

Synthesis and biological evaluation of benzimidazolyl substituted aloe-emodin derivatives

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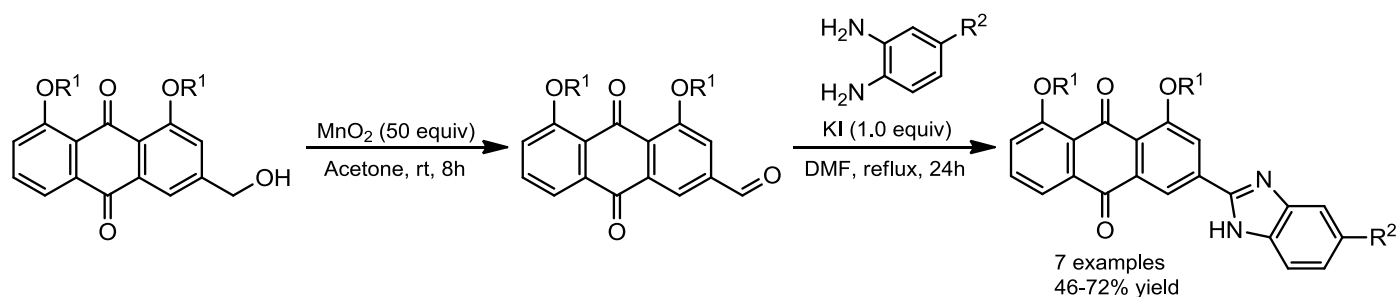
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Abstract

Benzimidazolic derivatives of the natural product aloe-emodin were prepared in good yields via aerobic condensation of the corresponding aldehydes with diamines in the presence of potassium iodide as auxiliary reagent. The required aldehydes were easily obtained by oxidation of aloe-emodin or its dimethyl-protected analogue. *In vitro* antimicrobial activity and cytotoxicity of the synthesized compounds were assessed.

Most of the compounds showed either low to moderate antiprotozoal activity, although it was quite aspecific. 3-(1H-Benzo[d]imidazol-2-yl)-1,8-dihydroxyanthracene-9,10-dione showed the highest specificity against *Leishmania infantum* with $IC_{50} = 4.06 \mu\text{M}$ and a selectivity index of 10.29.



Keywords: Aloe-emodin, benzimidazole, oxidative cyclization, antimicrobial, cytotoxicity

Introduction

The use of natural products as starting points for developing novel hit compounds is continuing to be a valuable strategy in the search for new bioactive compounds.¹⁻³ The anthraquinone moiety occurs in many biological active molecules with antiviral, antifungal, antibacterial and antiparasitic properties, such as chrysophanol **2** (Fig 1).⁴⁻⁶ One of these molecules is aloe-emodin **1** (3-hydroxymethyl-1,8-dihydroxy-9,10-antraquinone) (Figure 1), which is a well-known compound isolated from *Aloe vera* leaves and widely used as an intermediate in the preparation of therapeutically active compounds such as rhein **2** and diacerein **3**, and anthracycline type antibiotics.^{7,8} Aloe-emodin and derivatives thereof have been described to be useful in the treatment of cancer and psoriasis, and as antifungal, antiviral and antiplasmodial agents.^{5,9,10}

Although several transformations of aloe-emodin have been described,^{11,10,12-16} the transformation of the hydroxymethyl group to a heterocyclic moiety has received very little attention.^{17,18} Therefore, attempts were performed to synthesize benzimidazole derivatives of aloe-emodin to boost or tune its biological properties. Benzimidazoles occur as core structure in many pharmaceutical compounds with a diversity of bioactivities, including antiviral, antibacterial, antitumor, anti-Alzheimer, and anti-inflammatory activities.¹⁹⁻²²

