d, *J* = 15.7 Hz, 1H, THPB-13-*H*), 3.09 (d, *J* = 8.2 Hz, 2H, THPB-5-*H*), 3.04–2.97 (m, 1H, THPB-13-*H*), 2.65 (d, *J* = 15.7 Hz, 1H, THPB-6-*H*), 2.58 (d, *J* = 10.3 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.1, 148.8, 146.3, 146.2, 139.4, 135.9, 134.1, 133.3, 129.4, 129.3, 128.7, 128.6, 128.2, 127.6, 113.6, 108.4, 105.6, 100.9, 73.6, 64.5, 59.0, 56.0, 54.2, 51.2, 33.6, 29.3 ppm.

*4.1.38. Synthesis of 9-((3,4-Dichlorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**16e**)*

Compound **16e** was prepared according to the procedure described for compound **7a**, starting from compound **4** (1.5 g, 4.2 mmol), 1,2-dichloro-4-(chloromethyl)benzene (1.2 g, 6.4 mmol) and potassium carbonate (0.88 g, 6.4 mmol). The pure product **16e** (1.65 g, 76.6%) was obtained as yellow solid. Mp: 38–39 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.13 (s, 1H, C*H*O), 7.56 (d, *J* = 1.9 Hz, 1H, Ph-5-*H*), 7.45 (d, *J* = 8.2 Hz, 1H, Ph-2-*H*), 7.31 (s, 1H, THPB-11-*H*), 7.26 (d, *J* = 2.0 Hz, 1H, Ph-6-*H*), 6.75 (s, 1H, THPB-1-*H*), 6.57 (s, 1H, THPB-4-*H*), 5.92 (s, 2H, OCH_2O), 5.14 (d, *J* = 11.8 Hz, 1H, Ph-1-C*H*), 5.04 (d, *J* = 11.7 Hz, 1H, Ph-1-C*H*), 4.11 (d, *J* = 15.8 Hz, 1H, C*H*), 3.93 (s, 3H, OCH_3), 3.48 (dd, *J* = 11.1, 3.6 Hz, 1H, THPB-8-*H*), 3.40 (d, *J* = 15.9 Hz, 1H, THPB-8-*H*), 3.10 (td, *J* = 8.8, 3.7 Hz, 2H, THPB-13-*H*), 2.95 (dd, *J* = 16.8, 11.0 Hz, 1H, THPB-5-*H*), 2.65 (d, *J* = 16.6 Hz, 1H, THPB-5-*H*), 2.60–2.54 (m, 2H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.8 (*C*=O), 149.9, 148.5, 146.3, 141.3, 137.7, 132.5, 131.3, 130.5, 129.8, 127.4, 127.1, 113.9, 108.4, 105.5, 100.9, 72.8, 63.6, 59.0, 58.4, 55.9, 54.1, 53.4, 51.3, 50.6, 33.5, 29.1 ppm.

*4.1.39. Synthesis of 9-((2-Fluorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**16f**)*

Compound **16f** was prepared according to the procedure described for compound **7a**, starting from compound **4** (1.3 g, 3.7 mmol), 1-(chloromethyl)-2-fluorobenzene (0.80 g, 5.5 mmol) and potassium carbonate (0.76 g, 5.5 mmol). The pure product **16f** (1.28 g, 74.8%) was obtained as yellow solid. Mp: 182–183 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.22 (s, 1H, C*H*O), 7.51 (td, *J* = 7.5, 1.7 Hz, 1H, Ph-3-*H*), 7.35 (s, 1H, THPB-11-*H*), 7.34–7.31 (m, 1H, Ph-4-*H*), 7.17 (td, *J* = 7.5, 1.0 Hz, 1H, Ph-6-*H*), 7.11–7.07 (m, 1H, Ph-5-*H*), 6.77 (s, 1H, THPB-1-*H*), 6.58 (s, 1H, THPB-4-*H*), 5.92 (s, 2H, OCH_2O), 5.29 (d, *J* = 11.5 Hz, 1H, Ph-1-C*H*), 5.20 (d, *J* = 11.5 Hz, 1H, Ph-1-C*H*), 4.19 (d, *J* = 15.9 Hz, 1H, C*H*), 3.94 (s, 3H, OCH_3), 3.83 (dd, *J* = 16.9, 3.5 Hz, 1H, THPB-8-*H*), 3.48 (d, *J* = 3.3 Hz, 1H, THPB-8-*H*), 3.40 (d, *J* = 15.9 Hz, 1H, THPB-13-*H*), 3.13–3.07 (m, 2H, THPB-5-*H*), 3.03 (dd, *J* = 18.5, 7.0 Hz, 1H, THPB-13-*H*), 2.64 (dd, *J* = 17.0, 4.3 Hz, 1H, THPB-6-*H*), 2.58–2.52 (m, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 161.7, 160.1, 150.2, 148.8, 146.3, 146.1, 131.7, 130.7, 130.5, 130.2, 129.5, 127.7, 124.6, 124.2, 115.4, 113.5, 108.4, 105.6, 100.8, 68.9, 59.1, 56.0, 54.0, 51.2, 33.7, 29.5 ppm.

*4.1.40. Synthesis of 9-((4-Fluorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**16g**).*

Compound **16g** was prepared according to the procedure described for compound **7a**, starting from compound **4** (1.3 g, 3.7 mmol), 1-(chloromethyl)-2-fluorobenzene (0.80 g, 5.5 mmol) and potassium carbonate (0.76 g, 5.5 mmol). The pure product **16g** (1.24 g, 72.5%) was obtained as yellow solid. Mp: 164–165 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.22 (s, 1H, C*H*O), 7.43–7.40 (m, 2H, Ph-3,5-*H*), 7.35 (s, 1H, THPB-11-*H*), 7.09–7.05 (m, 2H, Ph-2,6-*H*), 6.77 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.93 (s, 2H, OCH_2O), 5.19 (d, *J* = 11.2 Hz, 1H, Ph-1-C*H*), 5.07 (d, *J* = 11.2 Hz, 1H, Ph-1-C*H*), 4.15 (d, *J* = 15.9 Hz, 1H, C*H*), 3.94 (s, 3H, OCH_3), 3.84 (dd, *J* = 14.0, 2.7 Hz, 1H, THPB-8-*H*), 3.49 (d, *J* = 8.6 Hz, 1H,

THPB-8-*H*), 3.39 (d, *J* = 15.8 Hz, 1H, THPB-13-*H*), 3.09 (dd, *J* = 14.0, 9.7 Hz, 2H, THPB-5-*H*), 3.03 (dd, *J* = 16.9, 11.3 Hz, 1H, THPB-13-*H*), 2.65 (d, *J* = 16.0 Hz, 1H, THPB-6-*H*), 2.57 (dd, *J* = 11.9, 8.6 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.6 (*C*=O), 163.5, 161.9, 150.1, 148.8, 146.3, 146.2, 133.2, 131.7, 130.1, 130.0, 129.4, 127.6, 115.5, 115.3, 113.6, 108.4, 105.6, 100.8, 73.8, 59.1, 58.4, 56.0, 54.2, 51.2, 33.7, 29.4 ppm.

*4.1.41. Synthesis of 9-(Benzyloxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino [3,2-a]isoquinoline-12-carbaldehyde (**16h**)*

Compound **16h** was prepared according to the procedure described for compound **7a**, starting from compound **4** (1.3 g, 3.7 mmol), (chloromethyl)benzene (0.70 g, 5.5 mmol) and potassium carbonate (0.76 g, 5.5 mmol). The pure product **16h** (1.11 g, 67.7%) was obtained as yellow solid. Mp: 175–176 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.21 (s, 1H, C*H*O), 7.44 (d, *J* = 7.3 Hz, 2H, Ph-2,6-*H*), 7.40–7.37 (m, 2H, Ph-3,5-*H*), 7.35–7.33 (m, 2H, Ph-4-*H*, THPB-11-*H*), 6.76 (s, 1H, THPB-1-*H*), 6.57 (s, 1H, THPB-4-*H*), 5.91 (s, 2H, OC*H*$_2$O), 5.22 (d, *J* = 11.3 Hz, 1H, Ph-1-C*H*), 5.11 (d, *J* = 11.3 Hz, 1H, Ph-1-C*H*), 4.16 (d, *J* = 15.9 Hz, 1H, C*H*), 3.93 (s, 3H, OC*H*$_3$), 3.82 (dd, *J* = 16.7, 3.0 Hz, 1H, THPB-8-*H*), 3.46 (dd, *J* = 11.2, 3.6 Hz, 1H, THPB-8-*H*), 3.38 (d, *J* = 15.9 Hz, 1H, THPB-13-*H*), 3.11–3.06 (m, 2H, THPB-5-*H*), 3.01 (dd, *J* = 16.9, 11.2 Hz, 1H, THPB-13-*H*), 2.65–2.61 (m, 1H, THPB-6-*H*), 2.56–2.51 (m, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.2, 149.1, 146.3, 146.1, 137.4, 131.7, 130.3, 130.1, 129.7, 129.3, 128.5, 128.2, 127.7, 126.9, 113.6, 108.4, 105.6, 100.8, 74.5, 59.0, 58.3, 56.0, 54.3, 51.2, 33.7, 29.5 ppm.

*4.1.42. Synthesis of 10-Methoxy-9-((4-nitrobenzyl)oxy)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinoline-12-carbaldehyde (**16i**)*

Compound **16i** was prepared according to the procedure described for compound **7a**, starting from compound **4** (1.3 g, 3.7 mmol), 1-(bromomethyl)-4-nitrobenzene (1.2 g, 5.5 mmol) and potassium carbonate (0.76 g, 5.5 mmol). The pure product **16i** (1.27 g, 70.1%) was obtained as yellow solid. Mp: 168–169 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.21 (s, 1H, C*H*O), 8.26 (d, *J* = 8.6 Hz, 1H, Ph-3-*H*), 8.18 (s, 1H, Ph-5-*H*), 7.63 (d, *J* = 8.5 Hz, 1H, Ph-2-*H*), 7.50 (s, 1H, Ph-6-*H*), 7.36 (s, 1H, THPB-11-*H*), 6.77 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.93 (s, 2H, OC*H*$_2$O), 5.33 (s, 1H, Ph-1-C*H*), 5.22 (d, *J* = 12.6 Hz, 1H, Ph-1-C*H*), 4.18 (d, *J* = 15.8 Hz, 1H, C*H*), 3.92 (s, 3H, OC*H*$_3$), 3.85 (dd, *J* = 16.5, 3.0 Hz, 1H, THPB-8-*H*), 3.50 (d, *J* = 13.6 Hz, 2H, THPB-13-*H*), 3.11 (dt, *J* = 24.7, 8.5 Hz, 2H, THPB-8-*H*, THPB-5-*H*), 3.01 (dd, *J* = 16.8, 11.3 Hz, 1H, THPB-5-*H*), 2.65 (d, *J* = 16.2 Hz, 1H, THPB-6-*H*), 2.59 (dd, *J* = 12.5, 9.3 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.7 (*C*=O), 150.5, 149.1, 146.3, 137.6, 131.7, 130.3, 130.1, 129.7, 129.2, 128.4, 127.7, 126.9, 115.6, 108.6, 106.1, 101.1, 79.6, 72.8, 62.5, 60.2, 59.0, 56.6, 55.3, 49.1, 33.8, 29.4 ppm.

*4.1.43. Synthesis of 2-((9-((2,4-Dichlorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo [4,5-g]isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**17a**)*

Compound **17a** was prepared according to the procedure described for compound **5**, starting from

compound **16a** (1.4 g, 2.7 mmol), hydrazinecarbothioamide (0.27 g, 3.0 mmol) and glacial acetic acid (0.15 eq). The pure product **17a** (1.18 g, 74.6%) was obtained as brown solid. Mp: 233–234 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.18 (s, 1H, N*H*), 8.44 (s, 1H, N=C*H*), 8.23 (s, 1H, N*H*), 8.06 (s, 1H, N*H*), 7.68 (d, *J* = 2.1 Hz, 1H, Ph-3-*H*), 7.67 (s, 1H, THPB-11-*H*), 7.63 (d, *J* = 8.3 Hz, 1H, Ph-5-*H*), 7.49 (dd, *J* = 8.3, 2.1 Hz, 1H, Ph-6-*H*), 6.93 (s, 1H, THPB-1-*H*), 6.67 (s, 1H, THPB-4-*H*), 5.97 (s, 1H, OC*H*O), 5.94 (s, 1H, OC*H*O), 5.11 (dd, *J* = 31.2, 12.1 Hz, 2H, Ph-1-CH_2), 4.01 (d, *J* = 15.7 Hz, 1H, C*H*), 3.89 (s, 3H, OCH_3), 3.48–3.43 (m, 2H, THPB-8-*H*), 3.27 (d, *J* = 15.7 Hz, 1H, THPB-13-*H*), 3.03–2.97 (m, 1H, THPB-13-*H*), 2.92–2.83 (m, 1H, THPB-5-*H*), 2.60 (d, *J* = 15.9 Hz, 1H, THPB-5-*H*), 2.49–2.45 (m, 1H, THPB-6-*H*), 2.39 (td, *J* = 11.1, 3.1 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 163.1, 161.8, 150.5, 146.3, 146.1, 145.0, 140.9, 139.1, 131.0, 130.8, 130.2, 128.7, 128.1, 127.0, 108.6, 106.3, 101.1, 79.6, 79.3, 72.5, 62.0, 58.2, 56.6, 54.2, 49.1, 33.7, 29.4 ppm.

