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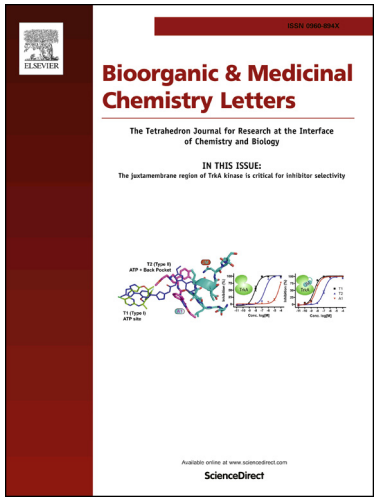

Novel benzimidazolyl tetrahydroprotoberberines: Design, synthesis, antimicrobial evaluation and multi-targeting exploration

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Graphical Abstract

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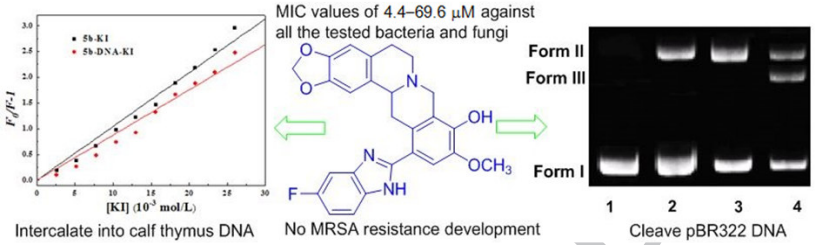

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[a]*Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Applied Chemistry of Chongqing Municipality, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China*
[b]*School of Chemistry and Chemical Engineering, Qiannan Normal University for Nationalities, Duyun 558000, China*

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ABSTRACT

A series of novel benzimidazolyl tetrahydroprotoberberines were conveniently designed and efficiently synthesized from berberine *via* direct cyclization of tetrahydroprotoberberine aldehyde and *o*-phenylene diamines under metal-free aerobic oxidation. All the new compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS spectra. The antimicrobial evaluation revealed that the 5-fluorobenzimidazolyl derivative **5b** was the most active antibacterial and antifungal molecule with broad spectrum in comparison to Berberine, Chloromycin, Norfloxacin and Fluconazole. It triggered almost no resistance development against MRSA even after 15 passages. Further studies demonstrated that compound **5b** could not only effectively interact with Topo IA by hydrogen bonds, but also intercalate into calf thymus DNA and cleave pBR322 DNA, which might be responsible for its powerful bioactivities.

The treatment of infectious diseases still remains a serious problem for scientific community despite of the rapid progress of biomedicinal science.[1] Several limitations such as toxicity, parenteral administration, and the decreased efficacy mainly due to microbial drug resistance of clinically used drugs force an immense need to search for new class of chemical entities with strong activity, low toxicity and different modes of action from currently clinical drugs.[2]

In the design of new drugs, hybridization approach may provide a more general method to obtain molecules with improved biological activities.[3] Especially the combination of two or more bioactive fragments could afford hybrids capable of overcoming multi-drug resistance.[4] This strategy has already given excellent results in drug discovery program[5] to overcome drug-resistance. Ciprofloxacin-1,2,4-triazole hybrids were demonstrated as potential antibacterial agents against drug-susceptible and drug-resistant bacteria.[6] Furthermore, our group has reported a series of berberine-azole hybrids as potential antibacterial and antifungal agents.[7]

Berberine is a well-known natural isoquinoline alkaloid present in roots and rhizomes of berberidaceae, ranunculaceae and papaveraceae *etc*.[7b] It plays a quite important role in the treatment of intestinal infections such as cholera, bacillary dysentery and acute gastroenteritis, which makes more and more researchers be interesting in developing berberine derivatives with antimicrobial ability. Tetrahydroprotoberberines, an important class of berberine derivatives, containing an isoquinoline backbone with methoxyl or hydroxyl group on benzene ring have been demonstrated to possess various biological activities.[8] Benzimidazole is an important heterocyclic structure in medicinal chemistry. It consists of a benzene ring fused an imidazole ring, which possesses larger conjugated system and higher electron-rich property than simple imidazole ring. So far a lot of benzimidazole compounds have been successfully developed as clinical drugs and have been widely used in the treatment of various diseases. Recently, literature has shown the great potentiality of benzimidazoles to inhibit the growth of bacteria and fungi.[9] Benzimidazole derivatives are able to compete with purines due to their structural similarity. This may inhibit the synthesis of nucleic acids, thereby killing microbial strains.[10] The different action mechanisms of benzimidazole type of antimicrobial molecules have attracted more and more attention to develop benzimidazoles as novel antimicrobial agents. On the basis of our previous findings

* Corresponding author. Tel.: +86-23-68254967; fax: +86-23-68254967; E-mail: zhouch@swu.edu.cn (Cheng-He Zhou); peng0703@sgmtu.edu.cn (Xin-Mei Peng)

regarding the antimicrobial properties of berberine derivatives and in consideration of the fact that benzimidazole could compete with purines and might inhibit the synthesis of nucleic acids, both scaffolds could bind DNA in different ways and, therefore, their combination could improve the efficacy as antimicrobial agents.