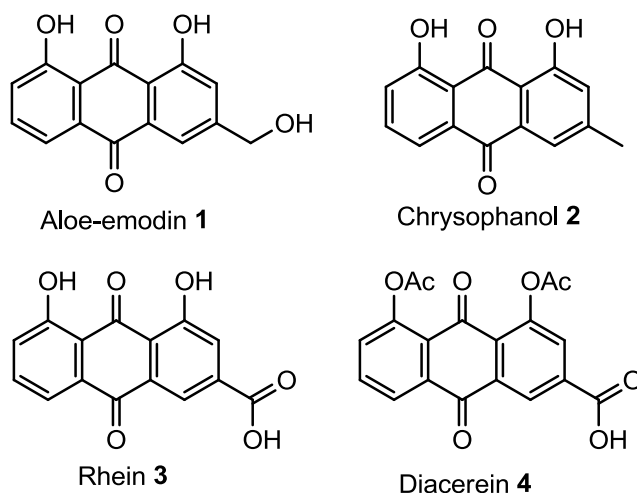


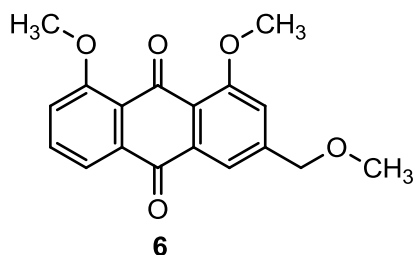
Figure 1. Structures of Aloe-emodin, chrysophanol, rhein and diacerein.

One of the general methods for the synthesis of benzimidazoles is the coupling of *o*-aryldiamines with carboxylic acids or their derivatives under dehydrating conditions.²³ Also the reaction of *o*-phenylenediamines with aldehydes in the presence of oxidants and catalysts has been described. Herein, we report the synthesis of benzimidazoles derived from aloe-emodin carbaldehyde and *o*-phenylenediamines via aerobic oxidation in the presence of potassium iodide as auxiliary reagent, following the conditions described by Chen *et al.*²⁴

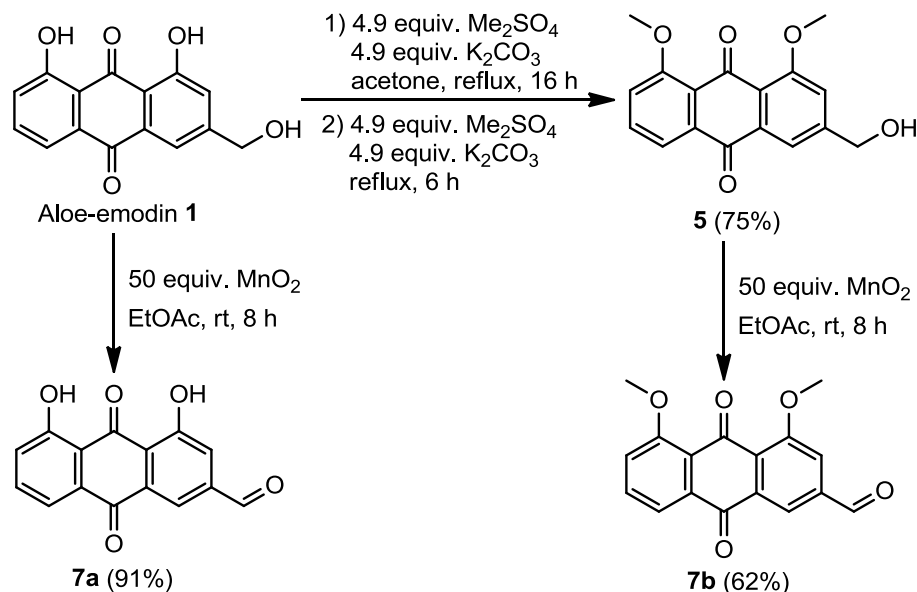
Results and Discussion

Aloe-emodin **1** was first methylated at the phenolic hydroxyl groups using dimethyl sulfate as reported in literature to afford compound **5** (Scheme 1). It was observed that an excess of dimethyl sulfate is required to allow an efficient dimethylation because of the low solubility of aloe-emodin in most organic solvents. In our hands optimal conditions used 9.8 equiv of Me₂SO₄ (added in two portions) at 0.04 M concentration of aloe-

emodin in acetone. Stirring the mixture longer than 24h in attempts to increase the yield resulted in significant amounts of the over-methylated compound **6** as side-product. Fortunately, anthraquinone **6** is much more soluble in acetone than the desired product, which can be purified by recrystallization from cold acetone.

