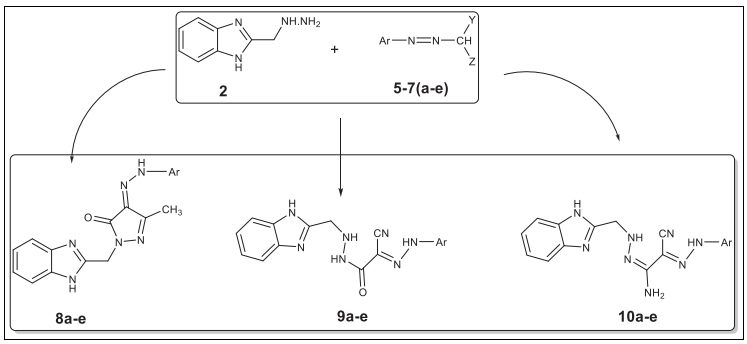
Synthesis and Pharmacological Investigations of Novel Pyrazolyl and Hydrazonoyl Cyanide Benzimidazole Entities

Mohamed E. Khalifa,[a]* Adil A. Gobouri,[a] Fahad M. Kabli,[a] Tariq A. Altalhi,[a] Abdulraheem S. A. Almalki,[a] and Amira M. Elemshaty[b]

[a]Department of Chemistry, Faculty of Science, Taif University, Taif 21974, Saudi Arabia
[b]Soil, Water & Environment Research Institute, Agricultural Research Center, Giza, Egypt
*E-mail: mohamedezzat200@hotmail.com; mohamedezzat@tu.edu.sa

Received October 22, 2018
DOI 10.1002/jhet.3522
Published online 00 Month 2019 in Wiley Online Library (wileyonlinelibrary.com).



Various novel benzimidazole entities linked to pyrazolyl and hydrazonoyl cyanide substrates carrying aryl and heteroaryl groups (**8a–e** to **10a–e**) were synthesized using new route syntheses and were focused on their pharmacological evaluation as one of the most important factors for the determination of the activity of these synthesized compounds. The obtained benzimidazoles' series were fully characterized and exhibited remarkable pharmacological activity upon *in vitro* screening for their antibacterial activity against strains of selected pathogenic Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) comparing with Ampicillin and Kanamycin as standard antibacterial agents and against human liver cancer cell lines (HepG2) as antitumor agents as well.

INTRODUCTION

Prevalence of infectious and malignant diseases has increased as much as the need for antimicrobial and antitumor agents, which are great importance for human survival [1]. The promising therapeutic potential of organic compounds containing benzimidazole scaffold as a core unit could be traced back to 19th century [2] and is found to exhibit various biological and pharmaceutical activities such as antibacterial [3], antifungal [4], antiviral [5,6], antioxidant [7], anticancer [8], analgesic [9], anti-inflammatory [10], antitubercular [11], antihelminthic [12], anticonvulsant [13], antiulcer [14], angiotensin-II receptor antagonists [15], antihypertensive [16], and to fluconazole against *Candida albicans* [4]. The combination of several types of hetero moieties into one vessel could produce a novel entity with enhanced bioactivities [17]. Based on the aforementioned reported findings, we have planned to synthesize a novel series of benzimidazole scaffolds derived from 2-(bromomethyl)-1*H*-benzimidazole (**1**) followed by their *in vitro* antibacterial as well as their antioxidant activity and antitumor activity against HepG2 cell line human hepatocyte carcinoma.

RESULTS AND DISCUSSION

Encouraged by our interest to design and synthesize potent biologically active compounds based on heterocyclic moieties containing sulfur and nitrogen compounds [18–22], benzimidazole derivatives have attracted our attention as important class of biological characteristics and clinical applications. Herein we report the synthesis of new benzimidazole derivatives starting from 2-(bromomethyl)-1*H*-benzimidazole (**1**) followed by functionalized reactions using new route syntheses and study their potential as bioactive compounds as described through this work.

Chemistry. In the current scenario, the starting compound 2-(bromomethyl)-1*H*-benzimidazole (**1**) was synthesized according to the reported method by the reaction of *o*-phenylenediamine with 2-bromoacetic acid [23–25]. A series of interesting reactions has been performed based on the reactivity of the 2-(bromomethyl)-1*H*-benzimidazole (**1**). Thus, reaction of 2-(bromomethyl)-1*H*-benzimidazole (**1**) with hydrazine hydrate gave the functionalized hydrazide derivative 2-(hydrazineylmethyl)-1*H*-benzimidazole (**2**) as depicted in Scheme 1, which is considered as

precursor for novel hydrazineyl and pyrazolone benzimidazoles.