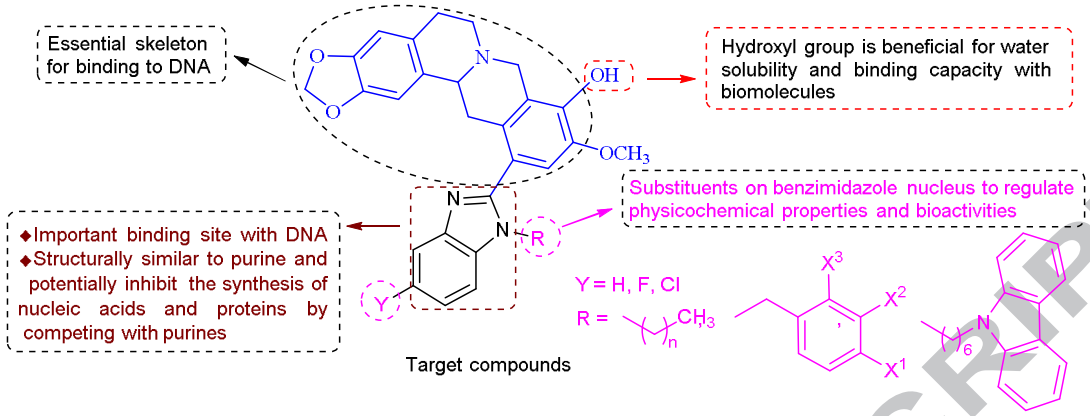
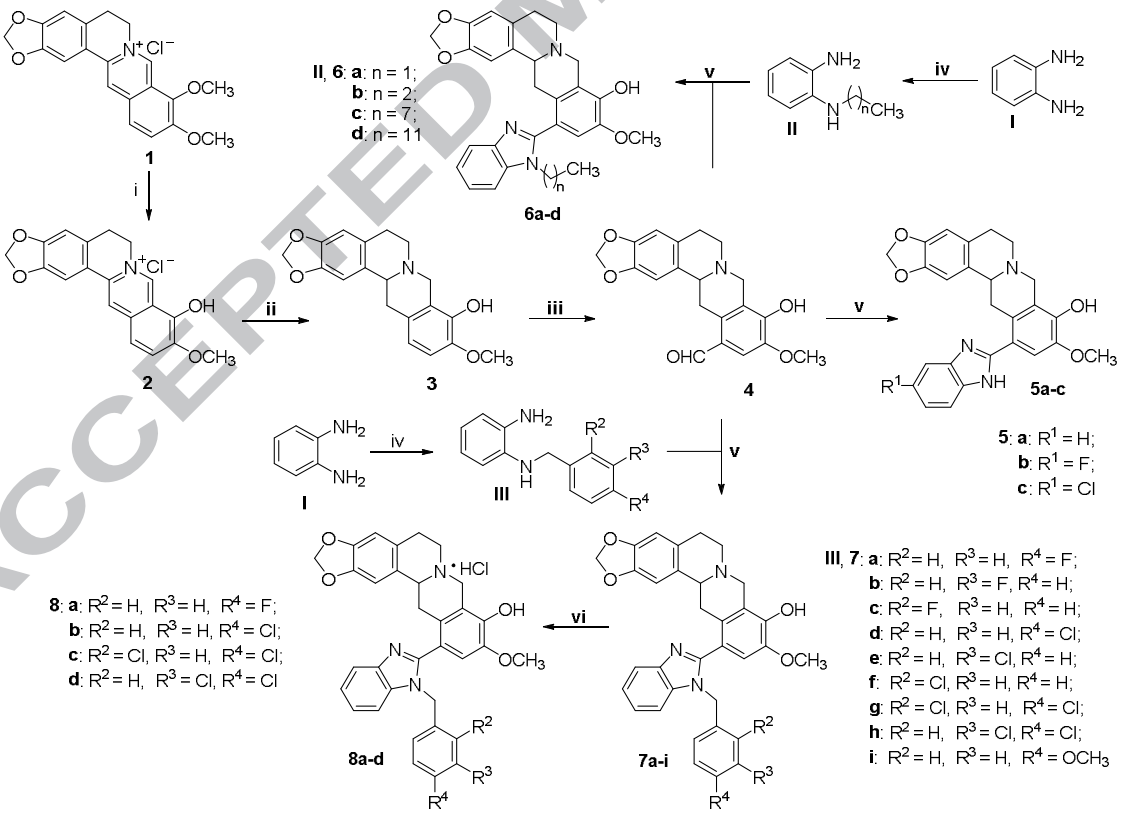


Figure 1. Basic design of benzimidazolyl tetrahydroprotoberberines.

Considering the above observations, and in continuation of our research in the field of heterocyclic compounds as novel potential antimicrobial agents, a series of benzimidazolyl tetrahydroprotoberberines were prepared via efficiently direct cyclization of tetrahydroprotoberberine aldehyde and *o*-phenylene diamines through metal-free aerobic oxidation in wet organic solvent.[11] Fluorine and chlorine atoms as well as substituted benzyl groups were introduced into benzimidazole ring with an aim to adjust bioactivities.[12] Aliphatic chain was incorporated to modulate molecular flexibility and regulate the lipid-water partition coefficient.[13] The modification by carbazole moiety was explored to be as comparison in activity due to its biological importance.[14] The further transformation of the prepared target compounds into their hydrochlorides was also investigated since some researches have proved that hydrochlorides could improve antimicrobial efficacy and broaden antimicrobial spectrum due to reinforcing water solubility.[15] The design was shown in Figure 1.



Scheme 1. Reagents and conditions: i) 190 °C/vacuum, EtOH/conc. HCl, 45 min; ii) $NaBH_4$, MeOH, r.t., 5 h; iii) a. HMTA/TFA, 120 °C, 5 h; b. 10% H_2SO_4, 90–100 °C, 2 h; iv) K_2CO_3, DMF, alkyl bromides or benzyl halides, r.t., 8 h; v) *o*-phenylene diamines/**II/III**, DMF/water, 80 °C, 12 h; vi) diethyl ether, HCl (g), 0 °C.

The target compounds **5–8**, **12** and **13** were synthesized according to the route shown in Schemes 1 and 2. Commercially available berberine chloride **1** was selected as starting material. Selective demethylation of compound **1** at 9-position was carried out in the presence of ethanol/hydrochloric acid solution at 190 °C under vacuum (20 mm Hg) for 45 minutes to easily afford berberrubine hydrochloride **2** with 79% yield, [16] which was further reduced by $NaBH_4$ in methanol to produce compound **3** in 62% yield.[17] The key intermediate **4** was obtained *via* direct formylation of compound **3** by hexamethelene tetramine (HMTA) in trifluoroacetic acid (TFA) and then hydrolysis with 10% H_2SO_4 at 90 °C for 5 h in excellent yield (82%) without further column purification. The target benzimidazolyl tetrahydroprotoberberines **5a–c**, **6a–d** and **7a–i** were effectively obtained in 25–48% yields by the cyclization of tetrahydroprotoberberine aldehyde **4** with a series of *o*-phenylene diamines under metal-free aerobic oxidation in DMF/water system at 80 °C for 12 h. Amongst, compounds **7a**, **7d**, **7g** and **7h** were further transformed into their corresponding hydrochlorides **8a–d** (70–85% yields) in diethyl ether by using dry hydrogen chloride gas at 0 °C.