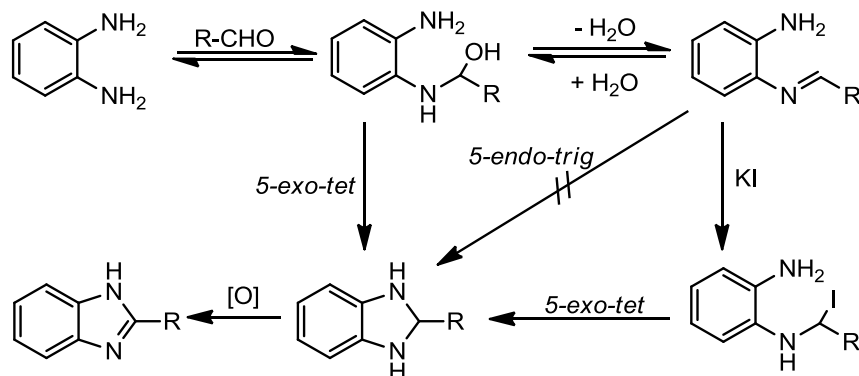


The synthesis of the required aldehydes **7a** and **7b** has been reported in literature using different oxidants, including chromium reagents like pyridinium chlorochromate (PCC), TEMPO/trichloroisocyanuric acid, VO(acac)₂/DABCO/O₂ and others.^{25,15,26,12} Yang-Ming *et al.* reported the oxidation of aloe-emodin **1** to aldehyde **7a** in the presence of manganese dioxide in refluxing acetone.²⁷ We synthesized aldehydes **7a** and **7b** by oxidation of aloe-emodin **1** or its dimethyl-protected analogue **5** with an excess of manganese dioxide in ethyl acetate (See Scheme 1) at room temperature, which resulted in the desired aldehyde **7a** (gram scale reaction) within 8 h in high yield (91 %). The same procedure was followed using dimethylated compound **5** to give **7b** but in lower yield.



Scheme 1. Oxidation of compounds **1** and **5** to afford compounds **7a-b**.

Having in hand the aldehydes **7a,b**, the corresponding benzimidazoles were prepared by reaction with aromatic diamines using potassium iodide as auxiliary reagent. A mechanism of this reaction was proposed by Chen *et al.*²⁴ According to these authors, KI promotes the cyclization of *N*-(*o*-aminoaryl)imines by attacking the C=N bond giving a tetrahedral intermediate which can undergo 5-exo-tet cyclization instead of a direct but less plausible a 5-endo-trig cyclization on the parent imine (Scheme 2).



Scheme 2. Mechanism proposed by Chen et al.²⁴

A mixture of the corresponding aldehyde and diamine was heated at 80 °C in DMF in the presence of 1 equiv of KI for 24 hours. Although KI is regenerated and could act as catalyst, it was found that raising the amount to 1 equiv gave superior results. The use of catalytic amounts of KI (10mol%) resulted in a longer reaction time and poor yields. The reaction using aldehyde **7a** resulted in the corresponding benzimidazolyl derivatives **8a-c** in 46-66% yield, while using aldehyde **7b** gave compounds **8d-g** in 50-72% yield (See Table 1). All compounds were purified by recrystallization or column chromatography, depending on their solubility. Compounds **8a** and **8b** have a poor solubility in common organic solvents such as chloroform and acetone, while compounds **8d** and **8f** were more soluble.

Table 1. Oxidative cyclization of aldehydes **7a-b** with aromatic diamines affording benzimidazoles **8a-g**

Compound	R ¹	R ²	Yield (%)
8a	-H	-H	66
8b	-H	-Br	58
8c	-H	-OCH ₃	46
8d	-CH ₃	-H	60
8e	-CH ₃	-Br	54
8f	-CH ₃	-OCH ₃	50
8g	-CH ₃	-Cl	72

The synthesis of benzimidazole **8d** was previously reported using sodium metabisulfite.¹⁷ The reaction was conducted at 120°C for 2 hours, no yield was reported. Compound **8a** was obtained by demethylation of compound **8d** with concentrated hydrobromic acid in acetic acid. Only the melting point of both compounds was reported.