*4.1.44. Synthesis of 2-((9-((2-Chlorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**17b**)*

Compound **17b** was prepared according to the procedure described for compound **5**, starting from compound **16b** (1.6 g, 3.4 mmol), hydrazinecarbothioamide (0.34 g, 3.7 mmol) and glacial acetic acid (0.15 eq). The pure product **17b** (1.35 g, 72.2%) was obtained as brown solid. Mp: 221–222 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.18 (s, 1H, N*H*), 8.44 (s, 1H, N=C*H*), 8.23 (s, 1H, N*H*), 8.07 (s, 1H, N*H*), 7.67 (s, 1H, Ph-3-*H*), 7.62–7.59 (m, 1H, Ph-4-*H*), 7.52–7.49 (m, 1H, Ph-6-*H*), 7.41–7.38 (m, 2H, Ph-5-*H*, THPB-11-*H*), 6.92 (s, 1H, THPB-1-*H*), 6.67 (s, 1H, THPB-4-*H*), 5.97 (d, *J* = 0.7 Hz, 1H, OC*H*O), 5.94 (d, *J* = 0.7 Hz, 1H, OC*H*O), 5.13 (dd, *J* = 28.6, 11.9 Hz, 2H, Ph-1-CH_2), 4.03 (d, *J* = 15.8 Hz, 1H, C*H*), 3.90 (s, 3H, OCH_3), 3.46–3.42 (m, 2H, THPB-8-*H*), 3.25 (d, *J* = 15.6 Hz, 1H, THPB-13-*H*), 2.98 (dd, *J* = 8.3, 2.5 Hz, 1H, THPB-13-*H*), 2.91–2.84 (m, 1H, THPB-5-*H*), 2.59 (d, *J* = 15.8 Hz, 1H, THPB-5-*H*), 2.49–2.45 (m, 1H, THPB-6-*H*), 2.38 (d, *J* = 3.1 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 162.5, 161.6, 150.6, 146.2, 146.0, 145.1, 141.1, 139.5, 131.0, 127.9, 124.9, 115.8, 115.7, 108.6, 108.3, 106.1, 101.1, 79.6, 67.7, 59.1, 56.5, 55.3, 53.9, 50.9, 49.1, 33.6, 29.4 ppm.

*4.1.45. Synthesis of 2-((9-((3-Chlorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**17c**)*

Compound **17c** was prepared according to the procedure described for compound **5**, starting from compound **16c** (0.70 g, 1.5 mmol), hydrazinecarbothioamide (0.15 g, 1.6 mmol) and glacial acetic acid (0.15 eq). The pure product **17c** (0.59 g, 71.4%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.20 (s, 1H, N*H*), 8.43 (s, 1H, N=C*H*), 8.22 (s, 1H, N*H*), 8.04 (s, 1H, N*H*), 7.45 (s, 1H, Ph-2-*H*), 7.38 (s, 1H, THPB-11-*H*), 7.33 (s, 2H, Ph-5,6-*H*), 7.21 (d, *J* = 6.4 Hz, 1H, Ph-4-*H*), 6.77 (s, 1H, THPB-1-*H*), 6.59 (s, 1H, THPB-4-*H*), 5.93 (s, 2H, OCH_2O), 5.20 (d, *J* = 11.6 Hz, 1H, Ph-1-C*H*), 5.08 (d, *J* = 11.6 Hz, 1H, Ph-1-C*H*), 4.20 (d, *J* = 15.7 Hz, 1H, C*H*), 3.94 (s, 3H, OCH_3), 3.85 (d, *J* = 5.2 Hz, 1H, THPB-8-*H*), 3.53 (s, 1H, THPB-8-*H*), 3.45 (s, 1H, THPB-13-*H*), 3.13 (s, 2H, THPB-5-*H*), 3.08–3.01 (m,

1H, THPB-13-*H*), 2.68 (d, *J* = 16.0 Hz, 1H, THPB-6-*H*), 2.60 (s, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.3 (*C*=S), 163.2, 161.5, 151.0, 146.2, 146.0, 145.4, 141.1, 137.6, 134.4, 131.2, 130.9, 128.9, 128.7, 128.0, 126.9, 115.5, 108.8, 101.6, 79.7, 74.2, 60.2, 56.8, 56.2, 55.3, 49.1, 33.4, 29.4 ppm.

*4.1.46. Synthesis of 2-((9-((4-Chlorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**17d**)*

Compound **17d** was prepared according to the procedure described for compound **5**, starting from compound **16d** (2.0 g, 4.2 mmol), hydrazinecarbothioamide (0.42 g, 4.6 mmol) and glacial acetic acid (0.15 eq). The pure product **17d** (1.75 g, 75.7%) was obtained as brown solid. Mp: 183–184 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.18 (s, 1H, N*H*), 8.43 (s, 1H, N=C*H*), 8.22 (s, 1H, N*H*), 8.06 (s, 1H, N*H*), 7.66 (s, 1H, THPB-11-*H*), 7.47 (q, *J* = 8.5 Hz, 4H, Ph-2,3,5,6-*H*), 6.93 (s, 1H, THPB-1-*H*), 6.68 (s, 1H, THPB-4-*H*), 5.97 (s, 1H, OC*H*O), 5.94 (d, *J* = 0.7 Hz, 1H, OC*H*O), 5.02 (dd, *J* = 24.3, 11.4 Hz, 2H, Ph-1-CH_2), 4.01 (d, *J* = 15.7 Hz, 1H, C*H*), 3.90 (s, 3H, OCH_3), 3.46–3.43 (m, 2H, THPB-8-*H*), 3.29 (d, *J* = 15.3 Hz, 1H, THPB-13-*H*), 3.03 (d, *J* = 6.2 Hz, 1H, THPB-13-*H*), 2.92–2.85 (m, 1H, THPB-5-*H*), 2.60 (d, *J* = 15.8 Hz, 1H, THPB-5-*H*), 2.47 (d, *J* = 11.7 Hz, 1H, THPB-6-*H*), 2.40 (dd, *J* = 12.4, 5.7 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 163.2, 161.6, 150.5, 146.2, 146.0, 145.4, 141.1, 134.7, 131.3, 130.9, 129.5, 128.1, 127.8, 115.5, 115.0, 108.6, 108.2, 106.1, 101.1, 79.6, 73.2, 59.0, 56.4, 54.1, 51.1, 33.8, 29.7 ppm.

*4.1.47. Synthesis of 2-((9-((3,4-Dichlorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo [4,5-g]isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**17e**)*

Compound **17e** was prepared according to the procedure described for compound **5**, starting from compound **16e** (0.70 g, 1.4 mmol), hydrazinecarbothioamide (0.14 g, 1.5 mmol) and glacial acetic acid (0.15 eq). The pure product **17e** (0.33 g, 40.7%) was obtained as brown solid. Mp: 194–195 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.18 (s, 1H, N*H*), 8.44 (s, 1H, N=C*H*), 8.23 (s, 1H, N*H*), 8.06 (s, 1H, N*H*), 7.74 (s, 1H, Ph-4-*H*), 7.66 (d, *J* = 8.5 Hz, 2H, Ph-3-*H*, THPB-11-*H*), 7.56 (d, *J* = 12.0 Hz, 1H, Ph-6-*H*), 7.47 (d, *J* = 8.0 Hz, 1H, Ph-5-*H*), 6.95 (s, 1H, THPB-1-*H*), 6.70 (s, 1H, THPB-4-*H*), 5.97 (d, *J* = 19.3 Hz, 2H, OCH_2O), 5.03 (q, *J* = 11.7 Hz, 2H, Ph-1-CH_2), 4.09 (d, *J* = 3.9 Hz, 1H, C*H*), 3.90 (s, 3H, OCH_3), 3.47 (d, *J* = 33.6 Hz, 2H, THPB-8-*H*), 3.33 (s, 4H, THPB-13-*H*, THPB-5-*H*), 2.91 (d, *J* = 18.4 Hz, 1H, THPB-6-*H*), 2.66 (s, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 181.9, 178.2 (*C*=S), 150.5, 146.3, 145.0, 144.4, 140.9, 139.2, 131.5, 131.0, 130.7, 130.3, 128.7, 128.1, 127.0, 108.6, 106.1, 101.2, 79.7, 79.5, 72.5, 62.0, 58.3, 56.6, 54.3, 49.1, 33.8, 29.7 ppm.

*4.1.48. Synthesis of 2-((9-((2-Fluorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**17f**)*

Compound **17f** was prepared according to the procedure described for compound **5**, starting from compound **16f** (0.95 g, 2.0 mmol), hydrazinecarbothioamide (0.20 g, 2.2 mmol) and glacial acetic acid

(0.15 eq). The pure product **17f** (0.53 g, 49.3%) was obtained as brown solid. Mp: 152–153 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.17 (s, 1H, N*H*), 8.43 (s, 1H, N=C*H*), 8.21 (s, 1H, N*H*), 8.05 (s, 1H, N*H*), 7.66 (s, 1H, Ph-4-*H*), 7.53 (t, *J* = 7.3 Hz, 1H, Ph-3-*H*), 7.42 (d, *J* = 6.1 Hz, 1H, THPB-11-*H*), 7.23 (d, *J* = 8.0 Hz, 2H, Ph-5,6-*H*), 6.92 (s, 1H, THPB-1-*H*), 6.67 (s, 1H, THPB-4-*H*), 5.96 (d, *J* = 19.0 Hz, 2H, OCH_2O), 5.10 (dd, *J* = 32.9, 11.3 Hz, 2H, Ph-1-CH_2), 3.98 (d, *J* = 15.7 Hz, 1H, C*H*), 3.90 (s, 3H, OCH_3), 3.49–3.41 (m, 2H, THPB-8-*H*), 3.22 (d, *J* = 15.1 Hz, 1H, THPB-13-*H*), 2.98 (s, 1H, THPB-13-*H*), 2.92–2.83 (m, 1H, THPB-5-*H*), 2.60 (d, *J* = 15.9 Hz, 1H, THPB-5-*H*), 2.47 (d, *J* = 14.4 Hz, 1H, THPB-6-*H*), 2.38 (s, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 161.8, 160.2, 150.6, 146.2, 146.0, 145.1, 141.1, 131.7, 131.0, 127.9, 124.9, 115.8, 115.7, 108.6, 108.3, 106.1, 101.1, 79.6, 67.7, 59.1, 56.5, 55.3, 53.9, 50.9, 49.1, 33.6, 29.4 ppm.

*4.1.49. Synthesis of 2-((9-((4-Fluorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**17g**)*

Compound **17g** was prepared according to the procedure described for compound **5**, starting from compound **16g** (0.32 g, 0.70 mmol), hydrazinecarbothioamide (0.070 g, 0.77 mmol) and glacial acetic acid (0.15 eq). The pure product **17g** (0.19 g, 52.1%) was obtained as brown solid. Mp: 153–154 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.17 (s, 1H, N*H*), 8.43 (s, 1H, N=C*H*), 8.21 (s, 1H, N*H*), 8.05 (s, 1H, N*H*), 7.66 (s, 1H, THPB-11-*H*), 7.53–7.47 (m, 2H, Ph-3,5-*H*), 7.21 (t, *J* = 8.7 Hz, 2H, Ph-2,6-*H*), 6.93 (s, 1H, THPB-1-*H*), 6.68 (s, 1H, THPB-4-*H*), 5.96 (d, *J* = 19.5 Hz, 2H, OCH_2O), 5.00 (dd, *J* = 24.6, 11.1 Hz, 2H, Ph-1-CH_2), 4.00 (d, *J* = 15.7 Hz, 1H, C*H*), 3.91 (s, 3H, OCH_3), 3.45 (d, *J* = 13.9 Hz, 1H, THPB-8-*H*), 3.36 (d, *J* = 9.4 Hz, 1H, THPB-8-*H*), 3.27 (d, *J* = 15.5 Hz, 1H, THPB-13-*H*), 3.02 (d, *J* = 7.0 Hz, 1H, THPB-13-*H*), 2.87 (d, *J* = 11.6 Hz, 1H, THPB-5-*H*), 2.60 (d, *J* = 15.6 Hz, 1H, THPB-5-*H*), 2.47 (d, *J* = 15.2 Hz, 1H, THPB-6-*H*), 2.40 (t, *J* = 10.1 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 163.2, 161.6, 150.6, 146.2, 146.0, 145.2, 141.1, 134.4, 131.3, 130.9, 129.5, 128.0, 127.8, 115.6, 115.5, 108.6, 108.2, 106.1, 101.1, 79.6, 73.2, 59.1, 56.5, 54.1, 51.0, 33.6, 29.4 ppm.