Compound **10** was synthesized from carbazole **9** and 1,6-dibromohexane in DMF in the presence of KOH at room temperature for 12 h in 59% yield,[18] which was reacted with *o*-phenylene diamine to give compound **11** in the yield of 52% using DMF as solvent and K_2CO_3 as base at room temperature for 8 h. The target molecule **12** was obtained by the cyclization of compound **11** and intermediate **4** in DMF/water system at 80 °C for 12 h with 28% yield. Further hydrochloride **13** was prepared in 80% yield by using dry hydrogen chloride gas at 0 °C in $CHCl_3$/diethyl ether.

Table 1. Screening of the optimal formylation conditions for intermediate **4**

Entry	Solvent	Temperature	HMTA Eq.[a]	Time	Yield
1	Water	80 °C	2	5h	-
2	25% AcOH	80 °C	2	6h	11%
3	50% AcOH	80 °C	1	8h	20%
4	80% AcOH	80 °C	1	8h	37%
5	AcOH	Reflux	1.2	5h	44%
6	TFA	Reflux	1.2	5h	82%

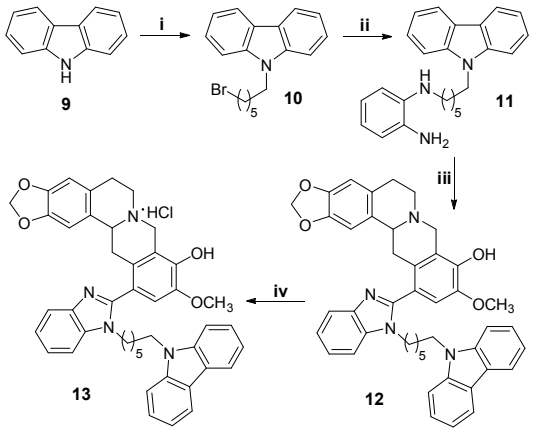
[a]HMTA Eq., HMTA Equivalent.

We here make the first report on an efficiently direct synthesis of tetrahydroprotoberberrubine-12-carbaldehyde **4**. Formylation of compound **3** was investigated by different conditions such as solvent, temperature, loading amount of HMTA, and reaction time (Table 1). Initially, the reaction was conducted under water medium, no reaction was observed. In the presence of acetic acid (different concentration), the formylation initiated but product **4** gave low yield. According to the overall observations, strong acid TFA made the formylation reaction go effectively (with product in 82% yield) under reflux for 5 h using 1.2 equivalent HMTA. In this reaction, TFA acted as not only the optimal solvent but also strong protonating agent.

The structures of all the new compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and HRMS (Supporting Information for details). The *in vitro* antimicrobial activities for intermediate **4** and all the target compounds were evaluated against four Gram-positive bacteria, six Gram-negative bacteria and five fungi using two fold serial dilution technique recommended by National Committee for Clinical Laboratory Standards (NCCLS)[19] with the positive control of clinical antimicrobial drugs Chloromycin, Norfloxacin and Fluconazole. The antibacterial and antifungal data as well as clog*P* values were depicted in Tables 2 and Table S1. Minimal inhibitory concentration (MIC, μM) is defined as the lowest concentration of the new compounds that completely inhibited the growth of bacteria or fungi.

The *in vitro* antibacterial evaluation (Table 2) demonstrated that some title compounds showed moderate to good activities against the tested strains. Amongst, compound **5b** displayed good antibacterial activities with MIC values ranging from 4.4 to 69.6 μM in comparison to reference drugs. It was more active against *B. subtilis* (MIC = 4.4 μM), *S. dysenteriae* (MIC = 17.4 μM) and *B. typhi* (MIC = 14.5 μM) than Chloromycin (MIC = 82.5 μM). Moreover, the anti-MRSA (MIC = 17.4 μM), anti-*E. coli* JM109 (MIC = 8.7 μM) and anti-*P. aeruginosa* (MIC = 11.6 μM) efficacies of compound **5b** were comparative or superior to those of Chloromycin (MIC = 41.3–99 μM) and Norfloxacin (MIC = 25.1–50.1 μM).

Table 2 showed valuable effects of the substituted benzyl groups at benzimidazole ring on biological activities. Most of the benzyl modified benzimidazolyl tetrahydroprotoberberines were more active towards the tested bacteria than aliphatic moiety substituted ones. Compound **7b** with 3-fluorobenzyl benzimidazole gave better inhibition against MRSA (MIC = 12.2 μM) and *E. coli* JM109 (MIC = 29.1 μM) than Chloromycin (MIC = 41.3 and 82.5 μM). Noticeably, compound **7d** with 4-chlorobenzyl benzimidazole showed remarkable antimicrobial activity against *B. subtilis*, *S. aureus*, *E. coli* JM109, *E. coli* DH52, *S. dysenteriae*, *P. aeruginosa*, *B. thyphi* and *B. proteus* in comparison with Chloromycin. Compound **7e** with 3-chlorobenzyl benzimidazole and compound **7f** with 2-chlorobenzyl benzimidazole displayed excellent inhibitory activity against *E. coli* JM109 with MIC value of 4.7 μM, which was more active than Chloromycin (MIC = 82.5 μM) and Norfloxacin (MIC = 50.1 μM). Regretfully, for hydrochlorides **8a–d**, only compound **8c** suggested good antibacterial ability against the tested strains in contrast with reference drug Chloromycin. Compound **12** exhibited more poor biological activities than compound **13** because the formation of salt improved the solubility of compound **13**.



Scheme 2. Reagents and conditions: i) KOH, 1,6-dibromohexane, DMF, r.t., 12 h; ii) K_2CO_3, DMF, *o*-phenylene diamine, r.t., 8 h; iii) compound **4**, DMF/water, 80 °C, 12 h; iv) $CHCl_3$/diethylether, HCl (g), 0 °C.