Table 2. Activities of synthesized compounds against *S. aureus*, *E. coli*, *C. albicans*, and *A. fumigatus* and cytotoxicity against Human Lung Fibroblasts (MRC-5)

Comp	IC ₅₀ (μM)				
	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>A. fumigatus</i>	MRC-5
std	0.04 ^a	0.58 ^a	0.74 ^b	0.31 ^c	8.32 ^d
5	>128.00	>128.00	>128.00	>128.00	25.40
7a	2.06	>128.00	>128.00	>128.00	11.96
7b	>128.00	>128.00	>128.00	>128.00	36.76
8a	64.91	>128.00	>128.00	>128.00	41.78
8b	33.37	>128.00	>128.00	>128.00	80.63
8c	>128.00	>128.00	>128.00	>128.00	>128.00
8d	>128.00	>128.00	>128.00	>128.00	2.00
8e	>128.00	>128.00	>128.00	>128.00	>128.00
8f	16.11	>128.00	>128.00	>128.00	<0.50
8g	>128.00	>128.00	>128.00	>128.00	32.00

^a Doxycycline. ^b Flucytosine. ^c Terbinafine. ^d Tamoxifen.

The antimicrobial activity and cytotoxicity of the synthesized compounds were tested *in vitro*. Results of antibacterial and antifungal activities are shown in **Table 2**.

Table 3. Activities of synthesized compounds against *T. cruzi*, *T. b. brucei*, *T. brucei rhodesiense*, and *L. infantum*

Comp.	IC ₅₀ (μM)			
	<i>L. infantum</i>	<i>T. cruzi</i>	<i>T. b. bruc</i>	<i>T. b. rhodes</i>
std	10.77 ^a	1.78 ^b	0.02 ^c	0.03 ^c
5	48.11	>128.00	>128.00	68.32
7a	4.06	4.36	4.12	4.03
7b	54.17	>128.00	63.50	75.30
8a	4.06	>128.00	46.25	29.58
8b	19.03	54.26	64.00	22.17
8c	64.91	>128.00	>128.00	68.90
8d	25.40	3.60	64.91	4.90
8e	>128.00	>128.00	>128.00	96.08
8f	3.41	<0.50	0.90	0.98
8g	21.53	48.11	>128.00	12.03

^a Miltefosine. ^b Benznidazol. ^c Suramine.

Cytotoxicity against MRC-5 cells (human fetal lung fibroblasts) was evaluated as well as to assess selectivity. Antibacterial activity was tested against both Gram-positive and Gram-negative bacteria, *S. aureus* and *E. coli* respectively. Compound **7a** (IC₅₀ = 2.06 μM) was the most active against *S. aureus*, followed by compounds **8f** (IC₅₀ = 16.11 μM) and **8b** (IC₅₀ = 33.37 μM) but still much less active than the standard doxycycline.

Antiprotozoal activity evaluation of the synthesized compounds against *Leishmania infantum*, *Trypanosoma cruzi*, *Trypanosoma brucei* and *Trypanosoma brucei rhodesiense* was also conducted. In general, compounds with the highest antiprotozoal activity (**7a**, **8f** and **8d**) also showed the highest cytotoxicity on MRC-5 cells, revealing this activity to be quite aspecific. Compound **8a** showed good activity ($IC_{50} = 4.06 \mu M$) against *L. infantum*, with a Selectivity Index (ratio of the cytotoxicity on MRC-5 (IC_{50}) to the antimicrobial activity (IC_{50})) of 10.29.

Conclusions

Benzimidazolic derivatives of aloe-emodin were obtained in good to moderate yields using a simple procedure. *In vitro* antimicrobial activity of the synthesized compounds was tested. The compounds showed no activity against *E. coli*, *C. albicans* or *A. fumigatus*. Compounds **7a** and **8f** were the most active against *S. aureus* but showed high cytotoxicity on MRC-5 cells. Most of the compounds showed either low to moderate antiprotozoal activity, although it was quite aspecific. Compound **8a** showed the highest specificity against *Leishmania infantum* with $IC_{50} = 4.06 \mu M$ and selectivity index of 10.29.

