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Regiospecific Reduction of 4,6-Dinitrobenzimidazoles: Synthesis, Characterization, and Biological Evaluation

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The regiospecific reduction of 4,6-dinitrobenzimidazole derivatives leading to the corresponding 4-amino-6-nitrobenzimidazoles was studied. The identification of the formed product structures was accomplished by spectroscopic and X-ray diffraction data. The anticancer and antiparasitic activities of the synthesized compounds were examined, and promising activities against *Toxoplasma gondii* and *Leishmania major* parasites were discovered for certain 4,6-dinitrobenzimidazoles in addition to moderate anticancer activities of the 4-amino-6-nitrobenzimidazole derivatives against *T. gondii* cells. However, the tumor cell experiments revealed a promising sensitivity of p53-negative colon cancer cells to these compounds.

Introduction

N-Heterocyclic compounds are an important compound class with versatile applications.[1] In particular, imidazole derivatives are prominent ligands for metal-NHC catalysts and for the design of biologically active organic compounds.[2–4] Indeed, heterocyclic organic compounds play an important role as drugs and for the development of new drug candidates for the treatment of various human diseases. Indoles such as Vinca alkaloids, quinolones such as camptothecins, and nucleotides such as 5-fluorouracil are currently applied for the treatment of cancer diseases.[5] *N*-Heterocycles are likewise valuable drugs against several infectious diseases. Various *N*-heterocyclic antiviral drugs such as ribavirin, zidovudine, remdesivir, and paxlovid became prominent examples in the course of the recent pandemics.[6] The quinoline alkaloids quinine, chloroquine, and mefloquine are efficient treatments for protozoal parasite infections such as malaria, while nitro-substituted *N*-heterocycles like metronidazole are clinically applied for the treatment of amoebiasis, giardiasis, etc.[7,8] The trypanocidal drugs fexinidazol (applied for the treatment of sleeping sickness) and benznidazole (a drug against Chagas disease) are further prominent examples of antiparasitic imidazoles.[9] The imidazole subgroup of benzimidazoles mimics natural purine bases and plays a significant role for the treatment of worm (albendazole, mebendazole) and viral infections (maribavir, Figure 1).[10] Albendazole and mebendazole were recently repurposed as anticancer compounds.[11,12] Various new substituted benzimidazoles exhibited anticancer and antiparasitic

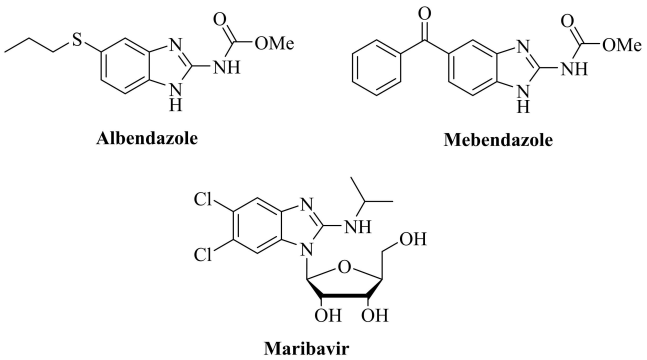

Figure 1. Structures of clinically applied anti-infective benzimidazoles.

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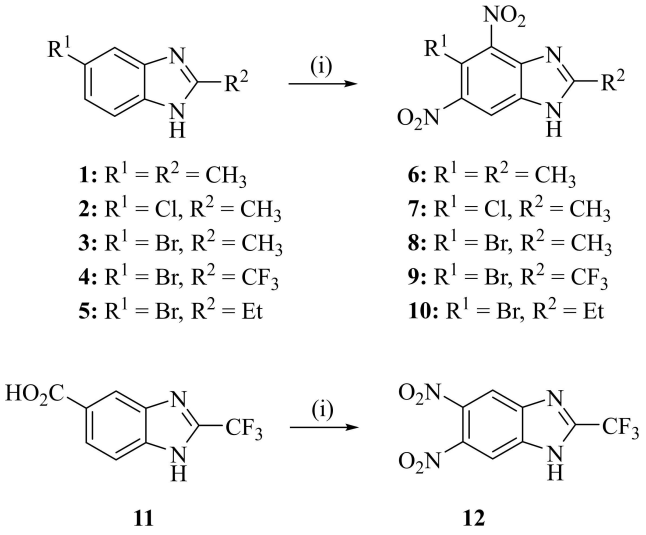
activities indicating a prospering field of medicinal chemistry research.[13–19] In this work, we report the regiospecific reduction of 4,6-dinitrobenzimidazoles to 4-amino-6-nitrobenzimidazole derivatives based on fundamental works published before.[20–29] We have previously studied the nitration of substituted benzimidazoles, and the structure of 2,5-dimethyl-4,6-dinitrobenzimidazole was elucidated by X-ray diffraction.[30,31] The compounds presented in this work were finally tested for their biological activities against cells of four human cancer cell lines, as well as against *Leishmania major* and *Toxoplasma gondii* parasites.