The *in vitro* antifungal data in Table S1 indicated that most of the synthesized intermediates and target compounds displayed effective potency against the tested five fungi. Compound **7d** was demonstrated to be the most active antifungal molecule against all the tested fungal strains in contrast with the clinically used Fluconazole. From the overall observations, the antifungal

activities of benzyl substituted benzimidazoles were more active than those of alkyl ones. Except for compounds **5c**, **8c–d**, **12** and **13**, all the tested compounds gave better inhibitory potency against *A. flavus* (MIC = 14.1–293.3 μM) than Fluconazole (MIC = 835.9 μM). Compounds **5b**, **6c** and **6d** showed the strongest inhibition towards *C. mycoderma* with MIC values ranging from 3.3 to 8.7 μM in comparison with Fluconazole (MIC = 26.1 μM). Compounds **5b** and **7d** showed strong activity towards *S. cerevisia* with MIC values of 8.7 and 14.1 μM respectively as compared to Fluconazole (MIC = 52.2 μM). The *in vitro* antifungal abilities of compounds **5c**, **8d**, **12** and **13** were quite poor, which were in accordance with their antibacterial activities.

To rationalize the observed antimicrobial activity and to understand the possible mechanism of the hybrids, molecular docking study of active compound **5b** with DNA topoisomerase IA (Topo IA) was undertaken. The crystal structure data was obtained from the Protein Data Bank. Compound **5b** possessed lowest binding energy (−13.74 KJ/mol). As shown in Figure 2, the results indicated that compound **5b** could interact with Topo IA through the formation of hydrogen bond between the hydroxyl group of compound **5b** and ASN 555 of Topo IA. On the other hand, HIS 365 and ASP 323 could form two hydrogen bonds with nitrogen atom at benzimidazole ring and –O-CH_2-O- group of compound **5b**, respectively. Furthermore, this molecule could also form electrostatic interactions by benzimidazole and berberine aromatic skeleton with ALA 554, ARG 161, ARG 114 and ASP 551. These hydrogen bonds might be the crucial reason that compound **5b** displayed strong inhibitory efficacy against the tested strains.

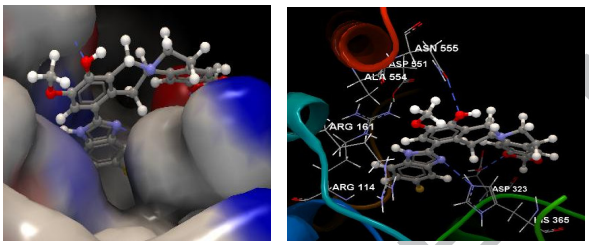


Figure 2. Docking results of compound **5b** with Topo IA (PDB code: 3PWT).

Because of the emergence of drug resistant pathogens, the efficacy of current antibiotics against infections has been diminished.[20] To investigate the drug resistance development of the tested strains against compound **5b**, the susceptible pathogen MRSA were employed. The new MIC values were determined every 24 h after propagation of bacterial cultures with fresh media and serially diluted concentrations of compound **5b**. To make comparative analysis, parallel cultures were exposed to 2-fold dilutions with the reference drugs Chloromycin and Norfloxacin as positive control. The experiment was repeated for 15 days. Results in Figure 3 showed the low propensity of bacterial pathogens to develop resistance towards compound **5b** as there was almost no change in the MIC values even after 15 passages.

The application of absorption spectroscopy is one of the most important techniques in DNA-binding studies. Generally, hyperchromism and hypochromism are important spectral features to distinguish the change of DNA double-helical structure.[24] In this work, with a fixed concentration of DNA, UV-vis absorption spectra were recorded with the increasing amount of compound **5b**. Figure 4 showed that the maximum absorption of DNA at 260 nm displayed proportional increase with the increasing concentration of compound **5b**. Simultaneously, the measured values of **5b**–DNA complex was a little greater than the absorption values of simply sum of free DNA and free compound **5b**, which was observed in the inset of Figure 4. These indicated that a weak hyperchromic effect existed between DNA and compound **5b**, which suggested the strong interaction of compound **5b** and DNA. The binding constant (K) of compound **5b** interacting with DNA was calculated to be K = 2.87 × 10^4 L/mol, R = 0.995, SD = 0.17 (R is the correlation coefficient. SD is standard deviation).

As is known, the interactions between drugs or bioactive small molecules and biomacromolecules are helpful to study the absorption, transportation, distribution, metabolism and possible action mechanism of agents.[21] Literature has reported that berberine and its derivatives could effectively interact with DNA.[22] Therefore, this work selected calf thymus DNA as a model to investigate the in vitro binding behavior of the most active compound **5b** with DNA at molecular level by UV-vis and fluorescence spectroscopic methods.[23]

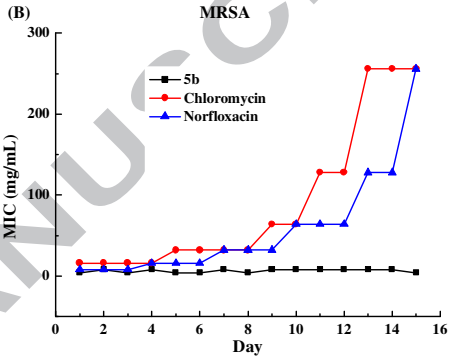


Figure 3. Evaluation of resistant development against compound **5b** in bacterial strain MRSA.

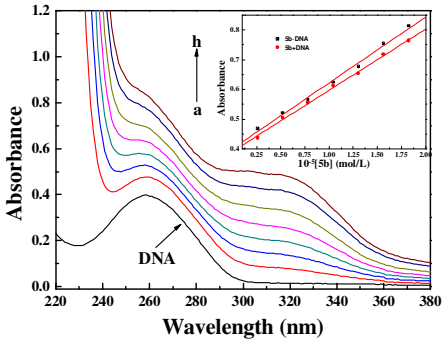


Figure 4. UV absorption spectra of DNA with different concentrations of compound **5b** (pH = 7.4, T = 290 K). Inset: comparison of absorption at 260 nm between the **5b**–DNA complex and the sum values of free DNA and free compound **5b**. c(DNA) = 4.52 × 10^{-5} mol/L, and c(compound **5b**) = 0–1.4 × 10^{-5} mol/L for curves a–h respectively at increment 0.2 × 10^{-5}.