4.1.50. Synthesis of 2-((9-(Benzyloxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (17h)

Compound **17h** was prepared according to the procedure described for compound **5**, starting from compound **16h** (1.2 g, 2.7 mmol), hydrazinecarbothioamide (0.27 g, 3.0 mmol) and glacial acetic acid (0.15 eq). The pure product **17h** (1.04 g, 74.8%) was obtained as brown solid. Mp: 189–190 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.21 (s, 1H, N*H*), 8.44 (s, 1H, N=C*H*), 8.28 (s, 1H, N*H*), 8.10 (s, 1H, N*H*), 7.76 (s, 1H, THPB-11-*H*), 7.50 (d, *J* = 7.2 Hz, 2H, Ph-2,6-*H*), 7.41 (t, *J* = 7.3 Hz, 2H, Ph-3,5-*H*), 7.38–7.35 (m, 1H, Ph-4-*H*), 7.05 (s, 1H, THPB-1-*H*), 6.80 (s, 1H, THPB-4-*H*), 6.02 (d, *J* = 19.9 Hz, 2H, OCH_2O), 5.11–5.05 (m, 2H, Ph-1-CH_2), 4.03 (dd, *J* = 14.2, 7.1 Hz, 1H, C*H*), 3.94 (s, 3H, OCH_3), 3.46–3.43 (m, 4H, THPB-8-*H*, THPB-13-*H*), 3.13 (s, 1H, THPB-5-*H*), 2.89 (s, 1H, THPB-5-*H*), 2.84 (s, 1H, THPB-6-*H*), 2.73 (s, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.3 (*C*=S), 163.2, 161.6, 151.0, 146.2,

146.0, 145.3, 141.1, 137.7, 134.4, 131.3, 130.9, 128.9, 128.7, 128.0, 126.9, 115.6, 108.7, 101.6, 79.7, 74.3, 60.2, 56.8, 56.5, 55.3, 49.1, 33.3, 29.7 ppm.

*4.1.51. Synthesis of 2-((10-Methoxy-9-((4-nitrobenzyl)oxy)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-12-yl)methylene)hydrazine-1-carbothioamide (**17i**)*

Compound **17i** was prepared according to the procedure described for compound **5**, starting from compound **16i** (1.3 g, 2.7 mmol), hydrazinecarbothioamide (0.27 g, 2.9 mmol) and glacial acetic acid (0.15 eq). The pure product **17i** (1.21 g, 79.6%) was obtained as brown solid. Mp: 199–200 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.17 (s, 1H, N*H*), 8.45 (s, 1H, N=C*H*), 8.27 (d, *J* = 8.6 Hz, 2H, Ph-3,5-*H*), 8.21 (s, 1H, N*H*), 8.04 (s, 1H, N*H*), 7.76 (d, *J* = 8.5 Hz, 2H, Ph-2,6-*H*), 7.67 (s, 1H, THPB-11-*H*), 6.94 (s, 1H, THPB-1-*H*), 6.68 (s, 1H, THPB-4-*H*), 5.96 (d, *J* = 18.6 Hz, 2H, OCH_2O), 5.17 (q, *J* = 12.7 Hz, 2H, Ph-1-CH_2), 4.08 (d, *J* = 4.9 Hz, 1H, C*H*), 3.89 (s, 3H, OCH_3), 3.48–3.45 (m, 4H, THPB-8-*H*, THPB-13-*H*), 3.08 (s, 1H, THPB-5-*H*), 2.89 (d, *J* = 7.2 Hz, 1H, THPB-5-*H*), 2.62 (d, *J* = 15.4 Hz, 1H, THPB-6-*H*), 2.47 (s, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.2 (*C*=S), 163.2, 161.6, 150.5, 147.6, 146.3, 145.9, 145.1, 141.0, 129.0, 128.1, 127.5, 123.9, 123.7, 115.6, 108.6, 106.1, 101.1, 79.6, 72.8, 62.5, 60.2, 59.0, 56.6, 55.3, 49.1, 33.2, 29.4 ppm.

*4.1.52. Synthesis of 9-((2,4-Dichlorobenzyl)oxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**18a**)*

Compound **18a** was prepared according to the procedure described for compound **6**, starting from compound **17a** (0.30 g, 0.51 mmol) and 2-chloroacetaldehyde (0.060 g, 0.77 mmol). The pure product **18a** (0.13 g, 40.5%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.59 (s, 1H, N*H*), 8.28 (s, 1H, N=C*H*), 7.73 (d, *J* = 8.1 Hz, 1H, Ph-3-*H*), 7.70 (d, *J* = 1.4 Hz, 1H, Ph-5-*H*), 7.52 (d, *J* = 7.1 Hz, 1H, Ph-6-*H*), 7.41 (s, 1H, THPB-11-*H*), 7.24 (s, 1H, thiazolyl-4-*H*), 7.19 (s, 1H, thiazolyl-5-*H*), 6.84 (s, 2H, THPB-1-*H*, THPB-4-*H*), 6.05 (s, 2H, OCH_2O), 5.18 (s, 2H, Ph-1-CH_2), 4.66 (s, 2H, THPB-8-*H*), 4.43 (s, 1H, THPB-13-*H*,), 4.08 (d, *J* = 15.6 Hz, 1H, C*H*), 3.90 (s, 3H, OCH_3), 3.81 (s, 1H, THPB-13-*H*,), 3.35 (d, *J* = 15.2 Hz, 2H, THPB-5-*H*,), 3.13–3.06 (m, 1H, THPB-6-*H*,), 2.88 (d, *J* = 14.0 Hz, 1H, THPB-6-*H*,) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.4, 150.7, 147.4, 144.8, 140.1, 133.5, 132.7, 130.8, 129.8, 129.4, 129.3, 128.6, 127.1, 126.0, 124.1, 112.0, 109.5, 108.4, 106.3, 101.8, 79.7, 73.4, 58.9, 56.5, 54.2, 51.4, 50.4, 49.1, 33.8, 29.4 ppm; HRMS (ESI) calcd. for $C_{30}H_{26}Cl_2N_4O_4S$ $[M + H]^+$, 609.1130; found, 609.1132.

*4.1.53. Synthesis of 9-((2-Chlorobenzyl)oxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**18b**)*

Compound **18b** was prepared according to the procedure described for compound **6**, starting from compound **17b** (0.30 g, 0.54 mmol) and 2-chloroacetaldehyde (0.064 g, 0.82 mmol). The pure product **18b** (0.13 g, 42.1%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.04 (s, 1H, N*H*), 8.54 (s, 1H, N=C*H*), 7.73 (d, *J* = 6.0 Hz, 1H, Ph-3-*H*), 7.56 (s, 1H, THPB-11-*H*), 7.53 (d, *J* = 6.9

Hz, 1H, Ph-4-*H*), 7.43 (s, 2H, Ph-5,6-*H*), 7.37 (d, *J* = 2.5 Hz, 1H, THPB-1-*H*), 7.19 (s, 1H, thiazolyl-4-*H*), 7.02 (s, 1H, THPB-4-*H*), 6.83 (s, 1H, thiazolyl-5-*H*), 6.05 (d, *J* = 4.8 Hz, 2H, OCH_2O), 5.21 (s, 2H, Ph-1-CH_2), 4.66 (d, *J* = 15.7 Hz, 2H, THPB-8-*H*), 4.45 (d, *J* = 15.3 Hz, 1H, THPB-13-*H*), 4.08 (d, *J* = 14.7 Hz, 1H, C*H*), 3.92 (s, 3H, OCH_3), 3.79 (s, 1H, THPB-5-*H*), 3.42 (d, *J* = 8.2 Hz, 2H, THPB-6-*H*), 3.13 (d, *J* = 13.4 Hz, 1H, THPB-5-*H*), 2.86 (d, *J* = 13.1 Hz, 1H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.4, 150.7, 147.4, 144.8, 135.1, 133.2, 132.7, 130.7, 130.4, 129.8, 129.4, 129.3, 127.9, 126.0, 124.1, 113.8, 109.8, 108.6, 106.3, 101.8, 79.7, 71.3, 62.7, 58.8, 56.7, 54.0, 51.4, 50.4, 33.6, 29.2 ppm; HRMS (ESI) calcd. for $C_{30}H_{27}ClN_4O_4S$ $[M + H]^+$, 575.1520; found, 575.1518.

*4.1.54. Synthesis of 9-((3-Chlorobenzyl)oxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**18c**)*

Compound **18c** was prepared according to the procedure described for compound **6**, starting from compound **17c** (0.063 g, 0.11 mmol) and 2-chloroacetaldehyde (0.013 g, 0.17 mmol). The pure product **18c** (0.026 g, 41.3%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.82 (s, 1H, N*H*), 8.34 (s, 1H, N=C*H*), 7.62 (s, 1H, Ph-2-*H*), 7.52 (d, *J* = 6.3 Hz, 2H, Ph-4-*H*, THPB-11-*H*), 7.45 (t, *J* = 9.2 Hz, 2H, Ph-5,6-*H*), 7.37 (s, 1H, THPB-1-*H*), 7.21 (s, 1H, thiazolyl-4-*H*), 6.99 (s, 1H, THPB-4-*H*), 6.85 (s, 1H, thiazolyl-5-*H*), 6.06 (d, *J* = 5.6 Hz, 2H, OCH_2O), 5.11 (s, 2H, Ph-1-CH_2), 4.67 (d, *J* = 6.6 Hz, 2H, THPB-8-*H*), 4.47 (d, *J* = 14.7 Hz, 1H, THPB-13-*H*), 4.09 (d, *J* = 13.6 Hz, 1H, C*H*), 3.94 (s, 3H, OCH_3), 3.86 (s, 1H, THPB-13-*H*), 3.40 (d, *J* = 8.7 Hz, 2H, THPB-5-*H*), 3.13 (d, *J* = 15.6 Hz, 1H, THPB-6-*H*), 2.92–2.87 (m, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.5, 150.7, 147.4, 144.8, 140.1, 133.5, 132.7, 130.8, 129.8, 129.4, 129.3, 128.6, 127.1, 126.0, 124.1, 112.1, 109.5, 108.6, 106.3, 101.8, 79.7, 73.4, 58.9, 56.5, 54.0, 51.4, 50.4, 49.1, 33.6, 27.2 ppm; HRMS (ESI) calcd. for $C_{30}H_{27}ClN_4O_4S$ $[M + H]^+$, 575.1520; found, 575.1521.

*4.1.55. Synthesis of 9-((4-Chlorobenzyl)oxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**18d**)*

Compound **18d** was prepared according to the procedure described for compound **6**, starting from compound **17d** (0.30 g, 0.54 mmol) and 2-chloroacetaldehyde (0.064 g, 0.82 mmol). The pure product **18d** (0.13 g, 40.5%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.58 (s, 1H, N*H*), 8.38 (s, 1H, N=C*H*), 7.56 (d, *J* = 7.3 Hz, 2H, Ph-3,5-*H*), 7.48 (d, *J* = 7.7 Hz, 3H, Ph-2,6-*H*, THPB-11-*H*), 7.32 (s, 1H, THPB-1-*H*), 7.19 (s, 1H, thiazolyl-4-*H*), 6.93 (s, 1H, THPB-4-*H*), 6.84 (s, 1H, thiazolyl-5-*H*), 6.05 (d, *J* = 4.0 Hz, 2H, OCH_2O), 5.11 (d, *J* = 5.5 Hz, 2H, Ph-1-CH_2), 4.68 (d, *J* = 16.3 Hz, 2H, THPB-8-*H*), 4.43 (s, 1H, THPB-13-*H*), 4.08 (d, *J* = 15.4 Hz, 1H, C*H*), 3.93 (s, 3H, OCH_3), 3.86 (s, 1H, THPB-13-*H*), 3.36 (d, *J* = 17.5 Hz, 2H, THPB-5-*H*), 3.13–3.07 (m, 1H, THPB-6-*H*), 2.90 (d, *J* = 14.8 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.4, 150.7, 147.4, 144.8, 136.6, 135.1, 133.2, 132.7, 130.5, 129.8, 129.4, 128.9, 127.9, 126.0, 109.8, 108.6, 106.3, 101.8, 79.7, 73.4, 71.3, 62.7, 58.8, 56.5, 54.0, 51.5, 50.4, 49.1, 33.7, 29.4 ppm; HRMS (ESI) calcd. for $C_{30}H_{27}ClN_4O_4S$ $[M + H]^+$, 575.1520;

found, 575.1521.

*4.1.56. Synthesis of 9-((3,4-Dichlorobenzyl)oxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**18e**)*

Compound **18e** was prepared according to the procedure described for compound **6**, starting from compound **17e** (0.15 g, 0.26 mmol) and 2-chloroacetaldehyde (0.030 g, 0.38 mmol). The pure product **18e** (0.054 g, 34.1%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.71 (s, 1H, N*H*), 8.34 (s, 1H, N=C*H*), 7.82 (s, 1H, Ph-5-*H*), 7.70 (s, 1H, Ph-2-*H*), 7.53 (d, *J* = 36.3 Hz, 2H, Ph-6-*H*, THPB-11-*H*), 7.37 (d, *J* = 18.8 Hz, 1H, THPB-1-*H*), 7.24 (d, *J* = 27.9 Hz, 1H, thiazolyl-4-*H*), 6.97 (s, 1H, THPB-4-*H*), 6.86 (s, 1H, thiazolyl-5-*H*), 6.07 (s, 2H, OCH_2O), 5.11 (s, 2H, Ph-1-CH_2), 4.74 (d, *J* = 13.6 Hz, 1H, C*H*), 4.68 (s, 1H, THPB-8-*H*), 4.47 (s, 2H, THPB-13-*H*), 3.93 (s, 4H, THPB-8-*H*, OCH_3), 3.41–3.33 (m, 2H, THPB-5-*H*), 3.13 (s, 1H, THPB-6-*H*), 2.90 (s, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.5, 150.7, 147.4, 144.8, 140.1, 138.8, 133.5, 132.7, 131.5, 131.2, 130.4, 129.8, 129.4, 129.3, 128.7, 127.1, 126.0, 124.1, 112.1, 109.5, 108.6, 101.8, 79.7, 72.7, 56.5, 54.0, 51.4, 49.1, 33.8, 27.9 ppm; HRMS (ESI) calcd. for $C_{30}H_{26}Cl_2N_4O_4S$ $[M + H]^+$, 609.1130; found, 609.1133.

