Supplementary Information

Aloe-emodin derived azoles as a new structural type of potential antibacterial agents: Design, synthesis and evaluation of the action on membrane, DNA and MRSA DNA isomerase

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1. Experimental protocols

1.1 General methods

X-6 melting point apparatus was used to measure the melting points. Precoated silica gel plates were used to perform TLC analysis. Bruker AV 600 spectrometer was used to record NMR spectra by treating tetramethylsilane (TMS) as an internal standard. IonSpec FT-ICR mass spectrometer with ESI resource is used to record the high-resolution mass spectra (HRMS). All fluorescence spectra were recorded on F-7000 Spectrofluorimeter (Hitachi, Tokyo, Japan) equipped with 1.0 cm quartz cells, the widths of both the excitation and emission slit were set as 2.5 nm, and the excitation wavelength was 280 nm. Fluorescence spectra were recorded at 290 K in the range of 300–550 nm. The UV spectrums were recorded at room temperature on a TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells. Tris and HCl were analytical purity. Sample masses were weighed on a microbalance with a resolution of 0.1 mg. All other chemicals and solvents were commercially available, and were used without further purification.

The stock solution of compound 4b was prepared in DMSO. Calf thymus DNA (Sigma Chemical Co., St. Louis, MO) was used without further purification, and its stock solution was prepared by dissolving an appropriate amount of DNA in doubly distilled water. The solution was allowed to stand overnight and stored at 4 °C in the dark for about one week. The concentration of DNA in stock solution was determined by UV absorption at 260 nm using a molar absorption coefficient \( \xi_{260} = 6600 \text{ L\cdot mol}^{-1}\cdot \text{cm}^{-1} \) (expressed as molarity of phosphate groups) by Bouguer-Lambert-Beer law. The purity of 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 indicated that DNA was sufficiently free from protein. NR stock solution was prepared by dissolving its solid (Sigma Chemical Co.) in doubly distilled water and was kept in a cool and dark place. All 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.

1.2. Antibacterial and antifungal assays

In comparison with the bacteria, the tested fungi were less sensitive to target aloe-emodin azoles 3-8. The target aloe-emodins gave no obvious suppression on C. albicans strains, and displayed low activity towards C. parapsilosis ATCC 22019, except for 2-mercaptotriazole derivative 7b (MIC = 16 μg/mL). In addition, as for C. tropicalis, aloe-emodins 4a,
4b and 8 exhibited moderate potency, which might be attributed to the existence of 1,2,4-triazole, non-substituted tetrazole, indole and 2-mercaptopbenzimidazole, respectively. Remarkably, all the aloe-emodin azoles showed better activities against *A. fumigatus* (MIC = 4–64 μg/mL) than clinical antifungal fluconazole (MIC = 256 μg/mL), except for 2-mercapto-1-methylimidazole 7a. It is worth noting that benzotriazolyl aloe-emodin 5e and 2-mercaptotriazole derivative 7b possessed prominent suppression on *A. fumigatus* strains with MIC value as low as 4 μg/mL. Therefore, the two compounds are promising to serve as anti-*A. fumigatus* agents to be further exploited.

Table S1. Antifungal data as MIC (μg·mL⁻¹) for compounds 3-8.

<table>
<thead>
<tr>
<th>Compds</th>
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*Fluconazole* 4 8 256 2

*C. a., Candida albicans; C. t., Candida tropicalis; A. f., Aspergillus fumigatus; C. p., C. p. 22019, Candida parapsilosis ATCC 22019.*

The prepared compounds were evaluated for their antibacterial activities against Gram-positive bacteria (MRSA, *Enterococcus faecalis, Staphylococcus aureus, Staphylococcus aureus ATCC 25923, and Staphylococcus aureus ATCC 29213*) and Gram-negative bacteria (*Klebsiella pneumoniae, Escherichia coli, Escherichia coli ATCC 25922, Pseudomonas aeruginosa, Pseudomonas aeruginosa ATCC 27853, and Acinetobacter baumannii*) with the positive control of clinical antibacterial drug norfloxacin. The bacterial suspension was adjusted with sterile saline to a concentration of 1 × 10⁵ CFU. Initially the compounds were dissolved in DMSO to prepare the stock solutions, then the tested compounds and reference drugs were prepared in Mueller-Hinton broth (Guangdong huaihai microbial sci. & tech co., Ltd, Guangzhou, Guangdong, China) to obtain the required concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 μg·mL⁻¹. These dilutions were inoculated and incubated at 37 °C for 24 h. Antibacterial screening demonstrated that some of the target compounds could effectively inhibit the growth of the tested bacteria and exhibit broad antimicrobial spectrum.