Results and Discussion

The dinitration of benzimidazoles has been realized by reaction of the starting compounds **1**–**5** with nitric acid according to previously reported procedures (Scheme 1).[30,31] Compounds **6**–**10** were obtained as solids in moderate to good yields. In contrast to that, the nitration reaction of 2-trifluoromethylbenzimidazole-5-carboxylic acid **11** underwent a decarboxylation step to 2-trifluoromethyl-5,6-dinitrobenzimidazole **12**, and did not produce the expected 2-trifluoromethyl-4,6-dinitrobenzimidazole-5-carboxylic acid (Scheme 1).



Scheme 1. Reagents and conditions: HNO_3, H_2SO_4, 0–5 °C, 1 h, 66–87 % for **6**–**10**, 40 % for **12**.

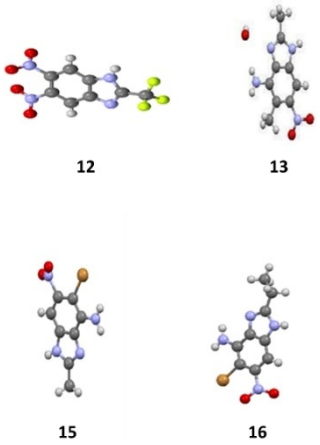
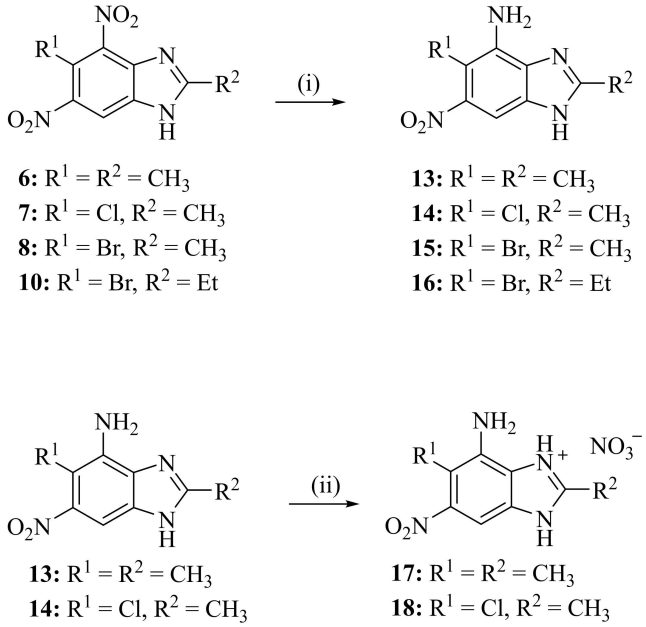


Figure 2. Ortep structures of nitrobenzimidazoles **12**, **13**, **15** and **16**.

The structure of 2-trifluoromethyl-5,6-dinitrobenzimidazole **12** was determined by X-ray diffraction of suitable monocrystals (Figure 2), and confirmed by spectroscopic data (NMR spectroscopy and mass spectrometry). Thus, this product was obtained by decarboxylation of the starting product before dinitration occurs. This reaction represents a novel procedure for the synthesis of 5,6-dinitrobenzimidazoles.