Neutral Red (NR) is a planar phenazine dye, whose binding mode with DNA is demonstrated to be intercalation. This work employed NR as a spectral probe to investigate the binding mode of **5b** with DNA. The absorption spectra of NR upon the addition of DNA were shown in Supporting Information. It was apparent that the absorption peak of the NR at around 460 nm gave gradual decrease with the increasing concentration of DNA, and a new band at around 530 nm developed. This was attributed to the formation of the new DNA–NR complex. An isosbestic point at 504 nm provided evidence of the formation of DNA–NR complex (Figure S3). The competitive binding between NR and

Table 2 ClogP values and antibacterial data as MIC (μM) for intermediate **4** and berberine-derived benzimidazoles[a,b,c]

Compds	clog*P*	Gram-positive bacteria				Gram-negative bacteria					
		MRSA	*B. subtilis*	*M. luteus*	*S. aureus*	*E. coli* JM109	*E. coli* DH52	*S. dysenteriae*	*P. aeruginosa*	*B. typhi*	*B. proteus*
3	2.91	1311.3±4.3	1573.6±2.1	1573.6±6.1	786.8±1.7	655.7±7.1	1573.6±1.7	1573.6±2.3	786.8±1.5	786.8±1.5	1311.3±4.3
4	2.66	301.8±4.6	482.9±9.1	301.8±4.6	724.4±0	241.5±4.6	241.5±4.6	120.7±2.3	301.8±4.6	301.8±4.6	482.9±9.1
5a	3.74	120.8±2.9	48.3±1.9	241.6±1.7	579.9±2.4	60.4±1.9	96.7±0.9	96.7±1.9	145±0.3	48.3±1.9	241.6±2.7
5b	3.90	17.4±0.5	4.4±0.8	46.4±1.1	58±0.6	8.7±0.8	23.2±1.9	17.4±0.6	11.6±0.7	14.5±0.9	69.6±0.5
5c	4.30	1075.8±7.8	717.2±5.6	896.5±6.6	1075.8±8.8	896.5±3.6	896.5±8.6	1075.8±9.9	358.6±5.3	448.3±5.3	896.5±8.6
6a	4.31	14.2±4.9	34.1±1.5	28.4±2.8	28.4±1.8	28.4±2.8	136.3±1.6	136.3±1.6	68.2±1.7	14.2±0.9	56.8±1.7
6b	4.80	55.2±1.1	110.3±1.2	110.3±1.2	44.1±1.1	110.3±1.2	110.3±1.2	264.7±2.7	66.2±1.8	27.6±1.6	22.1±1.6
6c	6.89	231.2±2.5	57.8±1.7	48.2±1.7	14.4±1.2	115.6±1.7	24.1±1.3	77.1±1.4	38.5±2.7	24.1±1.3	9.6±0.3
6d	8.56	174.9±2.6	35±1.2	105±0.9	26.2±0.7	26.2±0.3	87.5±1.3	174.9±2.6	17.5±0.6	8.7±0.8	5.5±0.9
7a	5.87	50.2±0.4	20.1±1.7	40.1±1.4	60.2±1.5	25.1±1.5	100.3±2.8	60.2±0.9	25.1±0.7	60.2±0.7	25.1±1.7
7b	5.87	12.2±0.2	19.4±1.4	19.4±0.8	48.5±1.8	29.1±0.5	38.8±2.8	116.4±0.7	48.5±1.8	48.5±1.8	38.8±1.8
7c	5.87	24.3±0.4	48.5±1.8	29.1±0.6	58.2±0.7	29.1±0.5	38.8±1.8	48.5±1.8	58.2±0.7	24.3±0.4	38.8±1.8
7d	7.27	18.9±1.2	11.8±1.1	28.3±0.9	9.4±1.1	37.7±1.3	56.5±0.7	18.9±1.2	23.6±1.2	9.4±1.1	11.8±1.1
7e	7.27	56.5±0.6	47.1±1.3	28.3±0.5	23.6±1.2	4.7±0.4	75.4±2.6	23.6±2.2	28.3±0.5	4.7±2.3	47.1±2.3
7f	7.27	23.6±2.2	188.4±8.3	23.6±1.2	9.4±0.8	4.7±0.7	23.6±1.2	28.3±0.7	23.6±1.2	37.7±2.3	47.1±1.3
7g	6.82	53.3±1.2	11.1±1.0	44.4±1.4	22.2±0.7	35.5±2.4	106.6±2.0	71.1±1.8	88.8±2.8	22.2±1.7	17.7±0.7
7h	6.82	22.2±1.7	22.2±1.7	106.6±3.3	35.5±2.4	44.4±1.4	44.4±2.4	44.4±1.4	35.5±1.4	53.3±1.8	22.2±2.7
7i	5.58	57±1.6	19±1.2	57±0.9	47.5±1.5	38±1.5	95±1.9	57±0.8	38±1.5	47.5±2.5	47.5±1.5
8a	6.19	218.4±2.9	182±4.1	182±4.2	109.2±6.0	182±2.5	182±2.5	145.6±5.1	182±3.7	218.4±8.9	182±3.6
8b	6.59	35.4±1.3	88.5±1.7	70.8±1.7	70.8±1.7	70.8±1.7	141.6±1.3	106.2±1.6	212.4±1.9	106.2±1.8	88.5±2.7
8c	7.14	20.9±0.9	33.5±1.5	20.9±1.2	20.9±1.2	20.9±1.2	20.9±1.2	50.2±2.1	67±2.3	6.3±0.6	20.9±1.2
8d	7.14	535.9±2.0	134±2.5	535.9±2.9	267.9±1.9	535.9±2.1	669.8±2.5	535.9±2.0	535.9±2.0	201±2.0	535.9±9.6
12	7.99	617.6±2.9	741.1±3.7	741.1±2.3	617.6±2.9	617.6±2.9	617.6±2.9	617.6±8.9	741.1±3.7	617.6±2.9	617.6±2.9
13	8.31	586.7±2.2	704±2.5	293.3±5.6	146.7±2.8	469.3±6.2	88±1.6	146.7±1.8	469.3±4.2	73.3±1.4	88±1.6
Berberine	–0.77	229.5±1.4	1147.3±5.4	1376.8±5.1	1376.8±5.1	1376.8±3.6	1147.3±7.4	1147.3±5.4	573.7±3.7	458.9±2.7	688.4±2.4
Chloromycin	–0.23	41.3±2.3	82.5±1.7	20.7±1.2	41.3±1.3	82.5±2.6	66±2.6	99±1.9	99±1.5	82.5±1.6	99±1.8
Norfloxacin	1.37	25.1±0.2	3.1±.03	4.2±1.8	1.1±0.5	50.1±0.9	50.1±0.9	5.3±1.8	41.8±0.8	10.4±3.6	12.5±0.5

[a]MIC, minimal inhibitory concentration. [b]*S. aureus*, *Staphylococcus aureus* (ATCC25923); MRSA, methicillin-resistant *Staphylococcus aureus* (N315); *S. dysenteriae*, *Shigella dysenteriae*; *B. subtilis*, *Bacillus subtilis*; *M. luteus*, *Micrococcus luteus* (ATCC4698); *B. proteus*, *Bacillus proteus* (ATCC13315); *E. coli*, *Escherichia coli* (JM109); *E. coli*, *Escherichia coli* (DH52); *P. aeruginosa*, *Pseudomonas aeruginosa*; *B. typhi*, *Bacillus typhi*. [c]clogP values were calculated using ChemBioDraw Ultra 14.0.