Experimental Section

General. Column chromatography purifications were conducted on silica gel 60 (40-63 μm ; Grace Davisil). TLC was carried out on plastic TLC sheets, precoated with silica gel 60F254 (Merck); the spots were visualized under UV light ($\lambda = 254 nm$). Melting points were acquired on a Buchi Melting Point B-540. IR absorption spectra were recorded on a Thermo Nicolet Avatar 370 FT-IR spectrophotometer. 1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 250 console and a Bruker Avance II 500 console at 250 or 63 MHz and 500 or 125 MHz, respectively. The deuterated solvent is mentioned in the analysis section and tetramethylsilane was used as an internal standard. Chemical shifts (δ) are given in parts per million (ppm). High Resolution Mass Spectrometry was conducted on a Waters Micromass QToF in ES+ mode, using reserpine as a reference. All reagents were purchased and used without further purifications. Unless explicitly mentioned, reactions were performed without specific drying of solvents or use of an inert atmosphere. Ethyl acetate for chromatography purposes was distilled prior to use.

Synthesis of 3-(hydroxymethyl)-1,8-dimethoxyanthracene-9,10-dione (5). This product was synthesized similarly to a literature procedure.¹¹ A solution of aloe-emodin (1 g, 3.7 mmol) in acetone (200 mL) was added with K_2CO_3 (2.5 g, 18.1 mmol) and dimethyl sulfate (1.73 mL, 18.2 mmol), and then refluxed overnight. The reaction mixture was added then with more K_2CO_3 (2.5 g) and dimethyl sulfate (1.73 mL) and refluxed for 6 hours. The reaction was monitored by TLC. After the completion of reaction, the yielded mixture was cooled to room temperature and filtered. The filtrate was dried under vacuum to afford a yellow solid. Recrystallization of the yellow solid from acetone gave compound **2** as yellow needles, yield 75%; m.p. 224-225 °C; 1H NMR (250 MHz, d_6 -DMSO) δ (ppm): 3.91 (s, 6H, $2 \times OCH_3$), 4.62 (s, 2H, CH_2O), 5.53 (1H, OH), 7.21 (1H, s, CH aromatic), 7.30 (1H, d, CH aromatic), 7.58 (1H, s, CH aromatic), 7.61 (1H, d, CH aromatic), 7.74 (1H, dd, CH aromatic). The obtained spectrum is consistent with literature data.¹²

General procedure for the synthesis of the aldehydes 7a-b. To a mixture of aloe-emodin (1 g, 3.7 mmol) (or compound **2**) and manganese dioxide (16.09 g, 185 mmol) was added 1000 mL of ethyl acetate. The reaction mixture was stirred for 8 hours, filtered, and the solvent was evaporated *in vacuo*. The product was purified by recrystallization from acetone.

4,5-Dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carbaldehyde (7a). Reddish solid, M.p.: 208-209 °C (lit. 208-210 °C), yield: 91.1%; ¹H NMR: (250 MHz, DMSO-*d*₆) δ (ppm): 11.93 (s, 1H, OH), 11.89 (s, 1H, OH), 10.11 (s, 1H, -CHO), 8.11 (d, 1H, CH aromatic), 7.86-7.72 (m, 3H, CH aromatic), 7.41 (d, 1H, CH aromatic). The obtained spectrum is consistent with literature data.¹²

4,5-Dimethoxy-9,10-dioxo-9,10-dihydroanthracene-2-carbaldehyde (7b). Yellow solid, yield 62.1%; m.p. 195-196 °C (lit. 198-200 °C); ¹H NMR (250 MHz, DMSO-*d*₆) δ (ppm): 3.92 (s, 3H, OCH₃), 4.00 (s, 3H, OCH₃), 7.56 (d, 1H, CH aromatic), 7.75 (m, 2H, 2×CH aromatic), 7.92 (s, 1H, CH aromatic), 8.18 (d, 1H, CH aromatic), 10.15 (s, 1H, CHO). The obtained spectrum is consistent with literature data.¹⁵

General procedure for the synthesis of the benzimidazoles 8a-g. An ortho-phenylenediamine derivative (1.0 mmol; 1.0 equiv) and an aldehyde **7** (1.0 mmol; 1.0 equiv) were dissolved in DMF (20 mL). Potassium iodide (1.0 mmol; 1.0 equiv) was added. The resulting reaction mixture was stirred at 80 °C in an open flask, and the reaction progress was monitored by TLC. On the completion of the reaction, the mixture was cooled to room temperature, poured into water (150 mL) and extracted with ethyl acetate (3 x 75 mL). The crude product obtained was purified by column chromatography on silica gel to afford the corresponding benzimidazole **8**; except compounds **8a-c**, which were purified by recrystallization due to their low solubility in common organic solvents.