Previous reports described the synthesis of aminonitrobenzimidazoles.[28,29] In this study, a new regiospecific method was developed for the synthesis of 4-amino-6-nitrobenzimidazoles using hydrogen gas in the presence of Pd on charcoal as catalyst in butanol as solvent (Scheme 2). The ^{1}H-NMR spectra of all products show a singlet corresponding to the aromatic proton as well as a large singlet corresponding to primary amine protons. In particular, the structures of compounds **13**, **15** and **16** were established by X-ray diffraction analyses (Figure 2). More information and detailed results of the X-ray diffraction and structure analysis data can be found in the *Supporting Information* (Tables S1–S12). The reduction of compound **9** did not produce a pure product. The reason for this failure may be attributed to the CF_3 group of **9**. The usage of butanol as solvent was mandatory for the regiospecific reduction since other alcohols such as methanol and ethanol led to diamino products. Non-polar solvents were not applied because of low solubility of the starting compounds.

Attempts to prepare copper(II) complexes from compounds **13** and **14** only led to the formation of the analogous nitrate salts **17** and **18** (Scheme 2). Copper complexes were not



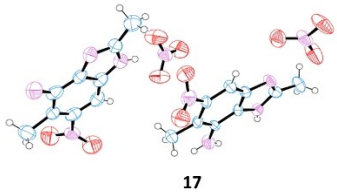
Scheme 2. Reagents and conditions: (i) H_2 (1 atm), cat. Pd/C (5 %), BuOH, r.t., 52–69 %; (ii) $Cu(NO_3)_2 \cdot H_2O$, EtOH, reflux, 24 h.

isolated. It is assumed that $Cu(OH)_2$ was formed under the described reaction conditions. Yet, crystals were obtained for **17** and **18**, and crystal structure analyses confirmed the formation of the nitrate salts (Figure 3).

The biological activities of the benzimidazoles were studied. Initially, the benzimidazole compounds were tested for their antiproliferative activities against HCT-116$^{p53-/-}$ colon carcinoma cells (Table 1). Compounds **7**, **8**, and **10** exhibited activities at doses below 50 μM in these cells, and **8** and **10** were only slightly less active than the positive control cisplatin. The other compounds were inactive. Based on their distinctly higher activity than **7**, compounds **8** and **10** were selected for testing in further human tumor cell lines, where they showed slightly lower activities than against the HCT-116$^{p53-/-}$ colon carcinoma cells. Both compounds were ca. twice as active against the p53-negative cells as against the p53-wildtype HCT-116 cells. This is of particular interest since p53 plays a crucial role as a tumor suppressor ("guardian of the genome"), and loss of p53 function in tumor cells was correlated with cancer resistance.[32] In terms of HCT-116 wild-type cells, compounds **8** and **10** were less active than cisplatin, which was inactive against HT-29 colorectal cancer cells, and, thus, the moderately active **8** and **10** were much more potent in HT-29 cells than cisplatin.[33]

The dinitrobenzimidazole compounds **6**–**10** and **12** as well as the amino-benzimidazoles **13**, **15**, and **16** were selected and tested for their antiparasitic activities against *T. gondii* parasites, *L. major* promastigotes, and *L. major* amastigotes (Table 2). Compounds **7**, **8**, and **10** showed distinct activity against *T. gondii* cells (EC_{50} = 5.5–9.3 μM) and *L. major* promastigotes (EC_{50} = 3.8–4.9 μM). **8** and **10** were also active against *L. major* amastigotes at concentrations below 10 μM. Although they displayed no selectivity and were less active than the approved antileishmanial drug amphotericin B (AmB) and the anti-*Toxoplasma* drug atovaquone (ATO), compounds **7**, **8** and **10** are suitable lead compounds for the generation of more active and selective analogs in future.

The amino-benzimidazoles **13**, **15**, and **16** revealed moderate activity against *T. gondii* parasites, while they were much less active against *L. major*. Considerable selectivities were not observed for these compounds either.



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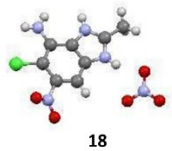

18

Figure 3. Ortep structures of nitrate salts **17** and **18**.

Table 1. Inhibitory concentrations IC_{50}[a] [μM] of substances **7**, **8**, and **10** when applied to cells of human U-87 MG likely glioblastoma, HT-29 colon adenocarcinoma, HCT-116 and HCT-116 p53–/– (p53 knockout mutant) colon carcinoma. Cisplatin served as positive control in the knockout cells.