*4.1.57. Synthesis of 9-((2-Fluorobenzyl)oxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**18f**)*

Compound **18f** was prepared according to the procedure described for compound **6**, starting from compound **17f** (0.15 g, 0.28 mmol) and 2-chloroacetaldehyde (0.033 g, 0.42 mmol). The pure product **18f** (0.057 g, 36.2%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.01 (s, 1H, N*H*), 8.56 (s, 1H, N=C*H*), 7.66 (t, *J* = 6.8 Hz, 1H, Ph-3-*H*), 7.57 (s, 1H, Ph-4-*H*), 7.45 (d, *J* = 6.2 Hz, 1H, THPB-11-*H*), 7.40 (d, *J* = 2.8 Hz, 1H, Ph-6-*H*), 7.27 (dd, *J* = 10.7, 7.4 Hz, 2H, THPB-1-*H*, Ph-5-*H*), 7.19 (s, 1H, thiazolyl-4-*H*), 7.04 (s, 1H, THPB-4-*H*), 6.83 (s, 1H, thiazolyl-5-*H*), 6.05 (d, *J* = 6.7 Hz, 2H, OCH_2O), 5.19 (s, 2H, Ph-1-CH_2), 4.67 (d, *J* = 8.9 Hz, 1H, THPB-8-*H*), 4.61 (d, *J* = 15.7 Hz, 1H, THPB-8-*H*), 4.41 (d, *J* = 15.2 Hz, 1H, THPB-13-*H*), 4.08 (d, *J* = 14.3 Hz, 1H, C*H*), 3.93 (s, 3H, OCH_3), 3.78 (s, 1H, THPB-13-*H*), 3.42 (d, *J* = 10.3 Hz, 2H, THPB-5-*H*), 3.12 (d, *J* = 13.0 Hz, 1H, THPB-6-*H*), 2.87 (d, *J* = 12.5 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.6, 150.8, 147.4, 144.9, 140.1, 133.5, 132.7, 131.5, 129.8, 129.4, 129.3, 128.6, 127.1, 126.0, 125.5, 124.5, 115.9, 112.1, 109.8, 108.6, 106.3, 101.8, 79.7, 73.4, 68.1, 56.6, 56.4, 49.1, 31.4, 26.8 ppm; HRMS (ESI) calcd. for $C_{30}H_{27}FN_4O_4S$ $[M + H]^+$, 559.1815; found, 559.1813.

*4.1.58. Synthesis of 9-((4-Fluorobenzyl)oxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**18g**)*

Compound **18g** was prepared according to the procedure described for compound **6**, starting from compound **17g** (0.10 g, 0.19 mmol) and 2-chloroacetaldehyde (0.022 g, 0.28 mmol). The pure product **18g** (0.038 g, 35.7%) was obtained as brown solid. Mp: > 250 ^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.68 (d, *J* = 49.5 Hz, 1H, N*H*), 8.44 (s, 1H, N=C*H*), 7.58 (s, 2H, Ph-3,5-*H*), 7.49 (s, 1H, THPB-11-*H*), 7.35 (s, 1H,

THPB-1-*H*), 7.22 (d, *J* = 24.9 Hz, 3H, Ph-2,6-*H*, thiazolyl-4-*H*), 6.97 (s, 1H, THPB-4-*H*), 6.84 (s, 1H, thiazolyl-5-*H*), 6.05 (s, 2H, OCH_2O), 5.09 (s, 2H, Ph-1-CH_2), 4.65 (s, 2H, THPB-8-*H*), 4.42 (s, 1H, THPB-13-*H*), 4.06 (s, 1H, C*H*), 3.94 (s, 3H, OCH_3), 3.39 (s, 3H, THPB-13-*H*, THPB-5-*H*), 3.11 (s, 1H, THPB-5-*H*), 2.90 (s, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.3, 161.7, 150.8, 147.4, 144.8, 140.1, 138.8, 133.8, 132.7, 131.5, 131.0, 130.4, 129.8, 129.4, 129.3, 128.7, 127.1, 126.0, 125.5, 115.8, 115.7, 112.1, 109.7, 108.6, 106.3, 101.8, 79.7, 73.6, 58.9, 56.6, 51.5, 50.3, 33.2, 28.9 ppm; HRMS (ESI) calcd. for $C_{30}H_{27}FN_4O_4S$ $[M + H]^+$, 559.1815; found, 559.1814.

*4.1.59. Synthesis of 9-(Benzyloxy)-10-methoxy-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**18h**)*

Compound **18h** was prepared according to the procedure described for compound **6**, starting from compound **17h** (0.12 g, 0.23 mmol) and 2-chloroacetaldehyde (0.027 g, 0.35 mmol). The pure product **18h** (0.041 g, 33.3%) was obtained as brown solid. Mp: > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.90 (s, 1H, N*H*), 8.52 (s, 1H, N=C*H*), 7.53 (d, *J* = 6.7 Hz, 3H, Ph-2,6-*H*, THPB-11-*H*), 7.43 (t, *J* = 6.8 Hz, 2H, Ph-3,5-*H*), 7.40–7.35 (m, 2H, Ph-4-*H*, THPB-1-*H*), 7.20 (s, 1H, thiazolyl-4-*H*), 7.02 (s, 1H, THPB-4-*H*), 6.84 (s, 1H, thiazolyl-5-*H*), 6.05 (d, *J* = 5.9 Hz, 2H, OCH_2O), 5.11 (s, 2H, Ph-1-CH_2), 4.66 (d, *J* = 14.8 Hz, 2H, THPB-8-*H*), 4.43 (d, *J* = 14.9 Hz, 1H, THPB-13-*H*), 4.08 (d, *J* = 14.2 Hz, 1H, C*H*), 3.94 (s, 3H, OCH_3), 3.84 (d, *J* = 5.7 Hz, 1H, THPB-13-*H*), 3.48 (t, *J* = 4.7 Hz, 1H, THPB-5-*H*), 3.35–3.32 (m, 1H, THPB-5-*H*), 3.12 (d, *J* = 13.4 Hz, 1H, THPB-6-*H*), 2.88 (d, *J* = 13.0 Hz, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.4, 150.7, 147.4, 144.9, 140.1, 137.6, 133.5, 132.7, 131.5, 129.8, 128.9, 128.6, 127.1, 125.9, 125.5, 124.1, 115.9, 112.1, 109.6, 108.6, 106.3, 101.8, 79.7, 74.4, 60.5, 58.5, 56.5, 49.1, 33.4, 28.8 ppm; HRMS (ESI) calcd. for $C_{30}H_{28}N_4O_4S$ $[M + H]^+$, 541.1910; found, 541.1913.

*4.1.60. Synthesis of 10-Methoxy-9-((4-nitrobenzyl)oxy)-12-((2-(thiazol-2-yl)hydrazono)methyl)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**18i**)*

Compound **18i** was prepared according to the procedure described for compound **6**, starting from compound **17i** (0.13 g, 0.23 mmol) and 2-chloroacetaldehyde (0.027 g, 0.35 mmol). The pure product **18i** (0.047 g, 35.0%) was obtained as brown solid. Mp: > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.64 (s, 1H, N*H*), 8.38 (s, 1H, N=C*H*), 8.29 (s, 2H, Ph-3,5-*H*), 7.81 (s, 2H, Ph-2,6-*H*), 7.47 (s, 1H, THPB-11-*H*), 7.32 (s, 1H, THPB-1-*H*), 7.20 (s, 1H, thiazolyl-4-*H*), 6.93 (s, 1H, THPB-11-*H*), 6.85 (s, 1H, thiazolyl-5-*H*), 6.06 (s, 2H, OCH_2O), 5.27 (d, *J* = 13.9 Hz, 2H, Ph-1-CH_2), 4.69 (d, *J* = 33.8 Hz, 3H, THPB-8-*H*, THPB-13-*H*), 4.44 (s, 1H, C*H*), 3.92 (s, 3H, OCH_3), 3.44–3.43 (m, 1H, THPB-13-*H*), 3.34 (s, 1H, THPB-5-*H*), 3.24 (s, 1H, THPB-5-*H*), 3.13–3.06 (m, 1H, THPB-6-*H*), 2.89 (s, 1H, THPB-6-*H*) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.5, 150.7, 147.4, 144.8, 140.1, 138.8, 133.5, 132.7, 131.5, 131.2, 130.4, 129.8, 129.2, 128.7, 127.1, 126.0, 124.0, 112.1, 109.5, 108.6, 101.8, 79.7, 74.4, 73.0, 60.5, 58.5, 56.5, 49.1, 33.8, 27.9 ppm; HRMS (ESI) calcd. for $C_{30}H_{27}N_5O_6S$ $[M + H]^+$, 586.1760; found, 586.1762.

4.2. Isolating genomic DNA from drug-resistant A. baumanii strain

An overnight *A. baumanii* culture (1 mL) was added into a microcentrifuge tube (1.5 mL), and the resulting system was centrifuged at 11500 × *g* for 1 min to pellet the cells. The supernatant was removed, the residue was thoroughly suspended in buffer GA (200 μL), then proteinase K (20 μL) and buffer GB (220 μL) were added and the resulting mixture was incubated for 10 min at 70 □, which aimed to weaken the cell wall so that efficient cell lysis could take place. After anhydrous ethanol (220 μL) was added into the mixture, some flocculate would appear and the system was vibrated for 15 s. The obtained flocculate was transferred into a spin column CB3 which was in a collection tube (2 mL) and centrifuged at 13400 × *g* for 30 s. The supernatant was poured out and the residue was thoroughly suspended in buffer GD (pretreatment with anhydrous ethanol, 500 μL), then the mixture was centrifuged at 13400 × *g* for 30 s, and the supernatant was removed again. The resulting residue was resuspended in buffer PW (pretreatment with anhydrous ethanol, 600 μL) and centrifuged at 13400 × *g* for 30 s, then the supernatant was pour out. The resulting DNA in the spin column CB3 was left at room temperature for 10–15 min to completely remove the residual anhydrous ethanol. Buffer TE (100 μL) was added into the above spin column CB3 and was left at room temperature for 2–5 min, then was centrifuged at 13400 × *g* for 2 min. At last, the pure DNA solution was collected into a microcentrifuge tube and stored at -20 □.

4.3. Bacterial membrane permeabilization assay

The overnight grown culture of drug-resistant *A. baumanii* cells (~1 × 10^4 CFU/mL) was harvested by centrifugation (4000 rpm, 5 min), washed, and resuspended in 5 mM glucose and 5 mM HEPES buffer (pH = 7.4) in 1:1 ratio. A volume of 10 μL of compound **9c** (12 × MIC) was added into a stock solution (2 mL) containing drug-resistant *A. baumanii* cells and PI (10 μM), and then incubated for 1 h at 30 ℃ with constant shaking (200 rpm). Fluorescence was monitored at excitation wavelength of 535 nm (slit width of 10 nm) and emission wavelength of 617 nm (slit width of 5 nm). As a measure of inner membrane permeabilization, the uptake of PI was monitored by the increase in fluorescence for 2 h. Fluorescence spectra were recorded on F-7000 Spectrofluorimeter (Hitachi, Tokyo, Japan), equipped with 1.0 cm quartz cells (T = 298 K).

Acknowledgments

This work was partially supported by National Natural Science Foundation of China (NSFC, nos. 21672173 and 21372186), Research Fund for International Young Scientists from International (Regional) Cooperation and Exchange Program (no. 81650110529), Chongqing Special Foundation for Postdoctoral Research Proposal (nos. Xm2016039, Xm2017184, Xm2017185).

References

[1] J.Y. Zhang, Y.P. Chen, K.P. Miller, M.S. Ganewatta, M. Bam, Y. Yan, M. Nagarkatti, A.W. Decho, C.B. Tang, Antimicrobial metallopolymers and their bioconjugates with conventional antibiotics against multidrug-resistant bacteria, J. Am. Chem. Soc. 136 (2014) 4873–4876.

[2] I. Levin-Reisman, I. Ronin, O. Gefen, I. Braniss, N. Shoresh, N.Q. Balaban, Antibiotic tolerance facilitates the evolution of resistance, Science 355 (2017) 826–830.

[3] Y.Y. Liu, Y. Wang, T.R. Walsh, L.X. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong, X. Huang, L.F. Yu, D. Gu, H. Ren, X. Chen, L. Lv, D. He, H. Zhou, Z. Liang, J.H. Liu, J. Shen, Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study, Lancet Infect. Dis. 16 (2016) 161−168.

[4] 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.

[5] S.E. Pidgeon, M.M. Pires, Vancomycin-dependent response in live drug-resistant bacteria *via* metabolic labeling, Angew. Chem. Int. Edit. 129 (2017) 8965–8969.