3-(1H-Benzo[d]imidazol-2-yl)-1,8-dihydroxyanthracene-9,10-dione (8a). Red solid, yield 66%; M.p. >350 °C; IR (cm⁻¹, neat): 3300, 1621, 1572, 1454, 1266, 1207, 1152, 1088, 1020, 814, 740, 724, 666; ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 13.33 (s, 1H, NH), 11.93 (s, 2H, OH), 8.51 (s, 1H, CH aromatic), 8.06 (s, 1H, CH aromatic), 7.74 (m, 5H, CH aromatic), 7.39 (s, 1H, CH aromatic), 7.26 (s, 1H, CH aromatic). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 191.93 (C=O), 181.90 (C=O), 162.42 (C-OH), 162.09 (C-OH), 149.34 (C=N), 138.59 (C aromatic), 138.28 (C aromatic), 138.10 (C aromatic), 134.82 (C aromatic), 133.97 (C aromatic), 125.23 (2×C aromatic), 121.06 (2×C aromatic), 120.14 (3×C aromatic), 117.65 (2×C aromatic), 117.06 (C aromatic), 116.76 (C aromatic). HRMS [C₂₁H₁₂N₂O₄+H]⁺ calculated 357.0876 found 357.0859

3-(5-Bromo-1H-benzo[d]imidazol-2-yl)-1,8-dihydroxyanthracene-9,10-dione (8b). Brown-yellow solid, yield 58%; m.p. 383-384 °C; IR (cm⁻¹, neat): 3300, 3072, 1623, 1455, 1384, 1263, 1206, 1156, 1013, 817, 759, 722, 667; ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.44 (s, 2H, OH), 8.41 (s, 1H, CH aromatic), 8.00 (s, 1H, CH aromatic), 7.88 – 7.65 (m, 3H, CH aromatic), 7.55 (d, 1H, CH aromatic), 7.35 (t, 2H, CH aromatic). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 191.27 (C=O), 181.14 (C=O), 161.59 (C-OH), 161.39 (C-OH), 149.81 (C=N), 137.47 (2×C aromatic), 137.03 (C aromatic), 134.16 (C aromatic), 133.25 (C aromatic), 124.57 (2×C aromatic), 120.58 (2×C aromatic), 119.50 (2×C aromatic), 117.02 (2×C aromatic), 116.64 (C aromatic), 116.07 (2×C aromatic). HRMS [C₂₁H₁₁BrN₂O₄+H]⁺ calculated 434.9981 found 434.9962

1,8-Dihydroxy-3-(5-methoxy-1H-benzo[d]imidazol-2-yl)anthracene-9,10-dione (8c). Black solid, yield 46%; m.p. 328-329 °C; IR (cm⁻¹, neat): 3321, 1620, 1454, 1385, 1284, 1211, 1158, 1091, 829, 760, 724, 666; ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 13.03 (s, 1H, NH), 11.88 (s, 2H, OH), 8.29 (s, 1H, CH aromatic), 7.86 (s, 1H, CH aromatic), 7.70 (d, 2H, CH aromatic), 7.36 (d+s, 2H, CH aromatic), 6.84 (d+s, 2H, CH aromatic), 3.75 (s, 3H, OCH₃). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 191.85 (C=O), 181.80 (C=O), 162.34 (C-OH), 162.03 (C-OH), 157.28 (C-OCH₃), 148.65 (C=N), 138.30 (C aromatic), 138.02 (2×C aromatic), 134.65 (C aromatic), 133.91 (C

aromatic), 125.14 (2×C aromatic), 120.38 (2×C aromatic), 120.11 (2×C aromatic), 117.43 (2×C aromatic), 116.63 (C aromatic), 113.89 (C aromatic), 56.08 (OCH₃). HRMS [C₂₂H₁₄N₂O₅+H]⁺ calculated 387.0982 found 387.0969