Compd.	U87	HT-29	HCT-116	HCT-116$^{p53-/-}$
Cisplatin	–	>100[b]	5.0 ± 0.6[b]	9.5 ± 0.6
7	–	–	–	31.0 ± 3.3
8	20.7 ± 2.1	26.5 ± 3.8	21.7 ± 3.5	13.3 ± 0.8
10	27.9 ± 0.9	24.1 ± 2.0	20.1 ± 3.0	10.5 ± 0.2

[a] Values are the means ± SD (standard deviation) of four independent experiments. They were derived from concentration–response curves obtained by measuring the percentage of vital cells relative to untreated controls after 72 h using MTT-assay. [b] Values determined by Muenzner et al.[33]

Conclusions

The presented regiospecific formation of 4-amino-6-nitrobenzimidazoles from the corresponding 4,6-dinitrobenzimidazole precursors is straightforward, and the structures of the products was confirmed by spectroscopic and X-ray diffraction methods. The 4-amino-6-nitrobenzimidazole derivatives displayed moderate activity against *T. gondii* parasites, and the design of more active derivatives and conjugates for the treatment of toxoplasmosis appears conceivable. The anticancer and antiparasitic activities of certain 4,6-dinitrobenzimidazoles were even more promising, considerable activities were observed both against *L. major* and against *T. gondii* parasites. More research needs to be conducted in order to identify the antiparasitic mode of action of these compounds. In addition, experiments with p53-negative colon cancer cells revealed a considerable sensitivity of these p53-mutant tumor cells to 4,6-dinitrobenzimidazoles. Hence, these compounds, and optimized derivatives of them,

Table 2. Inhibitory concentrations IC_{50} (in μM) of test compounds **6**–**I0**, **12**, **13**, **15**, and **16** when applied to cells of the Vero (African green monkey kidney epithelial) cell lines, effective concentrations EC_{50} when applied to promastigotes and amastigotes of *Leishmania major*, and cells of *Toxoplasma gondii*.[a] Amphotericin B (AmB) and atovaquone (ATO) were used as positive controls for the indicated cells.

Compd.	Promastigotes	Amastigotes	*T. gondii*	Vero
6	47.42	34.29	33.45	33.02
7	4.87	11.30	5.46	4.36
8	4.32	8.64	9.30	6.31
9	46.19	22.81	>35.21	>35.21
10	3.81	7.62	9.05	7.30
12	>45.43	>45.43	28.35	>45.43
13	>60.62	>60.62	18.19	15.52
15	>46.11	32.10	14.02	13.65
16	>43.84	31.92	12.98	19.82
ATO	–	–	0.07	9.5
AmB	0.83	0.47	–	7.7

[a] Values are the means of at least three independent experiments (SD ± 15%). They were obtained from concentration–response curves by calculating the percentage of treated cells in comparison to untreated controls after 72 h.

can play a significant role for the treatment of apoptosis-resistant cancers in future.

Experimental Section

Synthesis of dinitrobenzimidazoles – Typical procedure

92% H_2SO_4 (30 mL) was added dropwise to the benzimidazole starting compounds (0.025 mol). Under vigorous stirring, a cooled mixture of 65% HNO_3 (5 mL) and 92% H_2SO_4 (1 mL) was added. The reaction mixture was allowed to stand at 0–5 °C for 1 h, and poured in an ice-water mixture (1:1, v/v, 100 g) before NaCl (10 g) was added. The solution was maintained at 0–10 °C leading to the formation of crystalline solids, which were filtered off, and washed with cooled water. The crystals were dissolved in hot water, and the pH value of the resulting solution was adjusted to 7.5–8.0 with 3N NH_3. The precipitating dinitrobenzimidazoles were collected by filtration and recrystallized from ethanol. The purification of benzimidazole **12** was realized by repeated recrystallization from ethanol. Caution: H_2SO_4 and HNO_3 are very caustic acids.