The infrared (IR) spectrum of the hydrazide (**2**) revealed intense IR bands at 3358, 3244 cm^{-1} (NH, NH_2), 1547 cm^{-1} (NH bending), 1614 cm^{-1} (C═N), and 1418 cm^{-1} (CH_2 bending). The ^1H NMR spectrum of (**2**) displayed singlet signal at δ 4.65 ppm due to the methylene protons (2H, CH_2–NH), δ 7.12–7.46 ppm due to the aromatic protons, and singlet at δ 10.21 ppm due to the NH–benzimidazole proton.

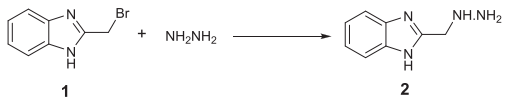
Thus, two-step reaction was performed in order to synthesize a new set of 2-((1*H*-benzimidazol-2-yl)methyl)-4-(2-arylhydrazineylidene)-3,5-disubstituted pyrazolyl derivatives using aryldiazenyl derivatives (**5a**–**e** to **7a**–**e**), but unexpected results were obtained as being explained later on. Firstly, various methods were reported for the preparation of the aryldiazenyl derivatives (**5a**–**e** to **7a**–**e**) [26–30]. In this study, we were going to prepare the target intermediate agents by azo coupling reaction of active methylene compounds (**3a**–**c**) (i.e., ethyl acetoactate, ethyl cyanoacetate, and malononitrile) with different freshly prepared aryl/heteryl diazonium salts (**4a**–**e**) in mild conditions (Scheme 2).

Secondly, the aryl/heteryl hydrazonyl derivatives (**5a**–**e** to **7a**–**e**) were allowed to react with 2-(hydrazineylmethyl)-1*H*-benzimidazole (**2**) in the presence of sodium ethoxide (Schemes 3–5). In traditional way, the Knorr pyrazole synthesis was reported widely using an acid catalyst [31], as the β-keto esters acted as Michael donors and hence underwent cycloaddition reaction with hydrazineyl derivative to obtain pyrazolone derivatives [32–34]. As a new route, using basic catalyst, the mechanism of the reaction was assumed to go on the pathway in order to afford the hydrazono intermediate before cyclization to obtain the target pyrazolone derivatives (**8a**–**e**) as basic heterocyclic nucleus as illustrated in Scheme 3 and Figure 1.

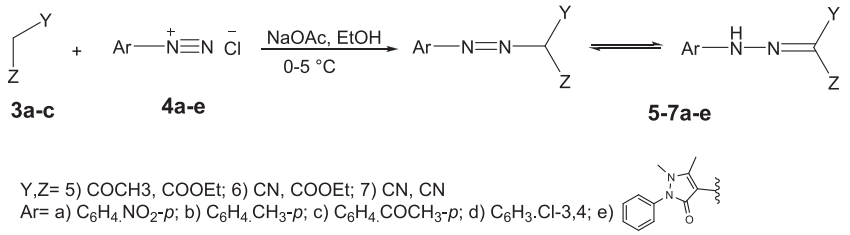
Unlikely the routine methodology, the condensation reaction of the hydrazide compound (**2**) with (**6a**–**e** and/or **7a**–**e**) was going without further internal cyclization to afford 2-oxo (and/or amino)acetohydrazonoyl cyanide derivatives (**9a**–**e** and **10a**–**e**) as shown by Schemes 4 and 5. To the best of our knowledge, the strongly deactivating action of nitrile group than acetyl group as Pi-acceptors might explain this phenomenon. The nitrile group tended to polarize the electron density from the adjacent Pi system and hence hindered its reduction and cyclization afterwards.

Generally, the IR spectra of derivatives (**8**–**10**) revealed characteristic IR bands at 3738–3740 cm^{-1} ($NH_{str.}$), 3264–3850 cm^{-1} (NH_2, **10a**–**e**), 2913–3164 (NH–hydrazo), 1653–1735 cm^{-1} (CO pyrazolone, **8a**–**e**) and 1642–1688 cm^{-1} (CO amide, **9a**–**e**), 2175–2228 cm^{-1} (CN, **9a**–**e** and **10a**–**e**). The ^1H NMR spectrum displayed singlet signal at 2.27–2.72 ppm due to the methyl protons (3H, CH_3 pyrazolone ring, **8a**–**e**) and singlet at 12.16–12.45 ppm due to the NH–benzimidazole proton.

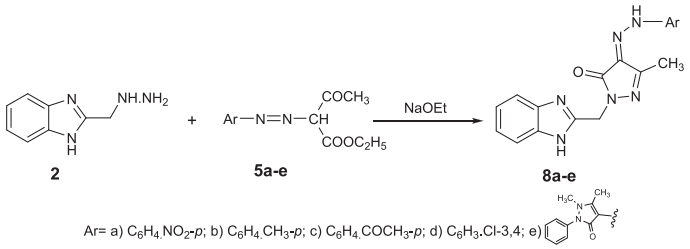
Scheme 1. Synthesis of 2-(hydrazineylmethyl)-1*H*-benzimidazole (**2**).