3-(1H-Benzo[d]imidazol-2-yl)-1,8-dimethoxyanthracene-9,10-dione (8d). Brown solid, yield 60%; m.p. 190-191°C; IR (cm⁻¹, neat): 3500, 3090, 2922, 1650, 1603, 1583, 1459, 1328, 1281, 1231, 1070, 1001, 910, 861, 792, 744, 716; ¹H NMR (500 MHz, DMSO- *d*₆) δ (ppm): 13.37 (s, 1H, NH), 8.52 (s, 1H, CH aromatic), 8.26 (s, 1H, CH aromatic), 7.78 (m, 3H, CH aromatic), 7.61 (d, 1H, CH aromatic), 7.58 (d, 1H, CH aromatic), 7.30 (t, 1H, CH aromatic), 7.26 (t, 1H, CH aromatic), 4.07 (s, 3H, OCH₃), 3.94 (s, 3H, OCH₃). ¹³C NMR (125 MHz, DMSO- *d*₆) δ (ppm): 183.74 (C=O), 181.45 (C=O), 159.93 (C-OCH₃), 159.47 (C-OCH₃), 150.02 (C=N), 144.37 (C3), 135.84 (C aromatic), 135.63 (C aromatic), 135.53 (C aromatic), 135.03 (C aromatic), 134.75 (C aromatic), 124.54 (C aromatic), 124.17 (C aromatic), 124.14 (C aromatic), 122.93 (C aromatic), 120.04 (C aromatic), 119.78 (C aromatic), 118.95 (C aromatic), 116.55 (C aromatic), 116.11 (C aromatic), 112.43 (C aromatic), 57.26 (OCH₃), 57.03 (OCH₃). HRMS [C₂₃H₁₆N₂O₄+H]⁺ calculated 385.1188 found 385.1183

3-(5-Bromo-1H-benzo[d]imidazol-2-yl)-1,8-dimethoxyanthracene-9,10-dione (8e). Brown-yellow solid, yield 54%; m.p. 275-276°C; IR (cm⁻¹, neat): 3090, 1655, 1604, 1584, 1445, 1322, 1274, 1239, 1066, 1011, 977, 916, 799, 755, 724; ¹H NMR (500 MHz, DMSO- *d*₆) δ (ppm): 13.39 (s, 1H, NH), 8.36 (s, 1H, CH aromatic), 8.09 (s, 1H, CH aromatic), 7.76 (s, 1H, CH aromatic), 7.72 – 7.63 (m, 2H, CH aromatic), 7.54 (d, 1H, CH aromatic), 7.48 (d, 1H, CH aromatic), 7.32 (d, 1H, CH aromatic), 4.01 (s, 3H, OCH₃), 3.90 (s, 3H, OCH₃). ¹³C NMR (125 MHz, DMSO- *d*₆) δ (ppm): 183.43 (C=O), 181.22 (C=O), 159.78 (C-OCH₃), 159.41 (C-OCH₃), 151.09 (C=N), 135.28 (C aromatic), 134.91 (C aromatic), 134.85 (C aromatic), 134.56 (C aromatic), 126.25 (C aromatic), 124.51 (C aromatic), 123.94 (C aromatic), 119.58 (2×C aromatic), 118.85 (2×C aromatic), 116.57 (2×C aromatic), 116.03 (2×C aromatic), 115.59 (C aromatic), 57.15 (OCH₃), 56.93 (OCH₃). HRMS [C₂₃H₁₅BrN₂O₄+H]⁺ calculated 463.0293 found 463.0288

1,8-Dimethoxy-3-(5-methoxy-1H-benzo[d]imidazol-2-yl)anthracene-9,10-dione (8f). Black solid, yield 50%; m.p. 190-191°C; IR (cm⁻¹, neat): 3152, 1662, 1585, 1445, 1304, 1277, 1234, 1159, 1065, 1011, 977, 912, 814, 752, 714; ¹H NMR (500 MHz, DMSO- *d*₆) δ (ppm): 13.21 (s, 1H, NH), 8.45 (s, 1H, CH aromatic), 8.18 (s, 1H, CH aromatic), 7.82 – 7.71 (m, 3H, CH aromatic), 7.63 (d, 1H, CH aromatic), 7.56 (d, 1H, CH aromatic), 7.48 (d, 1H, CH aromatic), 4.05 (s, 3H), 3.93 (s, 3H), 3.84 (s, 3H). ¹³C NMR (125 MHz, DMSO- *d*₆) δ (ppm): 183.11 (C=O), 180.76 (C=O), 159.25 (C-OCH₃), 158.78 (C-OCH₃), 156.77 (C-OCH₃), 148.44 (C=N), 138.31 (C aromatic), 135.92 (C aromatic), 135.14 (C aromatic), 134.83 (C aromatic), 134.33 (C aromatic), 134.06 (C aromatic), 123.48 (C aromatic), 119.96 (C aromatic), 119.09 (C aromatic), 118.26 (C aromatic), 115.65 (C aromatic), 115.45 (C aromatic), 115.16 (C aromatic), 114.94 (C aromatic), 113.64 (C aromatic), 56.53 (OCH₃), 56.34 (OCH₃), 55.50 (OCH₃). HRMS [C₂₄H₁₈N₂O₅+H]⁺ calculated 415.1294 found 415.1288