Likewise, all the prepared hybrids were tested for their antifungal properties against four fungi (*Candida albicans, Candida tropicalis, Aspergillus fumigatus, and Candida parapsilosis ATCC 22019*) with respect to clinical fluconazole. A spore suspension in sterile distilled water was prepared from one day old culture of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was observed to be 1–5×10⁵ spore·mL⁻¹. Eleven required concentrations
Minimal inhibitory concentration (MIC, μg·mL\(^{-1}\)) is defined as the lowest concentration of the new compounds that completely inhibit the growth of fungi, by means of standard two-fold serial dilution method in 96-well microtest plates (0.5–512 μg·mL\(^{-1}\)) of each tested compound were prepared by making dilutions in sterile RPMI 1640 medium using compound’s stock solutions. These dilutions were inoculated and incubated at 35 °C for 24 h according to the Clinical & Laboratory Standards Institute (CLSI). The tested microorganism strains were provided by the Clinical Laboratory Department, Sichuan Academy of Medical Sciences & Sichuan Provincial People’s Hospital. To ensure that the solvent had no effect on antifungal growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. All the fungi growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of fungi was regarded as minimal inhibitory concentration (MIC).

1.3. Resistance development assay

The representative compound 4b was selected to investigate the developing rate of bacterial resistance according to the reported method. We exposed a standard strain of resistant MRSA toward increasing concentrations of compound 4b from sub-MIC (0.5 × MIC, MIC is the minimal inhibitory concentration) for sustained passages and determined the new MIC values of compound 4b for each passage of MRSA. The initial MIC values of compound 4b and norfloxacin were determined against MRSA as mentioned above in antibacterial assay. For the next MIC experiment, the bacterial dilution was prepared by using the bacteria from sub-MIC concentration of the compound (0.5 × MIC). After a 12 h incubation period, again bacterial dilution was prepared by using the bacterial suspension from sub-MIC concentration of the compound (0.5×MIC) and assayed for the next MIC experiment. The process was repeated for 15 passages. The MIC values for compound 4b and norfloxacin against each passage of MRSA were determined.

1.4. Cytotoxicity

Cell viability upon the treatment of the prepared compounds were tested by using the CCK8 assay. Briefly, a suspension of cells (3000/well for normal human liver cell line LO2) were seeded in 96-well plates and cultured for 24 h. Then different concentrations ranging from 100 to 6.25 μM (PBS buffer containing 1% DMSO) for the compound 4b were added into the 96-well plates, in which cells were incubated for 72 h. Then, 10 μL of CCK8 solution was added to each well of the plates and incubated at 37 °C for 2 h. The absorbance of each well was measured at 450 nm in a microplate reader. At last, resultant OD\(_{450\text{ nm}}\) values were expressed as IC\(_{50}\) values. The anti-tumor growth effects of compound 4b \textit{in vitro} were studied \textit{via} measuring the viability of Hep-G2 cell using the above approach. The relative inhibition rates of cancer cell growth and IC\(_{50}\) values were calculated and plotted by GraphPad Prism6. Experiments were repeated at least three times.

![Figure S1](image-url). Cytotoxic assay of the active compound 4b on human hepatocyte LO2 cell line, IC\(_{50}\) = 26.62, selectivity index = 11.76 (left)
1.5. ROS generation

Intracellular ROS was measured using standard 2,7-dichlorofluoroscein diacetate (DCFH-DA) assay. Then 10⁶ CFU/mL of MRSA strain cells were treated with increasing concentrations of compound 4b for 6 h at 37 °C. Following treatment, both control and treated cells were washed with PBS and incubated with 10 μM DCFH-DA probe for 30 min in dark at 37 °C. The green fluorescence originating from the oxidative cleavage of DCFH-DA to DCF was measured with an excitation wavelength of 485 nm and emission wavelength of 528 nm. The fold increase in intracellular ROS production in cells treated with compound 4b in comparison to control cells was plotted.

![Figure S2. Changes of ROS level in MRSA treated by compound 4b (12× MIC).](image)

1.6. Bacterial membrane permeabilization

The overnight grown culture of MRSA was harvested (10000 rpm, 1 min), washed, and resuspended in 5 mM glucose and 5 mM HEPES buffer (pH = 7.4) in 1:1 ratio. Then an amount of 10 µL of test compound 4b (12 × MIC) was added to a cuvette containing 2 mL of bacterial suspension and 10 µM propidium iodide (PI). 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). A control experiment was performed by treating the preincubated bacterial and dye solution only with water (10 µL).

1.7. Fluorescence microscopy

The overnight grown culture of MRSA was treated with compound 4b (12 × MIC), and 0.1% Triton X-100 was used as positive control. All the treated and untreated cells (as negative control) were washed once with 1 × PBS (the images were captured with a 10 × objective in electron microscope) and stained with 4.5 mM PI for 15 min at 37 °C. Finally, the images were captured with a 10 × objective in fluorescence microscope using green light.