2,6-Dimethyl-5,7-dinitrobenzimidazole 6

Yield = 75%; m.p. 210–212 °C (Ethanol). ^{1}H-NMR (300 MHz, (D_6)DMSO): δ = 2.60 (s, 3H; CH_3), 2.65 (s, 3H; CH_3), 5.11 (broad s, 1H; NH), 8.28 ppm (s, 1H; CH_{aro}); ^{13}C-NMR (75.5 MHz, (D_6)DMSO): δ = 14.03 (CH_3), 14.80 (CH_3), 111.08 (Cq), 116.70 (CH_{aro}), 133.19 (Cq), 159.61 ppm (C=N); MS (70 eV): *m/z* (%): 236 $[M]^+$.

6-Chloro-2-methyl-5,7-dinitrobenzimidazole 7

Yield = 87%; m.p. 224–226 °C (Ethanol). ^{1}H-NMR (300 MHz, (D_6)DMSO: δ = 2.63 (s, 3H; CH_3), 5.11 (broad s, 1H; NH), 8.58 ppm (s, 1H; CH_{aro}); ^{13}C-NMR (75.5 MHz, (D_6)DMSO): δ = 14.86 (CH_3), 110.65 (Cq), 113.57 (CH_{aro}), 136.32 (Cq), 136.49 (Cq), 141.82 (Cq), 160.48 ppm (C=N); MS (70 eV): *m/z* (%): 257 $[M]^+$.

6-Bromo-2-methyl-5,7-dinitrobenzimidazole 8

Yield = 81%; m.p. 226–228 °C (Ethanol). ^{1}H-NMR (300 MHz, (D_6)DMSO): δ = 2.26 (s, 3H; CH_3), 8.52 (s, 1H; CH_{aro}), 13.63 ppm (s, 1H; NH); ^{13}C-NMR (75.5 MHz, (D_6)DMSO): δ = 14.86 (CH_3), 100.50 (Cq), 116.21 (CH_{aro}), 135.61 (Cq), 137.22 (Cq), 140.11 (Cq), 141.39 (Cq), 160.17 ppm (C=N); MS (70 eV): *m/z* (%): 301.9 $[M]^+$.

6-Bromo-2-trifluoromethyl-5,7-dinitrobenzimidazole 9

Yield = 71%; m.p. 170–172 °C (Ethanol). ^{1}H-NMR (300 MHz, (D_6)DMSO): δ = 4.22 (broad s, 1H; NH), 8.71 ppm (s, 1H; CH_{aro}); ^{13}C-NMR (75.5 MHz, (D_6)DMSO): δ = 100.49 (Cq), 116.18 (CH_{aro}), 121.90 (CF_3), 135.81 (Cq), 137.25 (Cq), 140.00 (Cq), 141.29 (Cq), 146.50 ppm (C=N); MS (70 eV): *m/z* (%): 355.9 $[M]^+$.

6-Bromo-2-ethyl-5,7-dinitrobenzimidazole 10

Yield = 66%; m.p. 206–208 °C (Ethanol). ^{1}H-NMR (300 MHz, (D_6)DMSO): δ = 1.29 (m, 3H; CH_3), 2.91 (m, 2H; CH_2), 3.58 (broad s, 1H; NH), 8.44 ppm (s, 1H; CH_{aro}); ^{13}C-NMR (75.5 MHz, (D_6)DMSO): δ = 12.10 (CH_3), 22.59 (CH_2), 98.29 (Cq), 109.71 (Cq), 113.46 (CH_{aro}), 137.01 (Cq), 139.66 (Cq), 144.32 (Cq), 165.30 ppm (C=N); MS (70 eV): *m/z* (%): 315.9 $[M]^+$.

2-Trifluoromethyl-5,6-dinitrobenzimidazole 12

Yield = 40%; m.p. 238–240 °C (Ethanol). ^{1}H-NMR (300 MHz, (D_6)DMSO): δ = 4.41 (broad s, 1H; NH), 8.66 ppm (s, 1H; CH_{aro}); ^{13}C-NMR (75.5 MHz, (D_6)DMSO): δ = 115.93 (CH_{ar}), 117.58 (Cq), 119.73 (Cq), 121.89 (CF_3), 139.43 (Cq), 139.95 (Cq), 146.89 (Cq), 147.21 (C=N), 147.53 ppm (C=O); MS (70 eV): *m/z* (%): 276 $[M]^+$.