3-(5-Chloro-1H-benzo[d]imidazol-2-yl)-1,8-dimethoxyanthracene-9,10-dione (8g). Orange solid, yield 72%; m.p. 266-267°C; IR (cm⁻¹, neat): 3467, 1658, 1585, 1445, 1325, 1273, 1241, 1066, 1012, 977, 913, 802, 755, 725; ¹H NMR (500 MHz, DMSO- *d*₆) δ (ppm): 8.46 (s, 1H, CH aromatic), 8.19 (s, 1H, CH aromatic), 7.76 (t, 1H, CH aromatic), 7.72 (d, 1H, CH aromatic), 7.67 (d, 1H, CH aromatic), 7.55 (d, 1H, CH aromatic), 7.29 (d, 1H, CH aromatic), 4.05 (s, 3H, OCH₃), 3.93 (s, 3H, OCH₃). ¹³C NMR (125 MHz, DMSO- *d*₆) δ (ppm): 182.87 (C=O), 180.66 (C=O), 159.20 (C-OCH₃), 158.79 (C-OCH₃), 150.50 (C=N), 134.81 (C aromatic), 134.36 (2×C aromatic), 133.97 (C aromatic), 133.86 (C aromatic), 127.40 (C aromatic), 124.17 (C aromatic), 123.41 (2C aromatic), 119.08 (C aromatic), 118.25 (2×C aromatic), 116.05 (2×C aromatic), 115.60 (2×C aromatic), 56.60 (OCH₃), 56.33 (OCH₃). HRMS [C₂₃H₁₅ClN₂O₄+H]⁺ calculated 419.0799 found 419.0793

Antimicrobial activity. Synthesized compounds were evaluated for antimicrobial activity in an integrated screening panel against the bacteria *Staphylococcus aureus* and *Escherichia coli*, the mold *Aspergillus fumigatus*, the yeast *Candida albicans*, and the protozoa *Trypanosoma cruzi*, *Trypanosoma brucei*, *Trypanosoma brucei rhodesiense* and *Leishmania infantum*, as previously described in Cos et al.⁴ The human MRC-5 cell line was also included to assess selectivity, as reported before.²⁸⁻³¹ The IC₅₀ values were determined from five 4-fold dilutions. The following positive controls were used: Tamoxifen (MRC-5, human fetal lung fibroblasts, ECACC 84100401) IC₅₀ 8.32 μM; doxycycline (*Staphylococcus aureus* ATCC 29213) IC₅₀ 0.04 μM; doxycycline (*Escherichia coli* ATCC 8739), IC₅₀ 0.58 μM; Flucytosine (*Candida albicans*, B59630) IC₅₀ 0.74 μM; terbinafin (*Aspergillus fumigatus* ATCC16404,) IC₅₀ 0.31 μM; suramine (*Trypanosoma brucei*, Squib 427) IC₅₀ 0.02 μM; suramine (*Trypanosoma brucei rhodesiense*, STIB-900) IC₅₀ 0.03 μM; benznidazol (*Trypanosoma cruzi*, Tulahuen CL2) IC₅₀ 1.78 μM; miltefosine (*Leishmania infantum*, MHOM/MA/67/ITMAP263) IC₅₀ 10.77 μM.

Statistical analysis. IC₅₀ calculations were performed using regression analysis [% inhibition vs. log (concentration)] using Sigma plot 13.0.

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Supplementary Material

Copies of ¹H and ¹³C NMR spectra of benzimidazoles **8a-g** are provided as Supplementary Material for this article.

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