[6] S. Siricilla, K. Mitachi, J.S. Yang, S. Eslamimehr, M.R. Lemieux, B. Meibohm, Y.D. Ji, M. Kurosu, A new combination of a pleuromutilin derivative and doxycycline for treatment of multidrug-resistant *Acinetobacter baumannii*, J. Med. Chem. 60 (2017) 2869–2878.

[7] S. Han, N. Caspers, R.P. Zaniewski, B.M. Lacey, A.P. Tomaras, X. Feng, K.F. Geoghegan, V. Shanmugasundaram, Distinctive attributes of β-Lactam target proteins in *Acinetobacter baumannii* relevant to development of new antibiotics, J. Am. Chem. Soc. 133 (2011) 20536–20545.

[8] W.M. Huggins, B.M. Minrovic, B.W. Corey, A.C. Jacobs, R.J. Melander, R.D. Sommer, D.V. Zurawski, C. Melander, 1,2,4-Triazolidine-3-thiones as narrow spectrum antibiotics against multidrug-resistant *Acinetobacter baumannii*, ACS Med. Chem. Lett. 8 (2017) 27−31.

[9] T.F. Durand-Réville, S. Guler, J. Comita-Prevoir, B. Chen, N. Bifulco, H. Huynh, S. Lahiri, A.B. Shapiro, S.M. McLeod, N.M. Carter, S.H. Moussa, C. Velez-Vega, N.B. Olivier, R. McLaughlin, N. Gao, J. Thresher, T. Palmer, B. Andrews, R.A. Giacobbe, J.V. Newman, D.E. Ehmann, B. de Jonge, J. O'Donnell, J.P. Mueller, R.A. Tommasi, A.A. Miller, ETX2514 is a broad-spectrum β-lactamase inhibitor for the treatment of drug-resistant Gram-negative bacteria including *Acinetobacter baumannii*, Nat. Microbiol. 2 (2017) 17104.

[10] L.N. Silva, K.R. Zimmer, A.J. Macedo, D.S. Trentin, Plant Natural products targeting bacterial virulence factors, Chem. Rev. 116 (2016) 9162–9236.

[11] L. Huang, Z.H. Luo, F. He, A.D. Shi, F.F. Qin, X.S. Li, Berberine derivatives, with substituted amino groups linked at the 9-position, as inhibitors of acetylcholinesterase/butyrylcholinesterase, Bioorg. Med. Chem. Lett. 20 (2010) 6649–6652.

[12] W.J. Shan, L. Huang, Q. Zhou, F.C. Meng, X.S. Li, Synthesis, biological evaluation of 9-N-substituted berberine derivatives as multi-functional agents of antioxidant, inhibitors of acetylcholinesterase, butyrylcholinesterase and amyloid-β aggregation, Eur. J. Med. Chem. 46 (2011) 5885–5893.

[13] M. Tillhon, L.M. Guaman Ortiz, P. Lombardi, A.I. Scovassi, Berberine: New perspectives for old remedies, Biochem. Pharmacol. 84 (2012) 1260−1267.

[14] S.L. Zhang, J.J. Chang, G.L.V. Damu, B. Fang, X.D. Zhou, R.X. Geng, C.H. Zhou, Novel berberine triazoles: Synthesis, antimicrobial evaluation and competitive interactions with metal ions to human serum albumin, Bioorg. Med. Chem. Lett. 23 (2013) 1008–1012.

[15] S.Q. Wen, J. Ponmani, 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. Kumar, Ekavali, K. Chopra, M. Mukherjee, R. Pottabathini, D.K. Dhull, Current knowledge and pharmacological profile of berberine: An update, Eur. J. Pharmacol. 761 (2015) 288–297.

[17] M.M. Kheir, Y.G. Wang, L. Hua, J. Hu, L.L. Li, F. Lei, L.J. Du, Acute toxicity of berberine and its correlation with the blood concentration in mice, Food Chem. Toxicol. 48 (2010) 1105–1110.

[18] Y.T. Liu, H.P. Hao, H.G. Xie, L. Lai, Q. Wang, C.X. Liu, G.J. Wang, Extensive intestinal first pass elimination and predominant hepatic distribution of berberine explain its low plasma levels in rats, Drug Metab. Dispos. 38 (2010) 1779–1784.

[19] M. Ishikawa, Y. Hashimoto, Improvement in aqueous solubility in small molecule drug discovery programs by disruption of molecular planarity and symmetry, J. Med. Chem. 54 (2011) 1539–1554.

[20] T. Takeuchi, S. Oishi, M. Kaneda, H. Ohno, S. Nakamura, I. Nakanishi, M. Yamane, J. Sawada, A. Asai, N. Fujii, Kinesin spindle protein inhibitors with diaryl amine scaffolds: Crystal packing analysis for improved aqueous solubility, ACS Med. Chem. Lett. 5 (2014) 566–571.

[21] L. Guo, J. Li, H. Lin, Y. Zhou, Y. Chen, F. Zhao, H.F. Sun, D. Zhang, H.L. Li, B.K. Shoichet, L. Shan, W.D. Zhang, X. Xie, H.L. Jiang, H. Liu, Design, synthesis, and biological evaluation of novel tetrahydroprotoberberine derivatives (THPBs) as selective α1A-adrenoceptor antagonists, J. Med. Chem. 59 (2016) 9489–9502.

[22] 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.

[23] C.H. Zhou, Y. Wang, Recent researches in triazole compounds as medicinal drugs, Curr. Med. Chem. 19 (2012) 239−280.

[24] X.L. Wang, K. Wan, C.H. Zhou, Synthesis of novel sulfanilamide-derived 1,2,3-triazoles and their evaluation for antibacterial and antifungal activities, Eur. J. Med. Chem. 45 (2010) 4631–4639.

[25] L.L. Dai, S.F. Cui, G.L.V. Damu, C.H. Zhou, Recent advances in the synthesis and application of tetrazoles, Chin. J. Org. Chem. 33 (2013) 224–244 (in Chinese).

[26] 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.

[27] G.A. Hampannavar, R. Karpoormath, M.B. Palkar, M.S. Shaikh, B. Chandrasekaran, Dehydrozingerone inspired styryl hydrazine thiazole hybrids as promising class of antimycobacterial agents, ACS Med. Chem. Lett. 7 (2016) 686–691.

[28] H. Mohammad, M. Cushman, M.N. Seleem, Antibacterial evaluation of synthetic thiazole compounds in vitro and in vivo in a Methicillin-resistant *Staphylococcus aureus* (MRSA) skin infection mouse model, PLoS One 10 (2015) e0142321.

[29] H.Z. Zhang, L.L. Gan, H. Wang, C.H. Zhou, New progress in azole compounds as antimicrobial agents, Mini-Rev. Med. Chem. 17 (2017) 122–166.

[30] X.M. Peng, L.P. Peng, S. Li, S.R. Avula, K.V. Kumar, S.L. Zhang, K.Y. Tam, C.H. Zhou, Quinazolinone azolyl ethanols: Potential lead antimicrobial agents with dual action modes targeting MRSA DNA, Future Med. Chem. 8 (2016) 1927–1940.

[31] Y. Ren, L. Zhang, C.H. Zhou, R.X. Geng, Recent development of benzotriazole-based medicinal drugs, Med. Chem. 4 (2014) 640–662.

[32] Md.K. Islam, S. Kim, H.K. Kim, S. Park, G.H. Lee, H.J. Kang, J.C. Jung, J.S. Park, T.J. Kim, Y.M. Chang, Manganese complex of ethylenediaminetetraacetic acid (EDTA)−benzothiazole aniline (BTA) conjugate as a potential liver-targeting MRI contrast agent, J. Med. Chem. 60 (2017) 2993−3001.

[33] L. Huang, T. Su, W.J. Shan, Z.H. Luo, Y. Sun, F. He, X.S. Li, Inhibition of cholinesterase activity and amyloid aggregation by berberine-phenyl-benzoheterocyclic and tacrine-phenylbenzoheterocyclic hybrids, Bioorg. Med. Chem. 20 (2012) 3038–3048.

[34] S.F. Cui, Y. Wang, J.S. Lv, G.L.V. Damu, C.H. Zhou, Recent advances in application of thiazole compounds, Sci. China Ser. B-Chem. 42 (2012) 1105−1131.

[35] E.S. Childress, Y. Kharel, A.M. Brown, D.R. Bevan, K.R. Lynch, W.L. Santos, Transforming sphingosine kinase 1 inhibitors into dual and sphingosine kinase 2 selective inhibitors: Design, synthesis, and *in vivo* activity, J. Med. Chem. 60 (2017) 3933−3957.

[36] L. Tan, Y. Tao, T. Wang, F. Zou, S. Zhang, Q. Kou, A. Niu, Q. Chen, W. Chu, X. Chen, H. Wang, Y. Yang, Discovery of novel pyridone-conjugated monosulfactams as potent and broad-spectrum antibiotics for multidrug-resistant Gram-negative infections, J. Med. Chem. 60 (2017) 2669−2684.

[37] D. Addla, S.Q. Wen, W.W. Gao, S.K. Maddili, L. Zhang, C.H. Zhou, Design, synthesis, and biological evaluation of novel carbazole aminothiazoles as potential DNA targeting antimicrobial agents, Med. Chem. Commun. 7 (2016) 1988–1994.

[38] A.A. Geronikaki, E.P. Pitta, K.S. Liaras, Thiazoles and thiazolidinones as antioxidants, Curr. Med. Chem. 20 (2013) 4460–4480.

[39] 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.

[40] Y. Cheng, S.R. Avula, W.W. Gao, D. Addla, V.K.R. Tangadanchu; L. Zhang, J.M. Lin, C.H. Zhou, 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.

[41] W.W. Gao, C.H. Zhou, Antimicrobial 2-aminothiazolyl quinolones: What is their potential in the clinic? Future Med. Chem. 9 (2017) 1461–1464.

[42] K.V. Sashidhara, K.B. Rao, P. Kushwaha, R.K. Modukuri, P. Singh, I. Soni, P.K. Shukla, S. Chopra, M. Pasupuleti, Novel chalcone-thiazole hybrids as potent inhibitors of drug resistant *Staphylococcus aureus*, ACS Med. Chem. Lett. 6 (2015) 809–813.

[43] E.B. Lindgren, M.A. de Brito, T.R.A. Vasconcelos, M.O. de Moraes, R.C. Montenegro, J.D. Yoneda, K.Z. Leal, Synthesis and anticancer activity of (*E*)-2-benzothiazole hydrazones, Eur. J. Med. Chem. 86 (2014) 12–16.

[44] A. Rouf, C. Tanyeli, Bioactive thiazole and benzothiazole derivatives, Eur. J. Med. Chem. 97 (2015) 911–927.

[45] L. Zhang, J.J. Chang, S.L. Zhang, G.L.V. Damu, R.X. Geng, C.H. Zhou, 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.

[46] J. Ponmani, 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.

[47] J. Ponmani, H.B. Liu, L. Gopala, Y. Cheng, X.M. Peng, R.X. Geng, C.H. Zhou, Novel benzimidazolyl tetrahydroprotoberberines: Design, synthesis, antimicrobial evaluation and multi-targeting exploration, Bioorg. Med. Chem. Lett. 27 (2017) 1737–1743.

[48] J.R. Duan, H.B. Liu, J. Ponmani, L. Gopala, S. Li, R.X. Geng, C.H. Zhou, Design, synthesis and biological evaluation of novel Schiff base-bridged tetrahydroprotoberberine triazoles as a new type of potential antimicrobial agents, Med. Chem. Commun. 8 (2017) 907–916.

[49] 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.

[50] Y. Zhou, Z.X. Li, S.Q. Zang, Y.Y. Zhu, H.Y. Zhang, H.W. Hou, T.C.W. Mak, A novel sensitive turn-on fluorescent Zn^{2+} chemosensor based on an easy to prepare C_3-symmetric Schiff-base derivative in 100% aqueous solution, Org. Lett. 14 (2012) 1214–1217.

[51] Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 7th ed.; National Committee for Clinical Laboratory Standards: Wayne, PA, 2006; M7-A7.

[52] X. Cao, Z. Sun, Y. Cao, R. Wang, T. Cai, W. Chu, W. Hu, Y. Yang, Design, synthesis, and structure-activity relationship studies of novel fused heterocycles-linked triazoles with good activity and water solubility, J. Med. Chem. 57 (2014) 3687–3706.

[53] W.W. Gao, S. Rasheed, V.K.R. Tangadanchu, Y. Sun, X.M. Peng, Y. Cheng, F.X. Zhang, J.M. Lin, C.H. Zhou, Design, synthesis and biological evaluation of amino organophosphorus imidazoles as a new type of potential antimicrobial agents, Sci. China Chem. 60 (2017) 769–785.

[54] J. Fujita, Y. Maeda, E. Mizohata, T. Inoue, M. Kaul, A.K. Parhi, E.J. LaVoie, D.S. Pilch, H. Matsumura, Structural flexibility of an inhibitor overcomes ddrug resistance mutations in *Staphylococcus aureus* FtsZ, ACS Chem. Biol. 12 (2017) 1947−1955.

[55] C. Ghosh, G.B. Manjunath, P. Akkapeddi, V. Yarlagadda, J. Hoque, D.S.S.M. Uppu, M.M. Konai, J. Haldar, Small molecular antibacterial peptoid mimics: The simpler the better, J. Med. Chem. 57 (2014) 1428–1436.