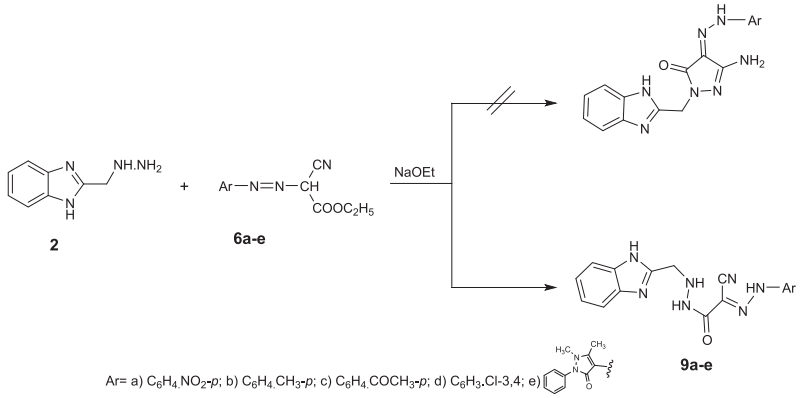
Scheme 2. Preparation of the aryldiazenyl derivatives (**5a**–**e** to **7a**–**e**).



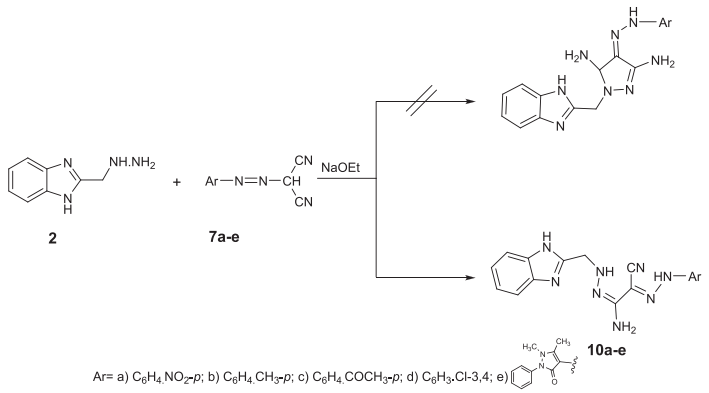
Scheme 3. Synthesis of 2-arylhydrazineylidene-3,5-disubstituted pyrazolyl derivatives (**8a**–**e**).



Scheme 4. Synthesis of 2-oxoacetohydrazonoyl cyanide derivatives (**9a**–**e**).



Scheme 5. Synthesis of 2-aminoacetohydrazonoyl cyanide derivatives (**10a**–**e**).



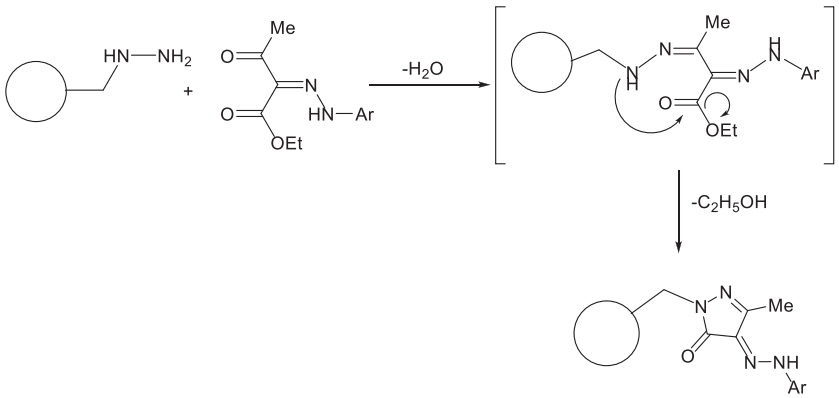


Figure 1. Mechanism of formation of compounds (**8a**–**e**).

PHARMACOLOGICAL EVALUATION

Antimicrobial performance. The novel synthesized compounds (**8** to **10a**–**e**, 100 μg/mL in DMSO) were subjected *in vitro* to antibacterial assay using disc diffusion method against two streams of pathogenic microorganisms *Escherichia coli* (Gram-negative bacteria) and *Staphylococcus aureus* (Gram-positive bacteria) in the media of nutrient agar [35]. The antibacterial activity was expressed by measuring the inhibition zone in mm, and the bactericides *Ampicillin* and *Kanamycin* respectively were served as positive reference standards (Fig. 1). It was shown that most of the tested compounds exhibited broad-spectrum antimicrobial activities against both Gram-positive bacteria and Gram-negative bacteria, with inhibition percentage in the range of 50% to 78%.