5b with DNA was observed in Figure S4. With the increasing concentration of compound **5b**, an apparent intensity increase was observed around 460 nm, which was opposite with the absorption of NR–DNA complex at the same wavelength. The various spectral changes suggested that compound **5b** could substitute NR in the DNA–NR complex.

To further explore the binding mode of compound **5b** with DNA, negatively charged I^- ion was employed due to the fact that negatively charged phosphate backbone of DNA is expected to repel a highly negatively charged quencher. Therefore, when a small molecule is protected from being quenched by anionic quencher, the binding mode is intercalation, whereas groove binding agents should be quenched readily.[25]

The K_{SV} values of compound **5b** quenched by I^- ion in the absence and presence of DNA were calculated to be 104.52 (R = 0.996) and 87.74 (R = 0.994) L mol^{-1}, respectively. It was apparent that when compound **5b** interacted with DNA, the quenching effect was decreased (Figure S7), which suggested the binding mode of compound **5b** to DNA was intercalation.

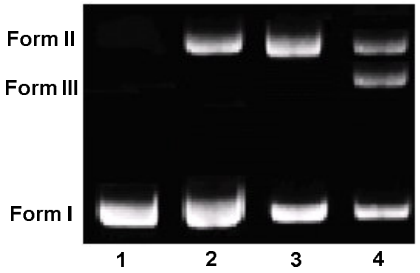


Figure 5. Cleavage of pBR322 DNA by various concentrations of compound **5b**. Lane 1 DNA control, Lane 2 DNA + 25 μM compound **5b**, Lane 3 DNA + 50 μM compound **5b**, Lane 4 DNA + 75 μM compound **5b**.

The DNA-cleaving activity of compound **5b** towards pBR322 DNA was studied under physiological conditions (37 °C and pH = 7.0). Figure 5 showed the GE patterns for the cleavage of pBR322 DNA by compound **5b** of varying concentrations. It can be seen that compound **5b** was capable of converting pBR322 DNA into Form II and that the cleaving activity showed a strong dependence on its concentration (Lane 4). When the concentration of compound **5b** was about 75 μM, almost all the Form I was converted to the Form II and Form III (a linear form, generated from cleavage of both Form I and Form II strand), which lent strong support that compound **5b** catalyzed the cleavage of pBR322 DNA plasmid by concentration dependence.

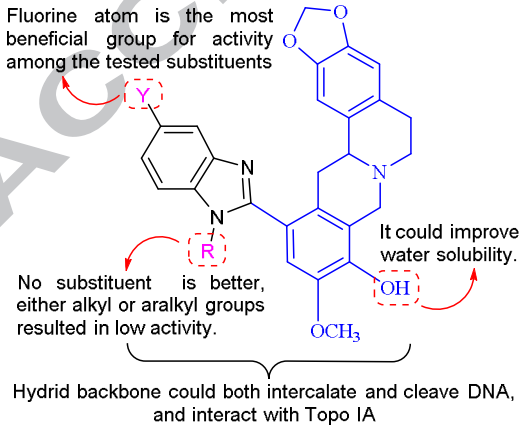


Figure 6. Preliminary summary for structure–activity relationship.

In conclusion, a series of novel benzimidazolyl tetrahydroprotoberberines have been successfully and efficiently prepared via efficient cyclization of tetrahydroprotoberberine aldehyde and *o*-phenylene diamines under metal-free aerobic oxidation. All the new compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR and HRMS spectra. The *in vitro* antimicrobial evaluation revealed that some title compounds showed good bioactivities against the tested strains. Compound **5b** bearing 5-fluorobenzimidazole moiety was found to be the most active antibacterial and antifungal molecule with broad spectrum in comparison with standard drugs Chloromycin, Norfloxacin and Fluconazole. More importantly, there was almost no MRSA resistance development even after 15 passages of bacterial incubation against compound **5b**. Further studies showed that this compound not only was able to effectively interact with Topo IA by hydrogen bonds, but also had the ability to intercalate into calf thymus DNA and cleave pBR322 DNA, which might be responsible for its powerful antimicrobial activities (Figure 6). Therefore, compound **5b** was potential to be developed as novel antimicrobial agent. Some properties including other antimicrobial mechanism explorations such as antibiofilm and bacterial membrane permeabilization activity as well as *in vivo* antibacterial and antifungal evaluation are currently underway in our laboratory.