[56] L. Zhang, D. Addla, J. Ponmani, A. Wang, D. Xie, Y.N. Wang, S.L. Zhang, R.X. Geng, G.X. Cai, S. Li, C.H. Zhou, Discovery of membrane active benzimidazole quinolones-based topoisomerase inhibitors as potential DNA-binding antimicrobial agents, Eur. J. Med. Chem. 111 (2016) 160–182.

[57] M.M. Konai, C. Ghosh, V. Yarlagadda, S. Samaddar, J. Haldar, Membrane-active phenylalanine-conjugated lipophilic norspermidine derivatives with selective antibacterial activity, J. Med. Chem. 57 (2014) 9409–9423.

[58] Y.Y. Chen, L. Gopala, R.R.Y. Bheemanaboina, H.B. Liu, Y. Cheng, R.X. Geng, C.H. Zhou, Novel naphthalimide aminothiazoles as potential multitargeting antimicrobial agents, ACS Med. Chem. Lett. 8 (2017) 1331–1335.

[59] X.F. Fang, D. Li, V.K.R. Tangadanchu, L. Gopala, W.W. Gao, C.H. Zhou, Novel potentially antifungal hybrids of 5-flucytosine and fluconazole: Design, synthesis and bioactive evaluation, Bioorg. Med. Chem. Lett. 27 (2017) 4964–4969.

[60] M. Gjorgjieva, T. Tomasic, M. Barancokova, S. Katsamakas, J. Ilas, P. Tammela, L.P. Masic, D. Kikelj, Discovery of benzothiazole scaffold-based DNA Gyrase B inhibitors, J. Med. Chem. 59 (2016) 8941–8954.

[61] B.T. Yin, C.Y. Yan, X.M. Peng, S.L. Zhang, S. Rasheed, R.X. Geng, C.H. Zhou, 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.

[62] 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.

[63] X.J. Fang, J. Ponmani, S.R. Avula, Q. Zhou, C.H. Zhou, Design, synthesis and biological evaluation of 5-fluorouracil-derived benzimidazoles as novel type of potential antimicrobial agents, Bioorg. Med. Chem. Lett. 26 (2016) 2584–2588.

[64] X.L. Li, Y.J. Hu, H. Wang, B.Q. Yu, H.L. Yue, Molecular spectroscopy evidence of berberine binding to DNA: Comparative binding and thermodynamic profile of intercalation, Biomacromolecules13 (2012) 873–880.

[65] L.L. Dai, H.Z. Zhang, S. Nagarajan, S. Rasheed, C.H. Zhou, Synthesis of tetrazole compounds as a novel type of potential antimicrobial agents and their synergistic effects with clinical drugs and interactions with calf thymus DNA, Med. Chem. Commun. 6 (2015) 147–154.

[66] Z.Z. Li, L. Gopala, V.K.R. Tangadanchu, W.W. Gao, C.H. Zhou, Discovery of novel nitroimidazole enols as *Pseudomonas aeruginosa* DNA cleavage agents, Bioorg. Med. Chem. 27 (2017) 4964–4969.

[67] W. Sun, R.A. Weingarten, M. Xu, N. Southall, S. Dai, P. Shinn, P.E. Sanderson, P.R. Williamson, K.M. Frank, W. Zheng, Rapid antimicrobial susceptibility test for identification of new therapeutics and drug combinations against multidrug-resistant bacteria, Emerg. Microbes Infec. 5 (2016) e116.

[68] 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.

[69] C.H. Zhou, J. Ponmani, X.M. Peng, Berberine-benzimidazole compounds or their pharmaceutically salts with preparation method and application thereof, China Patent CN 105130981 A 20151209.

Lists of table and scheme captions

Table 1 *In vitro* MIC (μmol/mL) data for hydroxyl aminothiazolyl berberine derivative **6**, alkyl ones **9a-g**, allyl one **12**, propargyl one **15a**, cyano one **15b** and substituted phenyl ones **18a-i** toward standard Gram-positive and Gram-negative bacterial strains.

Table 2 Drug combination study of hexyl aminothiazolyl berberine derivative **9c** with antibacterial drug norfloxacin.

Figure 1 Design of novel aminothiazolyl berberine derivatives.

Figure 2 *In vitro* antibacterial data as MIC (μmol/mL) for hydroxyl aminothiazolyl berberine derivative **6**, alkyl ones **9a-g**, allyl one **12**, propargyl one **15a**, cyano one **15b** and substituted phenyl ones **18a-i** against (a) drug-resistant Gram-positive MRSA (Methicillin-resistant *Staphylococcus. aureus* (N315)), *E. faecalis*; (b) drug-resistant Gram-negative *E. coli*, *P. aeruginosa*, *K. pneumonia*, *A. baumanii*. A = Berberine; B = Chloromycin; C = Norfloxacin. The standard deviation from three independent experiments is plotted.

Figure 3 Aqueous solubility of hydroxyl aminothiazolyl berberine derivative **6**, alkyl ones **9a-g**, allyl one **12**, propargyl one **15a**, cyano one **15b** and benzyl ones **18a-i**. A = Berberine. The standard deviation from three independent experiments is plotted.

Figure 4 Cell viability of human hepatocyte LO2 cells incubated with hexyl aminothiazolyl berberine derivative **9c** and 2,4-dichlorobenzyl one **18a** at different concentrations for 48 h. The standard deviation from three independent experiments is plotted.

Figure 5 Bactericidal kinetics of hexyl aminothiazolyl berberine derivative **9c** and 2,4-dichlorobenzyl one **18a** at 8 nmol/mL against drug-resistant *A. baumanii*. The standard deviation from three independent experiments is plotted.

Figure 6 Development of *A. baumanii* resistance to hexyl aminothiazolyl berberine derivative **9c** and 2,4-dichlorobenzyl one **18a**. The standard deviation from three independent experiments is plotted.

Figure 7 Bacterial membrane permeabilization of compounds (a) **9c** and (b) **18a** at concentrations of 0.024 μmol/mL against *A. baumanii*.

Figure 8 Three-dimensional conformations of aminothiazolyl berberine derivatives docked in DNA gyrase B (PDB code: 4DUH): **9c** (left) and **18a** (right).

Figure 9 (a) Absorption spectra of *A. baumanii* DNA with different concentrations of compound **9c** (pH = 7.4, T = 298 K). c(DNA) = 8.12×10^{-4} mol/L, c(compound **9c**) = 0–2.50×10^{-5} mol/L. Inset: comparison of absorption between the **9c**-DNA complex and that of the sum values of free DNA and free compound **9c** at 260 nm; (b) Absorption spectra of the competitive interaction between NR and **9c** with *A. baumanii* DNA. c(NR) = 2×10^{-5} mol/L, c(DNA) = 4.17×10^{-5} mol/L, and c(molecule **9c**) = 0–2.5×10^{-5} mol/L for curves a–i respectively. Inset: The wavelength range of 425–525 nm absorption spectra of competitive interaction between compound **9c** and NR with DNA.

Figure 10 (a) Gel electrophoresis assay of *A. baumanii* DNA; (b) Agarose gel electrophoresis patterns for the cleavage of drug-resistant *A. baumanii* DNA (1.31×10^{-6} mol/L) by compound **9c** in buffer (50 mM

Tris-HCl/50 mM NaCl, pH = 7.4) at 37 °C after 6 h of incubation. Lane 1, DNA control; Lane 2, DNA + buffer; Lane 3, DNA + buffer + compound **9c** (50 μM).

Scheme 1 Synthetic route of hydroxyl type of aminothiazolyl berberine derivative **6**.

Scheme 2 Synthetic route of aliphatic type of aminothiazolyl berberine derivatives **9a-g**, allyl one **12**, propargyl one **15a** and cyano one **15b**.

Scheme 3 Synthetic route of phenyl type of aminothiazolyl berberine derivatives **18a-i**.

Table 1. *In vitro* MIC (μmol/mL) data for hydroxyl aminothiazolyl berberine derivative **6**, alkyl ones **9a-g**, allyl one **12**, propargyl one **15a**, cyano one **15b** and substituted phenyl ones **18a-i** toward standard Gram-positive and Gram-negative bacterial strains. [a]

Compds	Gram-positive			Gram-negative	
	S. aureus ATCC 25923	*B. subtilis* ATCC 21216	*M. luteus* ATCC 4698	*P. aeruginosa* ATCC 27853	*E. coli* DH52
6	0.14	0.14	0.07	0.14	0.28
9a	0.07	0.53	0.53	0.07	0.07
9b	0.06	0.25	0.25	0.25	0.06
9c	0.03	0.12	0.06	0.06	0.06
9d	0.23	0.45	0.45	0.23	0.45
9e	0.11	0.22	0.22	0.22	0.22
9f	0.21	0.03	0.83	0.41	0.21
9g	0.13	0.26	0.26	0.13	0.13
12	0.03	0.52	0.13	0.26	0.13
15a	0.13	0.52	0.52	0.13	0.13
15b	0.13	0.26	0.26	0.26	0.26
18a	0.11	0.21	0.11	0.21	0.84
18b	0.11	0.22	0.89	0.45	0.45
18c	0.22	0.11	0.22	0.22	0.89
18d	0.45	0.45	0.22	0.22	0.11
18e	0.11	0.21	0.42	0.11	0.11
18f	0.06	0.46	0.23	0.46	0.23
18g	0.11	0.23	0.06	0.23	0.46
18h	0.24	0.47	0.24	0.24	0.95
18i	0.11	0.11	0.22	0.05	0.44
Berberine	0.34	0.69	0.69	0.69	0.69
Chloromycin	0.05	0.10	0.02	0.10	0.10
Norfloxacin	0.002	0.003	0.006	0.05	0.05

[a] *S. aureus*, *Staphylococcus aureus* (ATCC 25923); *B. subtilis*, *Bacillus subtilis* (ATCC 21216); *M. luteus*, *Micrococcus luteus* (ATCC 4698); *E. coli*, *Escherichia coli* DH52; *P. aeruginosa*, *Pseudomonas aeruginosa* (ATCC 27853).

Table 2 Drug combination study of hexyl aminothiazolyl berberine derivative **9c** with antibacterial drug norfloxacin.

Bacteria	Compound **9c**		
	MIC/μmol·mL^{-1}	Effect	FIC index
MRSA	0.007	synergism	0.50
E. faecalis	0.015	additivism	0.56
E. coli	0.030	synergism	0.31
P. aeruginosa	0.025	synergism	0.45
K. pneumonia	0.030	synergism	0.38
A. baumanii	0.0005	synergism	0.31

Fig. 1. Design of novel aminothiazolyl berberine derivatives.

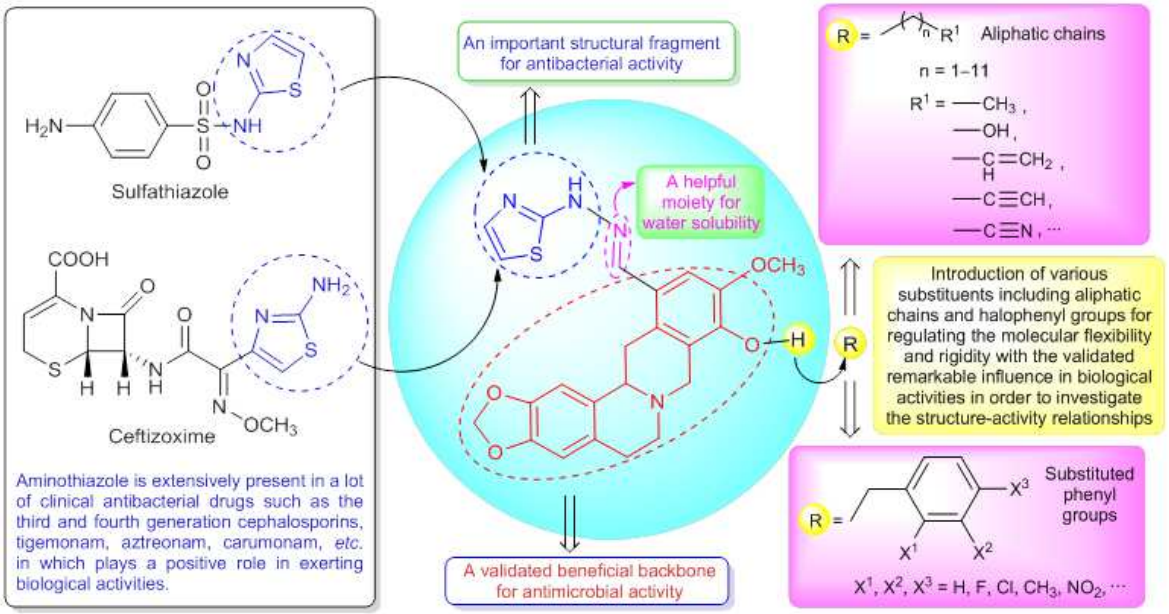


Fig. 2 *In vitro* antibacterial data as MIC (μmol/mL) for hydroxyl aminothiazolyl berberine derivative **6**, alkyl ones **9a-g**, allyl one **12**, propargyl one **15a**, cyano one **15b** and substituted phenyl ones **18a-i** against (a) drug-resistant Gram-positive MRSA (Methicillin-resistant *Staphylococcus. aureus* (N315)), *E. faecalis*; (b) drug-resistant Gram-negative *E. coli*, *P. aeruginosa*, *K. pneumonia*, *A. baumanii*. A = Berberine; B = Chloromycin; C = Norfloxacin. The standard deviation from three independent experiments is plotted.