1.8. Interactions with calf thymus DNA

DNA is an informational molecule encoding the genetic information, which makes it one of the objectives for the studies of biologically important small molecules such as antimicrobial drugs. Recently, DNA is increasingly investigated for interaction with small molecules in the rational design and construction of novel and efficient drugs. To explore the possible antimicrobial action mechanism, calf thymus DNA was selected as DNA model because of its medical importance, low cost and ready availability properties to study the binding behavior of compound 4b with calf thymus DNA on molecular level using neutral red (NR) dye as a spectral probe in vitro by UV-vis spectroscopic methods.
1.8.1. Absorption spectra of DNA in the presence of compound 4b

In this study, UV–vis absorption spectra were documented while the concentration of DNA being fixed with the consecutive and proportional increase of the concentration of compound 4b. As demonstrated in Fig. S3, the UV-vis absorption of DNA was first observed at 260 nm which showed gradual increase in absorbance associated by a slight red shift with the raising concentration of compound 4b. Moreover, the absorbance of 4b–DNA complex (inset of Fig. S2) was recorded less than that of the sum of free DNA and free compound 4b, which signified that a weak hypochromic effect has occurred between the CT-DNA and compound 4b. Both the hypochromic effect and hypochromic region preliminarily disclosed that the interaction mode of compound 4b was the intercalation into DNA, which were in accordance with the displayed spectral changes.

On the base of variations in the absorption spectra of DNA upon binding to compound 4b, equation (1) can be utilized to calculate the binding constant (K).

\[
\frac{A^0}{A - A^0} = \frac{\varepsilon_C}{\varepsilon_{D-C} - \varepsilon_C} + \frac{\varepsilon_C}{\varepsilon_{D-C} - \varepsilon_C} \times \frac{1}{K[Q]} \tag{1}
\]

\(A^0\) refers to the absorbance of DNA in the absence of compound 4b while \(A\) refers to the absorbance in the presence of 4b at 260 nm. In addition, \(\varepsilon_C\) and \(\varepsilon_{D-C}\) represents the absorption coefficients of compound 4b and compound 4b–DNA complex respectively. Also, [Q] stands for the volumetric molar concentration of compound 4b measured as mol·L\(^{-1}\) unit. The plot of \(A^0/(A - A^0)\) vs 1/[compound 4b] is plotted by making use of the absorption titration data and linear fitting (Fig. S4), binding constant, \(K = 3.421 \times 10^5\ L\cdot mol^{-1}\), (R = 0.990, SD = 0.008). Here, R is the correlation coefficient while SD refers to standard deviation. (R is the correlation coefficient, and SD is standard deviation).

![Absorption spectra of DNA with different concentrations of compound 4b](image)

**Figure S3.** UV absorption spectra of DNA with different concentrations of compound 4b (pH = 7.4, T = 290 K). Inset: comparison of absorption at 260 nm between the compound 4b–DNA complex and the sum values of free DNA and free compound 4b. \(c(DNA) = 5.25 \times 10^{-5}\) mol·L\(^{-1}\), and \(c(\text{compound 4b}) = 0–1.25 \times 10^{-5}\) mol·L\(^{-1}\) for curves a–g respectively at increment 0.25 \(\times 10^{-5}\).
1.8.2. Absorption spectra of NR interactions with DNA

To further understand the interaction between compound 4b and DNA, the absorption spectra of competitive interaction of compound 4b with Neutral Red (NR) were investigated. NR is a planar phenazine dye possessing a higher stability, lower toxicity and more convenient application, compared with other common probes. Furthermore, the binding of NR with DNA is an intercalative mode which has been sufficiently demonstrated. Therefore, NR was used as a spectral probe to investigate the binding mode of 4b with DNA in the present work. The absorption spectra of the NR dye upon the addition of DNA are depicted in Fig. S5. Apparently, the absorption peak of the NR at around 460 nm showed gradual decrease with the increasing concentration of DNA, and a new band at around 530 nm developed. This could be attributed to the formation of the new DNA–NR complex. An isosbestic point at 504 nm provided additional evidence of DNA–NR complex formation.

![Figure S5](image)

Figure S5. UV absorption spectra for NR and DNA (pH = 7.4, T = 298 K). $c$(NR) = $2 \times 10^{-5}$ mol·L$^{-1}$, and $c$(DNA) = $0–3.81 \times 10^{-5}$ mol·L$^{-1}$ at an increment of $0.48 \times 10^{-5}$ for curves a–i respectively.

1.9. Molecular docking

To further understand the mechanism of action of the synthesized compounds, highly active molecule 4b was docked
into the active site of MRSA DNA isomerase (PDB code: 2XCS). Sybyl 2.0 and Pymol 4.5.0 was used to perform the docking work. The grid size was set to be $45 \times 45 \times 45$ and the grid point spacing was set at default value 0.375 Å. The Lamarkian genetic algorithm (LGA) was applied for the conformational search.

1.10. General procedure and spectral data for some prepared compounds

1.10.1. Synthesis of 3-(chloromethyl)-1,8-dihydroxyanthracene-9,10-dione (2)

Aloe emodin (1) (2.70 g, 10 mmol) was dissolved in 200 mL anhydrous $N, N$-dimethylformamide, and sulfoxide chloride (20 mL, 275 mmol) was slowly dropped in an ice bath. After stirring for 1 h, the reaction was removed from the ice bath at room temperature. TLC was followed until the end of the reaction. The compound 2 was quenched with ice water, filtered, washed and recrystallized. Yield: 61.0%, melting point: 138—140 ºC.