Synthesis of aminonitrobenzimidazoles – Typical procedure

A solution of dinitrobenzimidazole (1 g) in butanol (20 ml) was stirred under hydrogen (1 atm) in the presence of Pd/C (5% Pd, 0.2 g). The progress of the reaction was monitored by TLC. The catalyst was filtered off, and the solvent was removed under reduced pressure. The residue was dried in an oven at 70 °C. The purification of the benzimidazoles **13**, **15**, and **16** was realized by repeated recrystallization from ethanol. Caution: Pd/C is a flammable solid, and H_2 a flammable gas.

7-Amino-2,6-dimethyl-5-nitrobenzimidazole 13

Yield = 52%; m.p. 200–202 °C (Ethanol). ^{1}H-NMR (300 MHz, (D_6)DMSO): δ = 1.90 (s, 3H; CH_3), 2.21 (s, 3H; CH_3), 5.44 (s, 2H; NH_2), 7.37 (s, 1H; CH_{aro}), 12.2 ppm (broad s, 1H; NH); ^{13}C-NMR (75.5 MHz, (D_6)DMSO): δ = 12.61 (CH_3), 13.54 (CH_3), 97.28 (Cq), 107.19 (CH_{aro}), 126.42 (Cq), 131.03 (Cq), 135.27 (Cq), 149.00 (Cq), 152.54 ppm (C=N); MS (70 eV): *m/z* (%): 206 $[M]^+$.

7-Amino-6-chloro-2-methyl-5-nitrobenzimidazole 14

Yield = 64%; m.p. 176–178 °C (Ethanol).^{1}H-NMR (300 MHz, (D_6)DMSO): δ = 2.53 (s, 3H; CH_3), 5.99 (s, 2H; NH_2), 7.41 (s, 1H; CH_{aro}), 12.66 (s, 1H; NH); ^{13}C-NMR (75.5 MHz, (D_6)DMSO): δ = 14.59 (CH_3), 143.73 (Cq), 153.46 ppm (C=N); MS (70 eV): *m/z* (%): 228 $[M]^+$.

7-Amino-6-bromo-2-methyl-5-nitrobenzimidazole 15

Yield = 58%; m.p. 114–116 °C (Ethanol). ^{1}H-NMR (300 MHz, (D_6)DMSO): δ = 2.46 (s, 3H; CH_3), 5.86 (s, 2H; NH_2), 7.34 ppm (s, 1H; CH_{aro}); ^{13}C-NMR (75.5 MHz, (D_6)DMSO): δ = 15.13 (CH_3), 146.15 (Cq), 153.8 ppm (C=N); MS (70 eV): *m/z* (%): 271.9 $[M]^+$.

7-Amino-6-bromo-2-ethyl-5-nitrobenzimidazole 16

Yield = 69%; m.p. 159–161 °C (Ethanol). ^{1}H-NMR (300 MHz, (D_6)DMSO): δ = 1.28 (t, ^3J = 5 Hz, 3H; CH_3), 2.82 (q, ^3J = 5 Hz, 2H; CH_2), 5.86 (s, 2H; NH_2), 7.35 ppm (s, 1H; CH_{aro}); ^{13}C-NMR (75.5 MHz, (D_6)DMSO): δ = 12.58 (CH_3), 22.51 (CH_2), 98.02 (Cq), 129.17 (Cq), 132.03 (Cq), 146.23 (Cq), 158.63 ppm (C=N); MS (70 eV): *m/z* (%): 285.9 $[M]^+$.

Synthesis of nitrate salts 17and 18 – Typical procedure

To a solution of (0.194 mmol) of **13** or **14** in 15 ml of ethanol, (0.097 mmol) of $Cu(NO_3)_2$,H_2O dissolved in 10 ml of ethanol was added dropwise at room temperature. After the addition was completed, the mixture was heated under reflux for 24 h. The solvent was removed. After some days, crystals were collected, washed with diethyl ether and dried.

4-Amino-2,5-dimethyl-6-nitrobenzimidazole mononitrate 17

Yield = 77%; m.p. 206–208 °C (Ethanol). IR(ATR) cm^{-1}: 3471; 3368; 2934; 1647; 158 ; 1531; 1337; 1177; 1142; 1018; 870; 828; 780; 747; 712; 685; 643; 622; 580; 566; 548; 537; 526.