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References and notes

1. a) Peng, X. M.; Cai, G. X.; Zhou, C. H. *Curr. Top. Med. Chem.* **2013**, *13*, 1963–2010; (b) Zhou, C.H.; Wang, Y. *Curr. Med. Chem.* **2012**, *19*, 239–280; (c) Zhang, L.; Peng, X.M.; Damu, G. L. V.; Geng, R.X.; Zhou, C.H. *Med. Res. Rev.* **2014**, *34*, 340–437.
2. a) Mandapati, K.; Gorla, S. K.; House, A. L.; McKenney, E. S.; Zhang, M.; Rao, S. N.; Gollapalli, D. R.; Mann, B. J.; Goldberg, J. B.; Cuny, G. D.; Glomski, I. J.; Hedstrom, L. *ACS Med. Chem. Lett.* **2014**, *5*, 846–850; b) Cui, S. F.; Addla, D.; Zhou, C. H. *J. Med. Chem.* **2016**, *59*, 4488-4510.
3. a) Viegas-Junior, C.; Danuello, A.; da Silva Bolzani, V.; Barreir, E. J.; Fraga, C. A. M. *Curr. Med. Chem.* **2007**, *14*, 1829–1852; b) Gong, H. H.; Addla, D.; Lv, J. S.; Zhou, C. H. *Curr. Top. Med. Chem.* **2016**, *16*, 3303–3364; c) Peng, X. M.; Peng, L. P.; Li, S.; Avula, S. R.; Kumar, K. V.; Zhang, S. L.; Tam, K. Y.; Zhou, C. H. *Future Med. Chem.* **2016**, *8*, 1927–1940.
4. Zhang, H. Z.; Jeyakkumar, P.; Kumar, K. V.; Zhou, C. H. *New J. Chem.* **2015**, *39*, 5776–5796.
5. a) Pavlović, D.; Mutak, S. *ACS Med. Chem. Lett.* **2011**, *2*, 331–336; b) Siddiqui, N.; Alam, Md. S.; Ali, R.; Yar, M. S.; Alam, O. *Med. Chem. Res.* **2016**, *25*, 1390–1402; c) Walsh, J. J.; Bell, A. *Curr. Pharm. Des.* **2009**, *15*, 2970–2985.
6. Plech, T.; Wujec, M.; Kosikowska, U.; Malm, A.; Rajtar B.; Polz-Dacewicz, M. *Eur. J. Med. Chem.* **2013**, *60*, 128–134.
7. a) Zhang, S. L.; Chang, J. J.; Damu, G. L. V.; Geng, R. X.; Zhou, C. H. *Med. Chem. Commun.* **2013**, *4*, 839–846; b) Zhang, S. L.; Chang, J. J.; Damu, G. L. V.; Fang, B.; Zhou, X. D.; Geng, R. X.; Zhou, C. H. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1008–1012; c) Zhang, L.; Chang, J. J.; Zhang, S. L.; Damu, G. L. V.; Geng, R. X.; Zhou, C. H. *Bioorg. Med. Chem.* **2013**, *21*, 4158–4169; d) Jeyakkumar, P.; Zhang, L.; Avula, S. R.; Zhou, C. H. *Eur. J. Med. Chem.* **2016**, *122*, 205–215.
8. a) Chu, H. Y.; Jin, G. Z.; Eitan, F.; Zhen, X. C. *Cell Mol. Neurobiol.* **2008**, *28*, 491–499; b) Cheng, P.; Wang, B.; Liu, X.; Liu, W.; Kang, W.; Zhou, J.; Zeng, J. *Nat. Prod. Res.* **2014**, *28*, 413–419; c) Parraga, J.; Cabedo, N.; Andujar, S.; Piqueras, L.; Moreno, L.; Galan, A.; Angelina, E.; Enriz, R. D.; Ivorra, M. D.; Sanz, M. J.; Cortes, D. *Eur. J. Med. Chem.* **2013**, *68*, 150–166; d)

Sun, H; Zhu, L; Yang, H; Qian, W; Guo, L; Zhou, S; Gao, B; Li, Z; Zhou, Y; Jiang, H; Chena, K.; Zhen, X.; Liu, H. *Bioorg. Med. Chem.* **2013**, *21*, 856–868.