1.10.2. Synthesis of 1,8-dihydroxy-3-((2-nitro-1H-imidazol-1-yl)methyl)anthracene-9,10-dione (3a)

A mixture of 2-nitroimidazole (84 mg, 0.75 mmol) and potassium carbonate (138 mg, 1.00 mmol) were stirred in acetonitrile (10 mL) at 65 ºC. Compound 2 (144 mg, 0.50 mmol) was added and reflux was performed at 80 ºC. Thin-layer chromatography was followed until the end of the reaction. After cooling to room temperature and neutralizing with glacial acetic acid until pH was neutral, the filtrate was then extracted and purified by column chromatography (Eluent: petroleum ether/ethyl acetate (10/1–10/3, V/V)), the compound 3a (40 mg) was obtained as yellow solid, yield: 22.4%, melting point: >250 ºC. $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 11.97 (s, 2H, anthraquinone-1,8-OH), 8.46 (s, 1H, imidazole-4-H), 8.15 (s, 1H, imidazole-5-H), 7.86 (d, $J = 7.7$ Hz, 1H, anthraquinone-5-H), 7.84 (s, 1H, anthraquinone-4-H), 7.83 (d, $J = 8.2$ Hz, 1H, anthraquinone-7-H), 7.78 (d, $J = 7.4$ Hz, 1H, anthraquinone-6-H), 7.44 (s, 1H, anthraquinone-2-H), 5.75 (s, 2H, CH$_2$); $^{13}$C NMR (151 MHz, DMSO-$d_6$) $\delta$ 191.98, 181.67, 161.93, 147.84, 146.20, 138.15, 137.96, 134.40, 133.73, 125.03, 123.50, 122.13, 119.89, 118.78, 116.36, 50.52; HRMS (ESI) calcd. for C$_{18}$H$_{11}$N$_3$O$_6$ [M+H]$^+$: 366.0726; found, 366.0725.

The preparation method of compound 3b-8 is the same as that of compound 3a.

1.10.3. Synthesis of 1,8-dihydroxy-3-((4-nitro-1H-imidazol-1-yl)methyl)anthracene-9,10-dione (3b)

Yellow solid, yield: 60.4%, melting point: >250 ºC. $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 11.90 (s, 2H, anthraquinone-1,8-OH), 8.59 (s, 1H, imidazole-5-CH), 8.09 (s, 1H, imidazole-2-CH), 7.81 (t, $J = 7.9$ Hz, 1H, 6-CH$_2$), 7.71–7.68 (m, 2H, anthraquinone-4,5-2CH), 7.40 (d, $J = 8.2$ Hz, 1H, anthraquinone-7-CH), 7.38 (d, $J = 10.0$ Hz, 1H, anthraquinone-2-CH), 5.46 (s, 2H, CH$_2$); $^{13}$C NMR (151 MHz, DMSO-$d_6$) $\delta$ 191.98, 181.67, 161.93, 147.84, 146.20, 138.15, 137.96, 134.40, 133.74, 125.01, 123.49, 122.13, 119.89, 118.78, 116.36, 50.52; HRMS (ESI) calcd. for C$_{18}$H$_{11}$N$_3$O$_6$ [M+H]$^+$: 366.0726; found, 366.0725.

1.10.4. Synthesis of 1,8-dihydroxy-3-((2-methyl-5-nitro-1H-imidazol-1-yl)methyl)anthracene-9,10-dione (3c)
Yellow solid, yield: 31.1%, melting point: \(>250\ ^\circ\mathrm{C}\); \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 11.95 (s, 2H, anthraquinone-1,8-O\(H\)), 10.14 (s, 1H, imidazole-4-\(H\)), 8.15 (s, 1H, anthraquinone-4-\(H\)), 7.87 (d, \(J = 8.1\) Hz, 1H, anthraquinone-5-\(H\)), 7.85 (d, \(J = 4.9\) Hz, 1H, anthraquinone-7-\(H\)), 7.79 (t, \(J = 7.1\) Hz, 1H, anthraquinone-6-\(H\)), 7.44 (d, \(J = 8.4\) Hz, 1H, anthraquinone-2-\(H\)), 5.33 (d, \(J = 4.6\) Hz, 1H, \(CH_2\)), 5.23 (s, 1H, \(CH_2\)), 1.24 (s, 3H, \(CH_3\)); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) \(\delta\) 161.87, 144.96, 137.96, 134.38, 125.02, 123.70, 119.90, 118.84, 116.42, 79.63, 40.53, 12.91; HRMS (ESI) calcd. for C\(_{16}\)H\(_{10}\)N\(_4\)O\(_4\) [M + H]+, 323.0780; found, 323.0782.