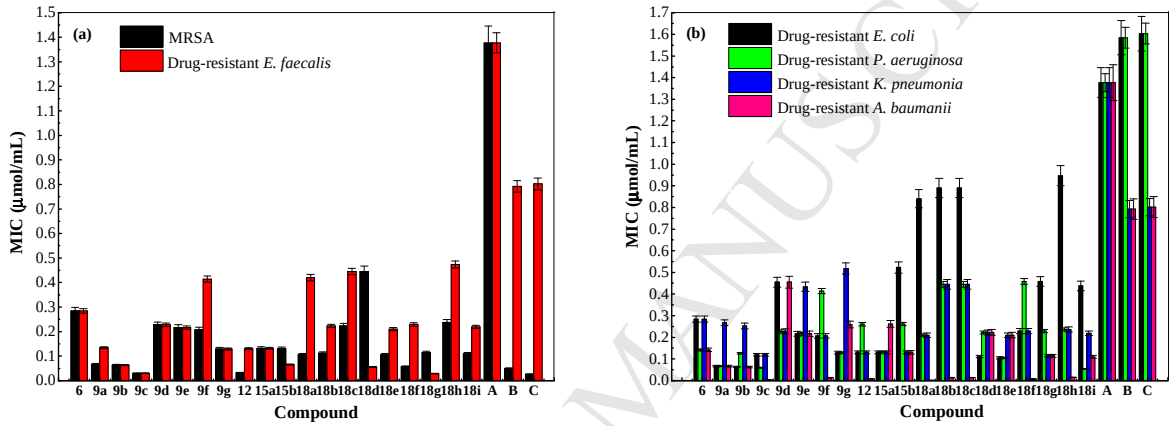


Fig. 3 Aqueous solubility of hydroxyl aminothiazolyl berberine derivative **6**, alkyl ones **9a-g**, allyl one **12**, propargyl one **15a**, cyano one **15b** and benzyl ones **18a-i**. A = Berberine. The standard deviation from three independent experiments is plotted.

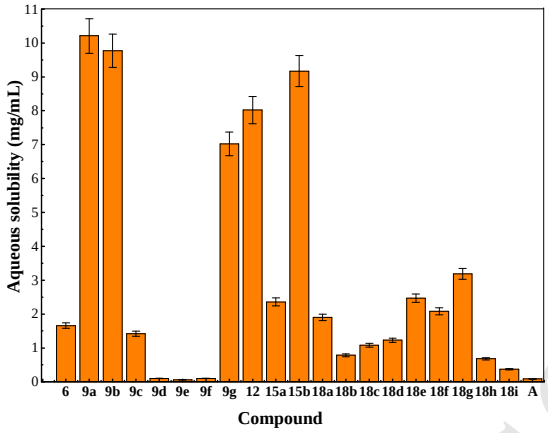


Fig. 4 Cell viability of human hepatocyte LO2 cells incubated with hexyl aminothiazolyl berberine derivative **9c** and 2,4-dichlorobenzyl one **18a** at different concentrations for 48 h. The standard deviation from three independent experiments is plotted.

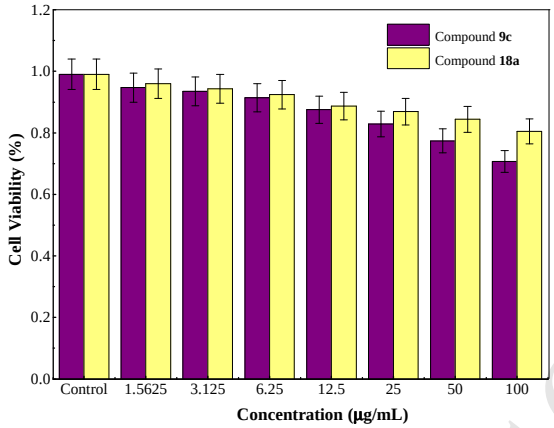


Fig. 5 Bactericidal kinetics of hexyl aminothiazolyl berberine derivative **9c** and 2,4-dichlorobenzyl one **18a** at 8 nmol/mL against drug-resistant *A. baumanii*. The standard deviation from three independent experiments is plotted.

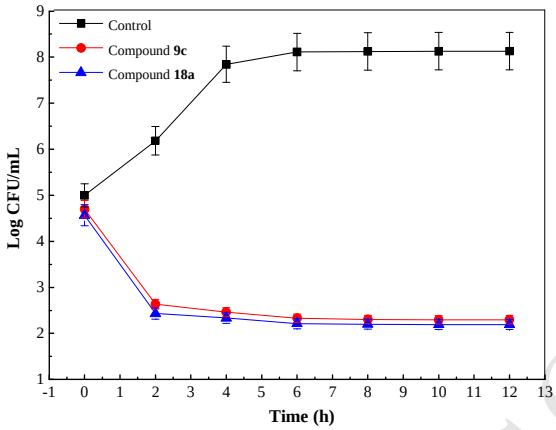


Fig. 6 Development of *A. baumanii* resistance to hexyl aminothiazolyl berberine derivative **9c** and 2,4-dichlorobenzyl one **18a**. The standard deviation from three independent experiments is plotted.

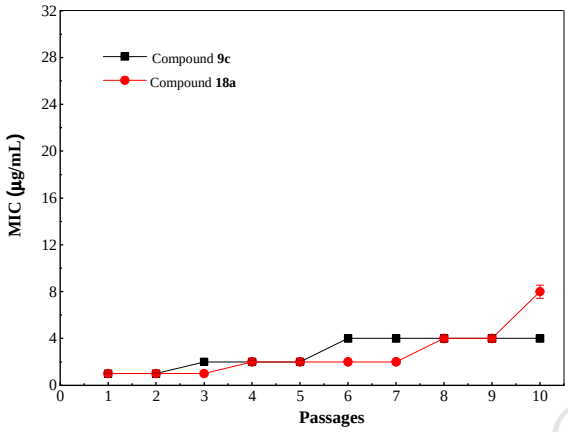


Fig. 7 Bacterial membrane permeabilization of compounds (a) **9c** and (b) **18a** at concentrations of 0.024 μmol/mL against *A. baumanii*.

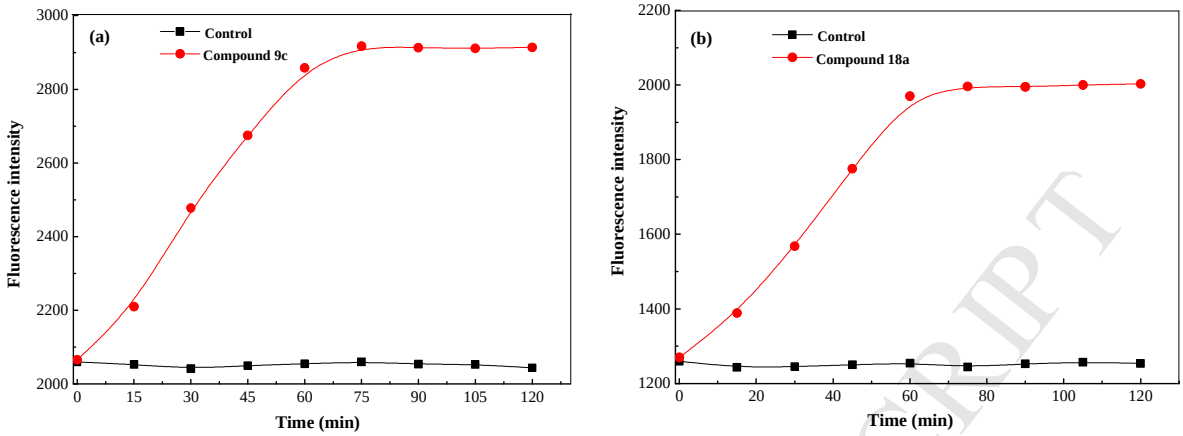


Fig. 8 Three-dimensional conformations of aminothiazolyl berberine derivatives docked in DNA gyrase B (PDB code: 4DUH): **9c** (left) and **18a** (right).

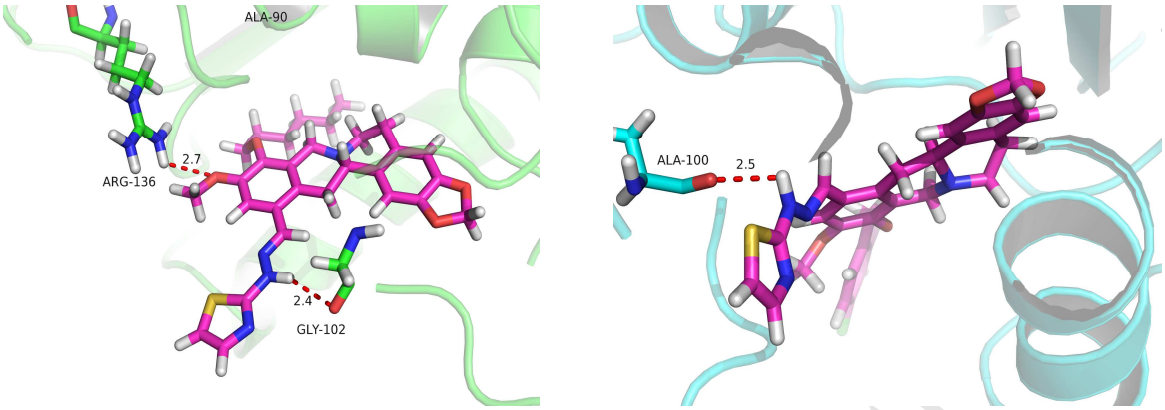


Fig. 9 (a) Absorption spectra of *A. baumanii* DNA with different concentrations of compound **9c** (pH = 7.4, T = 298 K). c(DNA) = 8.12 × 10^{-4} mol/L, c(compound **9c**) = 0–2.50 × 10^{-5} mol/L. Inset: comparison of absorption between the **9c**-DNA complex and that of the sum values of free DNA and free compound **9c** at 260 nm; (b) Absorption spectra of the competitive interaction between NR and **9c** with *A. baumanii* DNA. c(NR) = 2 × 10^{-5} mol/L, c(DNA) = 4.17 × 10^{-5} mol/L, and c(molecule **9c**) = 0–2.5 × 10^{-5} mol/L for curves a–i respectively. Inset: The wavelength range of 425–525 nm absorption spectra of competitive interaction between compound **9c** and NR with DNA.

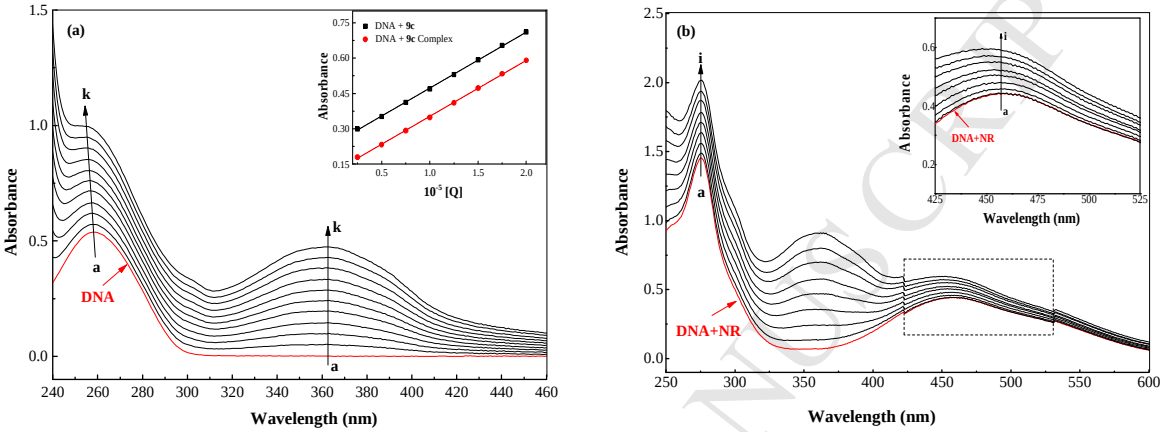
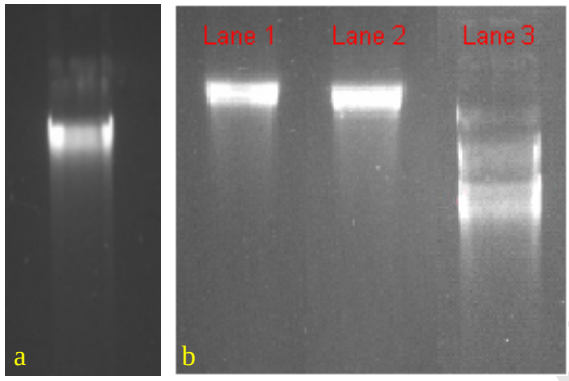
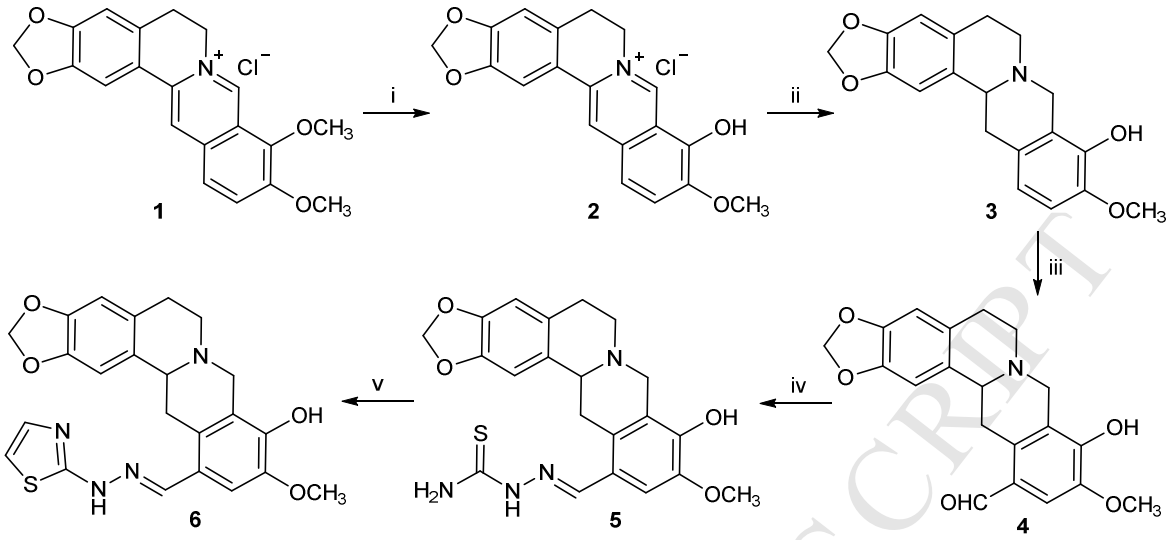