9. a) Zhang, S. L.; Damu, G. L. V.; Zhang, L.; Geng, R. X.; Zhou, C. H.. *Eur. J. Med. Chem.* **2012**, *55*, 164–175; b) Zhang, H. Z.; Damu, G. L. V.; Cai, G. X.; Zhou, C. H. *Eur. J. Med. Chem.* **2013**, *64*, 329–344.
10. a) Zhang, H. Z.; Lin, J. M.; Rasheed, S.; Zhou, C. H. *Sci. China Chem.* **2014**, *57*, 807–822; b) Fang, X. J.; Jeyakkumar, P.; Avula, S. R.; Zhou, Q.; Zhou, C. H. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2584–2588; c) Zhang, L.; Addla, D.; Jeyakkumar, P.; Wang, A.; Xie, D.; Wang, Y. N.; Zhang, S. L.; Geng, R. X.; Cai, G. X.; Li, S.; Zhou, C. H. *Eur. J. Med. Chem.* **2016**, *111*, 160–182.
11. Lee, Y. S.; Cho, Y. H.; Lee, S.; Bin, J. K.; Yang, J. H.; Chae, G. S.; Cheon, C. H. *Tetrahedron* **2015**, *71*, 532–538.
12. Yin, B. T.; Yan, C. Y.; Peng, X. M.; Zhang, S. L.; Rasheed, S.; Geng, R. X.; Zhou, C. H. *Eur. J. Med. Chem.* **2014**, *71*, 148–159.
13. Lv, J. S.; Peng, X. M.; Kishore, B.; Zhou, C. H. *Bioorg. Med. Chem. Lett*. **2014**, *24*, 308–313.
14. a) Zhang, F. F.; Gan, L. L.; Zhou, C. H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1881–1884; b) Wen, S. Q.; Jeyakkumar, P.; Avula, S. R.; Zhang, L.; Zhou, C. H. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2768–2773.
15. Zhang, Y. Y.; Zhou, C. H. *Bioorg. Med. Chem. Lett*. **2011**, *21*, 4349–4352.
16. Bodiwala, H. S.; Sabde, S.; Mitra, D.; Bhutani, K. K.; Singh, I. P. *Eur. J. Med. Chem.* **2011**, *46*, 1045–1049.
17. Ge, H. X.; Zhang, J.; Chen, L.; Kou, J. P.; Yu, B. Y. *Bioorg. Med. Chem.* **2013** *21*, 62–69.
18. Liu, Y. B.; Wang, C. Y.; Li, M. J.; Lai, G. Q.; Shen, Y. J. *Macromolecules* **2008**, *41*, 2045–2048.
19. a) Wang, X. L.; Wan, K.; Zhou, C. H. *Eur. J. Med. Chem.* **2010**, *45*, 4631–4639; b) Dhar, P.; Chan, P. Y.; Cohen, D. T.; Khawam, F.; Gibbons, S.; Snyder-Leiby, T.; Dickstein, E.; Rai, P. K.; Watal, G. *J. Agric, Food Chem.* **2014**, *62*, 3548−3552; c) Calabrese, E. C.; Castellano, S.; Santoriello, M.; Sgherri, C.; Quartacci, M. F.; Calucci, L.; Warrilow, A. G. S.; Lamb, D. C.; Kelly, S. L.; Milite, C.; Granata, I.; Sbardella, G.; Stefancich, G.; Maresca, B.; Porta, A. *J. Antimicrob. Chemother.* **2013**, *68*, 1111–1119; d) Bordallo-Cardona, M. Á.; Escribano, P.; de la Pedrosa, E. G. G.; Marcos-Zambrano, L. J.; Cantón, R.; Bouza, E.; Guinea, J. *Antimicrob. Agents Chemother.* **2017**, *61*, e01542−16.
20. Lohan, S.; Cameotra, S. S.; Bisht, G. S. *Eur. J. Med. Chem.* **2014**, *83*, 102−115.
21. a) Berti, F.; Bincoletto, S.; Donati, I.; Fontanive, G.; Fregonese, M.; Benedetti, F. *Org. Biomol. Chem.* **2011**, *9*, 1987–1999; b) Hu, Y. J.; Liu, Y.; Xiao, X. H. *Biomacromolecules* **2009**, *10*, 517–521.
22. a) Li, X. L.; Hu, Y. J.; Wang, H.; Yu, B. Q.; Yue, H. L. *Biomacromolecules* **2012**, *13*, 873–880; b) Addla, D.; Wen, S. Q.; Gao, W. W.; Maddili, S. K.; Zhang, L.; Zhou, C. H. *Med. Chem. Commun*. **2016**, *7*,1988–1994.
23. a) Dai, L. L.; Zhang, H. Z.; Nagarajan, S.; Rasheed, S.; Zhou, C. H. *Med. Chem. Commun*. **2015**, *6*, 147–154; b) Zhang, G. W.; Fu, P.; Wang, L.; Hu, M. M. *J. Agric. Food Chem.* **2011**, *59*, 8944–8952; c) Gong, H. H.; Baathulaa, K.; Lv, J. S.; Cai, G. X.; Zhou, C. H. *Med. Chem. Commun*. **2013**, *7*, 924–931.
24. Peng, L. P.; Nagarajan, S.; Rasheed, S.; Zhou, C. H. *Med. Chem. Commun*. **2015**, *6*, 222−229.
25. Peng, X. M.; Kumar, K. V.; Damu, G. L. V.; Zhou, C. H. *Sci. China Chem.* **2015**, *59*, 878−894.
26. Experimental: Melting points are determined on X-6 melting point apparatus and uncorrected. IR spectra were determined on a Bio-Rad FTS-185 spectrophotometer in the range of 400-4000 cm-1. 1H NMR and ^{13}C NMR spectra were recorded on a Bruker AV 600 or Varian 300 spectrometer using TMS as an internal standard. Chemical shifts were reported in parts per million (ppm), the coupling constants (*J*) were expressed in hertz (Hz) and signals were described as singlet (s), doublet (d), triplet (t), as well as multiplet (m). The mass spectra (MS) were recorded on LCMS-2010A and the high-resolution mass spectra (HRMS) were recorded on an IonSpec FT-ICR mass spectrometer with ESI resource. The following abbreviations were used HMTA: hexamethelene tetramine; TFA: trifluoroacetic acid; MIC: minimal inhibitory concentration; IC_{50}: 50% inhibiting concentration of drug; NR: Neutral Red. All chemicals and solvents were commercially available, and used without further purification. The concentration of DNA in stock solution was determined by UV absorption at 260 nm using a molar absorption coefficient ξ_{260} = 6600 L mol^{-1} cm^{-1} (expressed as molarity of phosphate groups) by Bouguer-Lambert-Beer law. The purity of the DNA was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of > 1.8 at A260/A280, which indicates that DNA was sufficiently free from protein. All of the solutions were adjusted with Tris-HCl buffer solution (pH = 7.4), which was prepared by mixing and diluting Tris solution with HCl solution. All chemicals were of analytical reagent grade, and doubly distilled water was used throughout.
27. *Synthesis of tetrahydroprotoberberrubine-12-benzimidazole (**5a**)* A solution of *o*-phenylenediamine (276 mg, 2.6 mmol) and intermediate **4** (1 g, 2.8 mmol) in wet DMF (DMF 9.0 mL, H_2O 1.0 mL) was stirred at 80 °C in an open flask, and the reaction progress was monitored by TLC. On the complete consumption of reactant, the reaction mixture was cooled to room temperature and added ice water (50 mL). The resulting product was isolated by filtration and dried, which was further purified by column chromatography on silica gel to afford the corresponding benzimidazole **5a** (490 mg). Yield: 40%; mp: 242–244 °C; IR (KBr) *v*: 3415 (NH and OH), 2911, 2840 (aliphatic C–H), 2762, 1621 (C=C) cm^{-1}, 1244 (C–O) cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.34 (s, 1H, Bim-N*H*), 9.16 (s, 1H, O*H*), 7.63 (d, *J* = 7.3 Hz, 1H, Bim-*H*), 7.49 (d, *J* = 7.3 Hz, 1H, Bim-*H*), 7.30 (s, 1H, 11-H), 7.21–7.14 (m, 2H, Bim-*H*), 6.78 (s, 1H, 1-*H*), 6.69 (s, 1H, 4-*H*), 5.93 (d, J = 7.7 Hz, 2H, OCH_2O), 4.10 (d, *J* = 15.7 Hz, 1H), 3.88 (s, 3H, OCH_3), 3.54 (dd, *J* = 16.5, 2.8 Hz, 1H), 3.39 (t, *J* = 11.5 Hz, 2H), 3.16–3.11 (m, 1H), 3.02 (dd, *J* = 16.4, 11.3 Hz, 1H), 2.97–2.91 (m, 1H), 2.63 (d, *J* = 15.7 Hz, 1H), 2.47 (d, *J* = 11.2 Hz, 1H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ: 152.6, 146.1, 145.9, 145.1, 144.2, 143.9, 134.9, 131.5, 128.1, 127.9, 123.6, 122.3, 121.6, 120.8, 119.1, 112.1, 111.4, 108.5, 106.3, 100.9, 59.5, 56.5, 54.4, 50.9, 36.1, 29.6 ppm; HRMS (ESI): calcd for $C_{26}H_{23}N_3O_4$ $[M+H]^+$, 442.1767; found, 442.1768.