1.10.5. Synthesis of 3-((1H-1,2,4-triazol-1-yl)methyl)-1,8-dihydroxyanthracene-9,10-dione (4a)

Yellow solid, yield: 46.2%, melting point: \(>250\ ^\circ\mathrm{C}\); \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 12.05 (s, 1H, anthraquinone-8-O\(H\)), 11.98 (s, 1H, anthraquinone-1-O\(H\)), 8.21 (s, 1H, triazole-5-\(H\)), 8.04 (s, 1H, triazole-3-\(H\)), 7.84 (d, \(J = 7.2\) Hz, 1H, anthraquinone-5-\(H\)), 7.70 (d, \(J = 2.4\) Hz, 2H, anthraquinone-6,4-\(H\)), 7.32 (d, \(J = 8.4\) Hz, 1H, anthraquinone-7-\(H\)), 7.09 (s, 1H, anthraquinone-2-\(H\)), 5.45 (s, 2H, \(CH_2\)); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 194.62–188.82, 162.99, 152.81, 145.98–141.91, 137.47, 135.29–131.60, 124.93, 122.89, 118.60, 52.67; HRMS (ESI) calcd. for C\(_{17}\)H\(_{11}\)N\(_3\)O\(_4\) [M+H]+: 322.0828; found, 322.0819.

1.10.6. Synthesis of 3-((1H-tetrazol-1-yl)methyl)-1,8-dihydroxyanthracene-9,10-dione (4b)

Yellow solid, yield: 49.2%, melting point: \(>250\ ^\circ\mathrm{C}\); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 12.04 (s, 1H, anthraquinone-8-O\(H\)), 11.95 (s, 1H, anthraquinone-1-O\(H\)), 8.59 (s, 1H, tetrazole-5-\(H\)), 7.84 (s, 1H, anthraquinone-4-\(H\)), 7.77 (d, \(J = 7.5\) Hz, 1H, anthraquinone-5-\(H\)), 7.70 (s, 1H, anthraquinone-7-\(H\)), 7.32 (dd, \(J = 8.5, 2.4\) Hz, 1H, anthraquinone-6-\(H\)), 7.18 (s, 1H, anthraquinone-2-\(H\)), 5.90 (s, 2H, \(CH_2\)); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 161.87, 144.96, 137.96, 134.38, 125.02, 123.70, 119.90, 118.84, 116.42, 55.32; HRMS (ESI) calcd. for C\(_{16}\)H\(_{10}\)N\(_4\)O\(_4\) [M+H]+, 323.0780; found, 323.0783.

1.10.7. Synthesis of 1,8-dihydroxy-3-((5-methyl-1H-tetrazol-1-yl)methyl)anthracene-9,10-dione (4c)

Yellow solid, yield: 42.1%, melting point: \(>250\ ^\circ\mathrm{C}\); \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 11.90 (s, 2H, anthraquinone-1,8-O\(H\)), 7.81 (s, 1H, anthraquinone-4-\(H\)), 7.71 (s, 1H, anthraquinone-5-\(H\)), 7.53 (d, \(J = 1.3\) Hz, 1H, anthraquinone-6-\(H\)), 7.40 (s, 1H, anthraquinone-7-\(H\)), 7.22 (s, 1H, anthraquinone-2-\(H\)), 5.82 (s, 2H, \(CH_2\)), 2.55 (s, 3H, \(CH_3\)); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) \(\delta\) 161.89, 119.89, 49.43, 14.54; HRMS (ESI) calcd. for C\(_{17}\)H\(_{13}\)N\(_4\)O\(_4\) [M+H]+: 337.0937; found, 337.0938.

1.10.8. Synthesis of 3-((1H-benzo[d]imidazol-1-yl)methyl)-1,8-dihydroxyanthracene-9,10-dione (5a)

Yellow solid, yield: 61.1%, melting point: \(>250\ ^\circ\mathrm{C}\); \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 11.90 (s, 2H, anthraquinone-1,8-O\(H\)), 7.81 (s, 1H, benzimidazole-2-\(CH\)), 7.78 (s, 1H, anthraquinone-4-\(CH\)), 7.71 (d, \(J = 7.2\) Hz, 1H, anthraquinone-5-\(CH\)), 7.67 (s, 1H, benzimidazole-4-\(CH\)), 7.57–7.53 (m, 2H, anthraquinone-6,7-2\(CH\)), 7.37 (s, 1H, anthraquinone-2-\(CH\)), 7.23 (dd, \(J = 8.9, 3.4\) Hz, 3H, benzimidazole-5,6,7-3\(CH\)), 5.69 (s, 2H, \(CH_2\)); \(^{13}\)C NMR (151 MHz,
DMSO-\(d_6\) δ191.88, 181.57, 161.89 (d, \(J = 15.8\) Hz), 146.02, 137.15, 125.03, 123.43, 122.12, 119.87, 118.78, 119.89, 49.43; HRMS (ESI) calcd. for C\(_{22}\)H\(_{14}\)N\(_2\)O\(_4\) [M+H]\(^+\): 371.1032; found, 371.1031.