Fig. 10 (a) Gel electrophoresis assay of *A. baumanii* DNA; (b) Agarose gel electrophoresis patterns for the cleavage of drug-resistant *A. baumanii* DNA (1.31×10^{-6} mol/L) by compound **9c** in buffer (50 mM Tris-HCl/50 mM NaCl, pH = 7.4) at 37 °C after 6 h of incubation. Lane 1, DNA control; Lane 2, DNA + buffer; Lane 3, DNA + buffer + compound **9c** (50 μM).

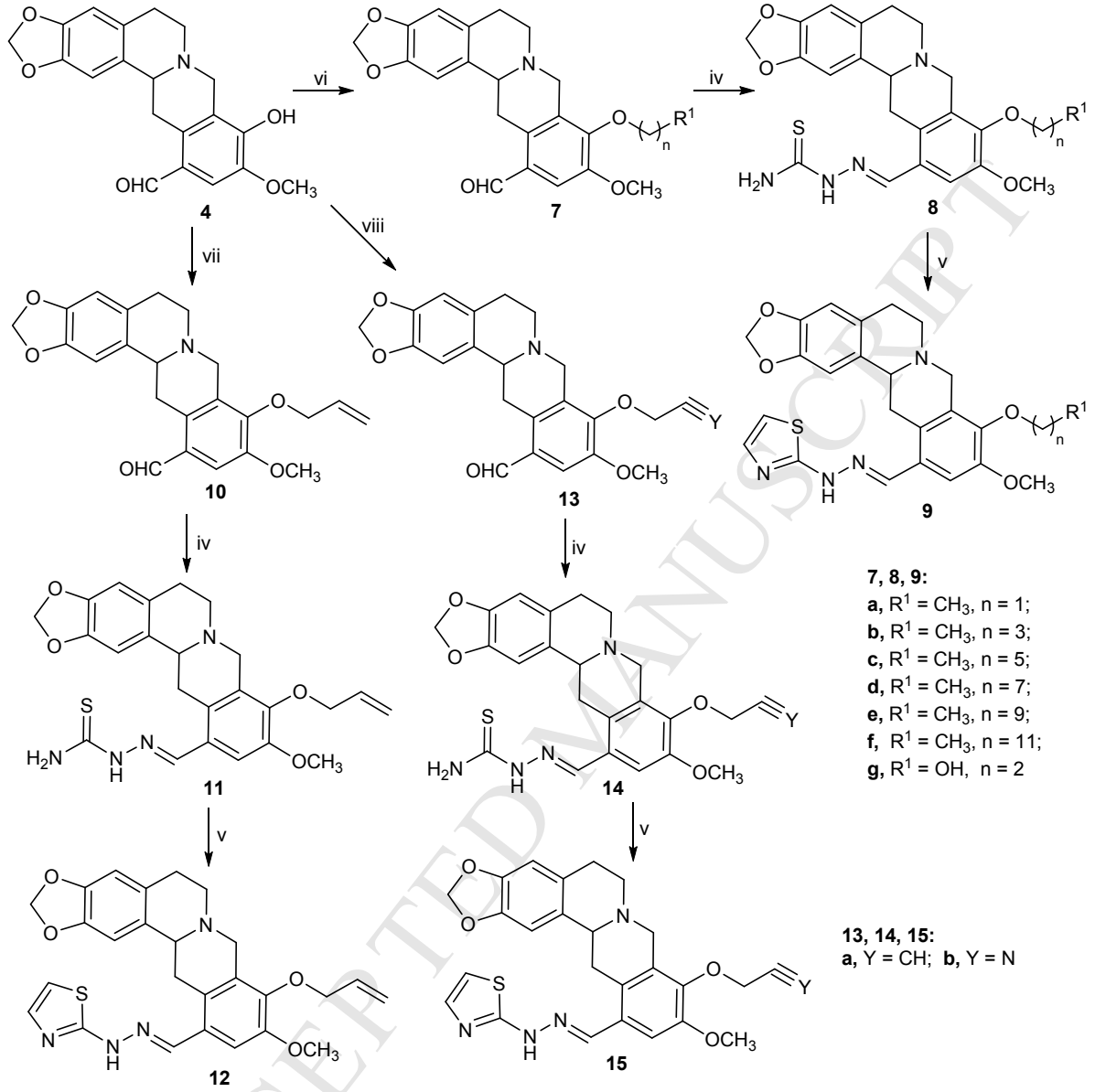


Scheme 1 Synthetic route of hydroxyl type of aminothiazolyl berberine derivative **6**.



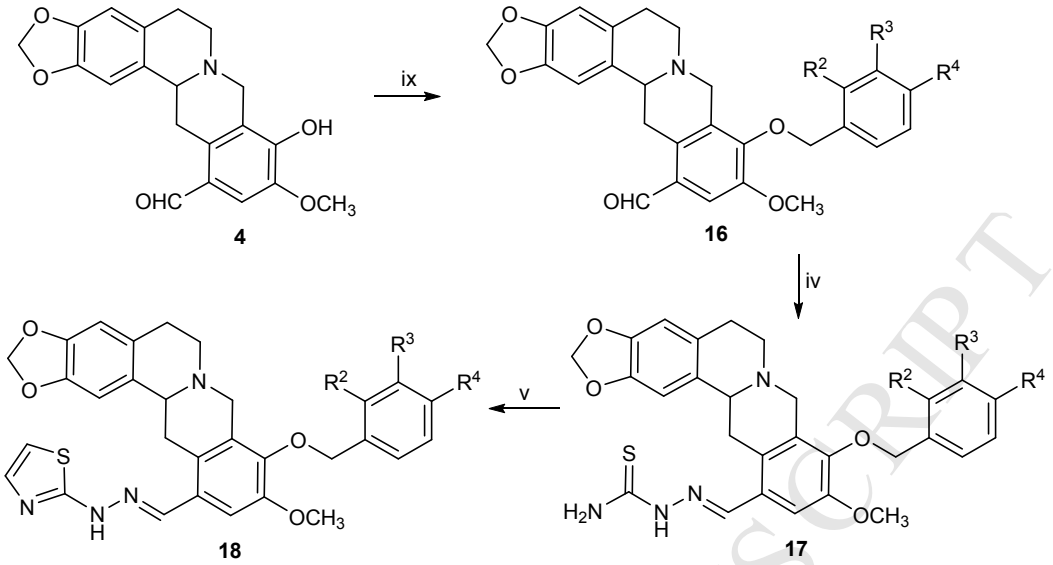
Reagents and conditions: (i) 190 ^{o}C/vacuum, EtOH/conc. HCl; (ii) $NaBH_4$, MeOH, r.t.; (iii) a. HMTA/TFA, 120 ^{o}C; b. 10% H_2SO_4 aq., 90−100 ^{o}C; (iv) hydrazinecarbothioamide, glacial acetic acid, EtOH, 80 ^{o}C; (v) 2-chloroacetaldehyde, EtOH, 80 ^{o}C.

Scheme 2 Synthetic route of aliphatic type of aminothiazolyl berberine derivatives **9a-g**, allyl one **12**, propargyl one **15a** and cyano one **15b**.



Reagents and conditions: (iv) hydrazinecarbothioamide, glacial acetic acid, EtOH, 80 ^{o}C; (v) 2-chloroacetaldehyde, EtOH, 80 ^{o}C; (vi) alkyl bromides, K_2CO_3, DMF, 80 ^{o}C; (vii) 3-bromoprop-1-ene, K_2CO_3, DMF, 80 ^{o}C; (viii) 3-bromoprop-1-yne or 2-chloroacetonitrile, K_2CO_3, DMF, 80 ^{o}C.

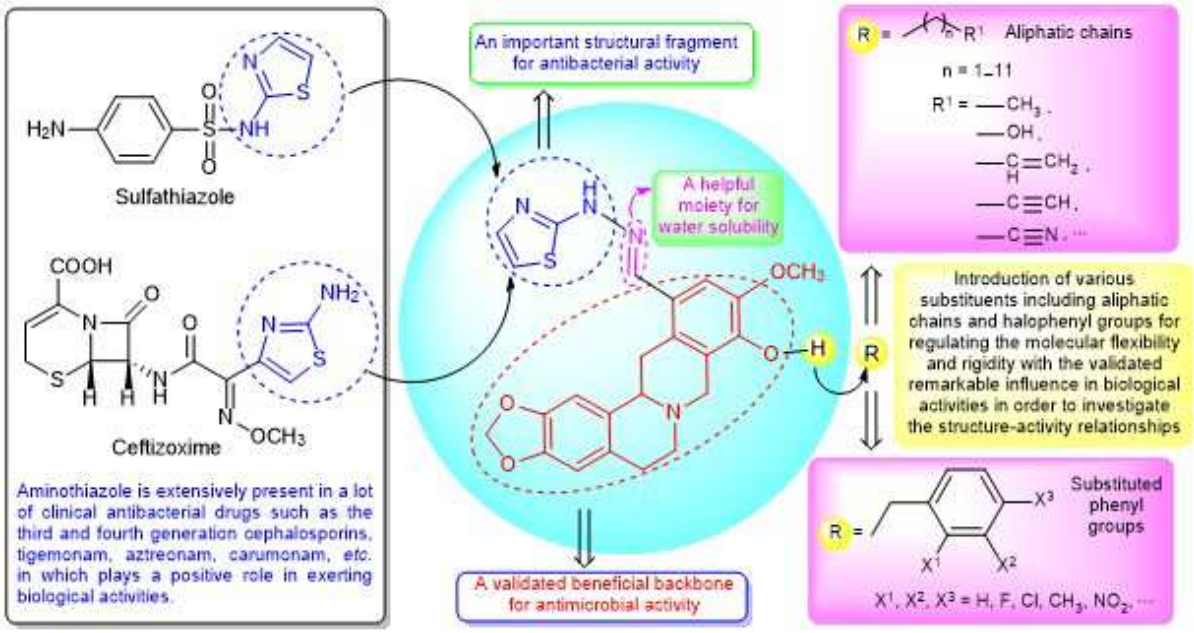
Scheme 3 Synthetic route of phenyl type of aminothiazolyl berberine derivatives **18a-i**.



16, 17, 18:

a, R^2 = Cl, R^3 = H, R^4 = Cl; **b,** R^2 = Cl, R^3 = H, R^4 = H
c, R^2 = H, R^3 = Cl, R^4 = H; **d,** R^2 = H, R^3 = H, R^4 = Cl
e, R^2 = H, R^3 = Cl, R^4 = Cl; **f,** R^2 = F, R^3 = H, R^4 = H
g, R^2 = H, R^3 = H, R^4 = F; **h,** R^2 = H, R^3 = H, R^4 = H
i, R^2 = H, R^3 = H, R^4 = NO_2

[a] Reagents and conditions: (iv) hydrazinecarbothioamide, glacial acetic acid, EtOH, 80 °C; (v) 2-chloroacetaldehyde, EtOH, 80 °C; (ix) substituted benzyl chlorides, K_2CO_3, DMF, 80 °C.



- Novel aminothiazolyl berberine derivatives as potentially antimicrobial agents were developed.
- Compounds **9c** and **18a** exhibited good activities (MIC = 2 nmol/mL) against clinically drug-resistant Gram-negative *A. baumanii*.
- Compounds **9c** and **18a** showed low cytotoxicity, rapidly bactericidal effects and quite slow development of resistance.
- Compounds **9c** and **18a** could bind with DNA gyrase through hydrogen bonds and disturb the *A. baumanii* membrane.
- Molecule **9c** could not only intercalate but also cleave *A. baumanii* DNA.
- Combination use of **9c** with clinical drug could enhance the efficiency and broaden antibacterial spectrum.