4-Amino-5-chloro-2-methyl-6-nitrobenzimidazole mononitrate 18

Yield = 71%; m.p. 184–186 °C(Ethanol). IR(ATR) cm^{-1}: 3350; 3200; 2757; 2629; 1652; 1625; 1574; 1533; 1442; 1410; 1386; 1369; 1344; 1255; 1168; 1024; 882; 829; 781; 747; 650; 623; 559; 539.

Tumor cell culture conditions

KB-V1Vbl (ACC-149) MDR cervix carcinoma, U-87 MG likely glioblastoma, HT-29 (ACC-299), HCT-116 (ACC-581) and HCT-116p53–/– (p53 knockout mutant) colon carcinoma, were cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, and 1% antibiotic-antimycotic at 37 °C, 5% CO_2, and 95% humidity. To keep KB-V1Vbl cells resistant, 340 nM vinblastine was added to the cell culture medium 24 h after every passage. Cells were grown at 5% CO_2, and 95% humidity. Only mycoplasma-free cell cultures were used.

MTT assay with tumor cells

The tumor cells (5×10^4 cells/ml, 100 µl/well) were grown in 96-well plates for 24 h. Then, they were treated with various concentrations of compounds **2**, **3**, **6**, **18**, or vehicle (DMSO) for 72 h under cell culture conditions. After the addition of 12.5 µl of a 0.5% MTT solution in PBS the cells were incubated for 2 h at 37 °C so that MTT could be converted to formazan crystals. The plates were centrifuged (300×g, 5 min, 4 °C), the medium withdrawn, and the formazan dissolved in 25 µl of DMSO containing 10% SDS and 0.6% acetic acid for at least 1 h at 37 °C. The absorbance of formazan (λ = 570 nm), and background (λ = 630 nm) was measured with a microplate reader (Tecan infinite F200). The IC_{50} values were derived from dose-inhibition curves as the means ± SD of four independent experiments with respect to vehicle treated control cells set to 100%. These data were used for a curve-fitting using the function "log(inhibitor) vs. normalized response – Variable slope" without constraints (GraphPad Prism 7).

Evaluation of benzimidazoles against *Toxoplasma gondii*

Vero cells (ATCC® CCL81™, USA) were used as host cells for the cultivation of *T. gondii* tachyzoites (obtained from Dr. Saeed El-Ashram, State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, China) with the benzimidazoles as described.[34] RH strain tachyzoites of *T. gondii* were cultivated in 96 well plates by using RPMI 1640 with 10% fetal bovine serum (FBS). Vero cells were used as host cells. Different concentrations of compounds were added after 4 h from the onset of the cell infection for assessing their activity and then incubated for 72 h under the required culturing conditions. Atovaquone (ATO) was applied as positive control. Inverted microscope was used for identifying cell viability via following equation:

Inhibition (%) = (1 − infected cells of experimental/infected cells of control) × 100.

Evaluation of benzimidazoles against *Leishmania major*

As reported previously, *L. major* promastigotes isolated from a Saudi person (February 2016), and *L. major* amastigotes isolated from infected BALB/c mice, were cultured and used for compound testing.[35] BALB/c mice were received from the Pharmaceutical College of the King Saud University, KSA, and kept according to the ethical guidelines of the committee of research ethics, Deanship of Scientific Research, Qassim University, permission number 20-03-20. Both forms of *L. major* amastigotes and promastigotes were cultivated in 96 well plates by using RPMI 1640 with 10% fetal bovine serum (FBS). Mouse macrophage cells were used as host cells for developing amastigotes. For assessing the activity of benzimidazoles after 72 h, different concentrations of them were added, while amphotericin B (AmB) was applied as positive control. For the detection of viable parasites, MTT (tetrazolium salt of (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) was added and evaluated spectrophotometry by ELISA reader (spectrophotometer) at 570 nm.

Toxicity evaluation of benzimidazoles by MTT assay

Vero cells were cultured and applied for MTT tests of the benzimidazoles as described previously.[35,36] For assessing the cytotoxicity of benzimidazoles, 96 well plates were used for 3 d incubation of the cells at different concentrations in RPMI 1640 with 5% FBS. Then, colorimetric assay by spectrophotometer was used at 540 nm for the detection of cell toxicity (IC_{50}).

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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