1.10.9. Synthesis of 3-((2-ethyl-1H-benzo[d]imidazol-1-yl)methyl)-1,8-dihydroxyanthracene-9,10-dione (5b)

Yellow solid, yield: 31.5%, melting point: >250 °C; \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) δ 7.81–7.77 (m, 1H, anthraquinone-6-H), 7.67 (d, \(J = 6.8\) Hz, 1H, anthraquinone-5-H), 7.63 (dd, \(J = 5.2, 3.7\) Hz, 1H, benzoimidazole-4-H), 7.48 (dd, \(J = 5.3, 3.7\) Hz, 1H, benzoimidazole-7-H), 7.20 (dd, \(J = 9.0, 5.2\) Hz, 2H, benzoimidazole-5, 6-H), 6.97 (s, 1H, anthraquinone-2-H), 5.68 (s, 2H, CH\(_2\)), 2.74 (dd, \(J = 12.2, 6.1\) Hz, 2H, CH\(_3\)CH\(_2\)), 1.30 (t, \(J = 7.4\) Hz, 3H, CH\(_3\)); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) δ 191.98, 181.67, 161.93, 147.02, 138.15, 137.96, 125.01, 123.49, 122.13, 119.89, 118.78, 50.52, 21.54, 11.81; HRMS (ESI) calcd. for C\(_{24}\)H\(_{18}\)N\(_2\)O\(_4\) [M+H]\(^+\): 399.1345; found, 399.1346.

1.10.10. Synthesis of 3-((5,6-dimethyl-1H-benzo[d]imidazol-1-yl)methyl)-1,8-dihydroxyanthracene-9,10-dione (5c)

Yellow solid, yield: 28.8%, melting point: >250 °C; \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) δ 11.90 (s, 1H, anthraquinone-8-OH), 11.88 (s, 1H, anthraquinone-1-OH), 8.33 (s, 1H, benzoimidazole-2-H), 7.82–7.77 (m, 1H, anthraquinone-6-H), 7.68 (dd, \(J = 7.5, 1.0\) Hz, 1H, anthraquinone-5-H), 7.52 (t, \(J = 4.4\) Hz, 1H, anthraquinone-7-H), 7.46 (s, 1H, anthraquinone-4-H), 7.37 (d, \(J = 1.1\) Hz, 1H, benzoimidazole-4-H), 7.31 (s, 1H, anthraquinone-2-H), 7.16 (d, \(J = 1.5\) Hz, 1H, benzoimidazole-7-H), 5.62 (s, 2H, CH\(_2\)), 1.26–1.23 (s, 6H, CH\(_3\)); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) δ 191.09, 180.21, 161.46, 161.06, 144.73, 140.89, 139.44, 136.90, 136.10, 134.05, 132.97, 132.5, 132.05, 131.22, 130.46, 129.87, 123.51, 120.64, 118.82, 118.13, 116.67, 114.37, 114.22, 112.95, 108.89, 48.17, 18.92, 18.76; HRMS (ESI) calcd. for C\(_{24}\)H\(_{18}\)N\(_2\)O\(_4\) [M+H]\(^+\): 399.1346; found, 399.1346.

1.10.11. Synthesis of 1,8-dihydroxy-3-((5-nitro-1H-benzo[d]imidazol-1-yl)methyl)anthracene-9,10-dione (5d)

Yellow solid, yield: 27.2%, melting point: >250 °C; \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) δ 11.91 (s, 1H, anthraquinone-8-OH), 11.88 (s, 1H, anthraquinone-1-OH), 7.88 (s, 1H, benzimidazole-5-C), 7.83–7.80 (m, 1H, anthraquinone-5-C), 7.71 (d, \(J = 7.4\) Hz, 1H, benzimidazole-7-C), 7.54 (d, \(J = 1.2\) Hz, 1H, anthraquinone-4-C), 7.40 – 7.39 (m, 1H, anthraquinone-6-C), 7.30 (s, 1H, anthraquinone-4-C), 7.18 (s, 1H, anthraquinone-2-C), 5.39 (s, 2H); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) δ 192.01, 181.76, 162.04, 161.83, 148.28, 137.92, 134.23, 133.77, 124.98, 122.74, 119.88, 118.34, 116.45, 49.39, 21.34; HRMS (ESI) calcd. for C\(_{22}\)H\(_{13}\)N\(_3\)O\(_6\) [M+H]\(^+\): 416.0883; found, 416.0886.

1.10.12. Synthesis of 3-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,8-dihydroxyanthracene-9,10-dione (5e)

Yellow solid, yield: 22.1%, melting point: >250 °C; \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) δ 12.02 (s, 1H, 8-OH), 11.98 (s, 1H, anthraquinone-1-OH), 7.88 (dd, \(J = 6.6, 3.1\) Hz, 2H, benzotriazole-4,7-C), 7.82 (d, \(J = 7.5\) Hz, 2H, anthraquinone-4,5-C), 7.68 (t, \(J = 8.0\) Hz, 1H, anthraquinone-6-C), 7.41 (dd, \(J = 6.6, 3.1\) Hz, 2H, benzotriazole-5,6-C), 7.30 (d, \(J = 8.4\) Hz, 1H, anthraquinone-7-C), 7.17 (s, 1H, anthraquinone-2-C), 5.97 (s, 2H, CH\(_2\)); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) δ 192.64, 181.19, 162.88, 162.65, 144.89, 144.53, 137.38, 134.18, 133.47, 126.86,
124.80, 123.34, 120.18, 119.23, 118.20, 115.73, 59.38; HRMS (ESI) calcd. for C_{21}H_{13}N_{3}O_{4} [M+H]^+: 372.0984; found, 372.0985.

1.10.13. Synthesis of 3-((9H-carbazol-9-yl)methyl)-1,8-dihydroxyanthracene-9,10-dione (6)  
Yellow solid, yield: 19.1%, melting point: >250 °C; ^1H NMR (600 MHz, CDCl₃) δ 11.98 (s, 1H, anthraquinone-8-OH), 11.95 (s, 1H, anthraquinone-1-OH), 8.13 (d, J = 7.7 Hz, 2H, carbazolyl-4,5-2CH), 7.80 (d, J = 7.5 Hz, 1H, carbazolyl-1,8-2CH), 7.77 (s, 1H, anthraquinone-4-CH), 7.66 (t, J = 7.5 Hz, 1H, anthraquinone-6-CH), 7.31 (d, J = 8.2 Hz, 2H, anthraquinone-5,7-2CH), 7.29 (d, J = 1.7 Hz, 1H, carbazolyl-1-CH), 7.27 (d, J = 7.9 Hz, 2H, carbazolyl-3,6-CH), 6.86 (s, 1H, anthraquinone-2-CH), 5.56 (s, 2H, CH₂); ^13C NMR (151 MHz, DMSO-d₆) δ 192.89–190.02, 182.50–179.33, 162.08–161.37, 140.56, 138.02–137.62, 134.33–133.22, 130.32–129.75, 126.53, 125.12–124.55, 122.85, 122.14–121.43, 120.96, 118.04–116.91, 116.61–112.85, 109.90, 45.75; HRMS (ESI) calcd. for C_{27}H_{17}NO_{4} [M+H]^+: 420.1236; found, 420.1233.

1.10.14. Synthesis of 1,8-dihydroxy-3-(((1-methyl-1H-imidazol-2-yl)thio)methyl)anthracene-9,10-dione (7a)  
Yellow solid, yield: 51.0%, melting point: >250 °C; ^1H NMR (600 MHz, DMSO-d₆) δ 11.94 (s, 1H, anthraquinone-8-OH), 7.81 (t, J = 7.9 Hz, 1H, anthraquinone-1-OH), 7.71 (d, J = 7.5 Hz, 1H, anthraquinone-5-H), 7.63 (s, 1H, anthraquinone-4-H), 7.39 (d, J = 8.3 Hz, 1H, imidazole-5-CH), 7.24 (s, 1H, imidazole-4-CH), 7.16 (s, 1H, anthraquinone-7-CH), 6.98 (s, 1H, anthraquinone-2-CH), 4.31 (s, 2H, CH₂), 3.45 (s, 3H, imidazole-1-CH₃); ^13C NMR (151 MHz, DMSO-d₆) δ 194.32–187.39, 183.70–179.03, 161.79, 151.40–147.41, 137.86, 133.79, 129.70, 124.57, 120.13, 115.28, 40.57–39.31, 37.97; HRMS (ESI) calcd. for C_{19}H_{14}N_{2}O_{4}S [M+H]^+: 367.0753; found, 367.0752.

1.10.15. Synthesis of 3-(((4H-1,2,4-triazol-3-yl)thio)methyl)-1,8-dihydroxyanthracene-9,10-dione (7b)  
Yellow solid, yield: 43.1%, melting point: >250 °C; ^1H NMR (600 MHz, CDCl₃) δ 12.02 (s, 1H, anthraquinone-8-OH), 12.01 (s, 1H, anthraquinone-1-OH), 7.98 (s, 1H, triazole-1-H), 7.38 (s, 1H, triazole-5-H), 7.26 (d, J = 25.9 Hz, 3H, anthraquinone-4,5,6-3H), 6.95 (s, 1H, anthraquinone-7-CH), 6.86 (s, 1H, anthraquinone-2-CH), 4.19 (s, 2H, CH₂), 3.45 (s, 3H, imidazole-1-CH₃); ^13C NMR (151 MHz, CDCl₃) δ 164.65–161.79, 149.67–145.37, 137.36, 135.29–132.12, 120.21, 119.76, 116.61–114.28, 44.60; HRMS (ESI) calcd. for C_{17}H_{11}N_{3}O_{4}S [M+H]^+: 354.0549; found, 354.0542.

1.10.16. Synthesis of 1,8-dihydroxy-3-(((1-methyl-1H-tetrazol-5-yl)thio)methyl)anthracene-9,10-dione (7c)  
Yellow solid, yield: 60.1%, melting point: >250 °C; ^1H NMR (600 MHz, CDCl₃) δ 11.92 (s, 1H, anthraquinone-8-OH), 11.94 (s, 1H, anthraquinone-1-OH), 7.87 (d, J = 1.5 Hz, 1H, anthraquinone-5-H), 7.83 (dd, J = 7.5, 0.9 Hz, 1H, anthraquinone-6-H), 7.70 (s, 1H, anthraquinone-4-H), 7.31 (dd, J = 8.4, 0.9 Hz, 1H, anthraquinone-7-H), 7.26 (s, 1H, anthraquinone-2-H), 4.61 (s, 2H, CH₂), 3.91 (s, 3H, tetrazole-5-CH₃); ^13C NMR (151 MHz, DMSO-d₆) δ 191.90, 181.62, 161.89, 144.55, 137.95, 134.39, 133.69, 125.03, 123.25, 119.89, 118.47, 116.38, 31.40, 31.11; HRMS (ESI) calcd. for
\[ \text{C}_{17}\text{H}_{12}\text{N}_{4}\text{O}_{4}\text{S} [\text{M+H}]^+: 369.0658; [\text{M+Na}]^+: 391.0477; \text{found, 391.0478}. \]

1.10.17. Synthesis of 3-(((1H-benzo[d]imidazol-2-yl)thio)methyl)-1,8-dihydroxyanthracene-9,10-dione (8)

Yellow solid, yield: 34.1%, melting point: >250 °C; \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 12.51 (s, 1H, benzimidazole-1-NH), 11.92 (s, 1H, anthraquinone-8-OH), 11.87 (s, 1H, anthraquinone-1-OH), 7.82–7.78 (m, 1H, anthraquinone-4-H), 7.70 (dd, \(J = 7.5, 3.5\) Hz, 1H, anthraquinone-6-H), 7.53–7.49 (m, 2H, benzimidazole-4,7-2H), 7.48 (s, 1H, anthraquinone-5-H), 7.38 (dd, \(J = 8.4, 2.5\) Hz, 1H, anthraquinone-7-H), 7.24–7.17 (m, 3H, anthraquinone-2-H, benzimidazole-5,6-2H), 4.74 (d, \(J = 13.6\) Hz, 2H, CH\(_2\)); \(^1\)\(^3\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 192.53, 181.97–178.81, 162.72, 153.73–150.87 (m), 145.99, 137.34, 124.83, 124.50, 120.24, 115.39, 34.67; HRMS (ESI) calcd. for \(\text{C}_{22}\text{H}_{14}\text{N}_{2}\text{O}_{4}\text{S} [\text{M+H}]^+: 403.0753; \text{found, 403.0753}. \)

1.11. Some representative Spectra

\(^1\)H NMR spectrum of compound 3b

\(^1\)\(^3\)C NMR spectrum of compound 3b
HRMS spectrum of compound 3b

Chemical Formula: C_{12}H_{11}N_2O_3
Exact Mass: 365.0648
[M+H]^+: 366.0726

^1H NMR spectrum of compound 4a
$^{13}$C NMR spectrum of compound 4a

HRMS spectrum of compound 4a
$^1$H NMR spectrum of compound 4b

$^{13}$C NMR spectrum of compound 4b
HRMS spectrum of compound 4b

1H NMR spectrum of compound 5a
$^{13}$C NMR spectrum of compound 5a

HRMS spectrum of compound 5a
Chemical formula: $\text{C}_{22}\text{H}_{14}\text{N}_{2}\text{O}_{4}$

Exact mass: 370.0954

$[\text{M+H}]^+ : 371.1032$

$^1\text{H}$ NMR spectrum of compound 5b

$^{13}\text{C}$ NMR spectrum of compound 5b
HRMS spectrum of compound 5b

LXY-36  30-Sep-2018
ZCH-1662  11 (0.190) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:27) TOF MS ES+

Chemical formula: C_{24}H_{19}N_{2}O_{4}
Exact mass: 398.1287
[M+H]^+: 399.1345

^1H NMR spectrum of compound 5c
$^{13}$C NMR spectrum of compound 5c

HRMS spectrum of compound 5c
Chemical formula: C_{24}H_{16}N_{2}O_{4}
Exact mass: 398.1287
[M+H]^+: 399.1345

\(^1\)H NMR spectrum of compound 6

\(^1\)C NMR spectrum of compound 6
HRMS spectrum of compound 6

Chemical Formula: $C_{26}H_{21}NO_4$  
Exact Mass: 419.1158  
$[M+H]^+$: 420.1236

$^1$H NMR spectrum of compound 7a
$^{13}$C NMR spectrum of compound 7a

HRMS spectrum of compound 7a
Chemical Formula: C\textsubscript{19}H\textsubscript{14}N\textsubscript{2}O\textsubscript{4}S

Exact Mass: 366.0674

[M+H]\textsuperscript{+}: 367.0753