Presence of strong electron withdrawing group (e.g., compound **9d**) showed higher activity against *E. coli* (Gram-negative bacteria), while most of the synthesized compounds exhibited moderate to good activity against *S. aureus* (Gram-positive bacteria).

The plotted data in Figure 2 showed that most of the tested compounds exhibited variable antimicrobial activities from nil to moderate to high depending on their chemical structure. It is obviously clear that the presence of strong electron withdrawing group (e.g., nitro group) linked to the phenyl function (e.g., compounds **8d**, **9d**, and **10d**) showed higher activity against Gram-positive bacteria (i.e., *S. aureus*) than the positive control (Kanamycin). Introducing a phenyl function enhanced the reactivity against *S. aureus* (Gram-positive bacteria), where the action of phenyl nucleus was referred to its ability to form unstable complexes on the cell membrane and hence inactivating intracytoplasm enzymes [36,37]. Most of the synthesized compounds exhibited nil to moderate activity against *E. coli* (Gram-negative bacteria) comparing with its positive control (Ampicillin).

Antioxidant potential of the synthesized compounds. The method DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay incorporating a metastable free radical was used to provide accurate expression for the antioxidant activity of the synthesized compounds comparing with the intracellular scavenging activity of Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) as standard agent [38]. That is capable of accepting hydrogen radicals from antioxidants in solution and the reaction between DPPH, and antioxidant can be monitored by the decrease in absorbance of the violet colored free radical. Inhibition percentages of DPPH radical by tested compounds were plotted in Figure 3, and the measure of drug's potency in terms of half-maximal effective concentration (EC_{50}) values was plotted in Figure 4, which referred to the compound concentration where 50% of its maximal effect was observed. The resonance phenomena of double bonds and lone pair electrons on nitrogen may lead to radical formation in more than one site, especially on the benzene ring attached to the nitro group (highly electron withdrawing group) that enabled the benzene ring to convert to a radical form and forms a new covalent bond with another radical (e.g., **8a**, **8b**, **8d**, **8e**, **10b**, **10c**, and **10e** – the highest DPPH values and the lowest EC_{50} values). This may explain the good antioxidant and scavenging activities of the tested compounds. The presence of pyrazole and benzimidazole rings in one compound (e.g., **8a**–**e**) and fewer rings in others gave them the advantage over other structures. This conclusion was also supported by previously reported results [39].

Cytotoxicity evaluation. All synthesized compounds were screened *in vitro* for possible potential antitumor molecules against human liver cancer cell line (HepG2) and showed variable anticancer activity. Neutral red (NR) technique was used to evaluate the cytotoxic effects of gradient concentration (100 to 500 µg/mL) of tested compounds in terms of cell viability, where the lower values showed higher antitumor potency (Fig. 5). The

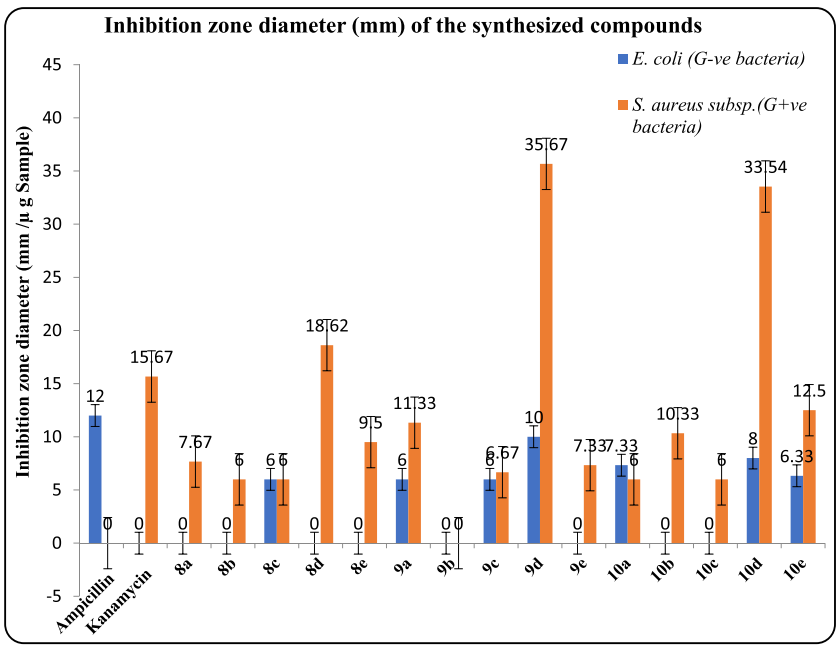


Figure 2. Antimicrobial activity of the novel benzimidazole derivatives against selected pathogenic bacteria. [Color figure can be viewed at wileyonlinelibrary.com]

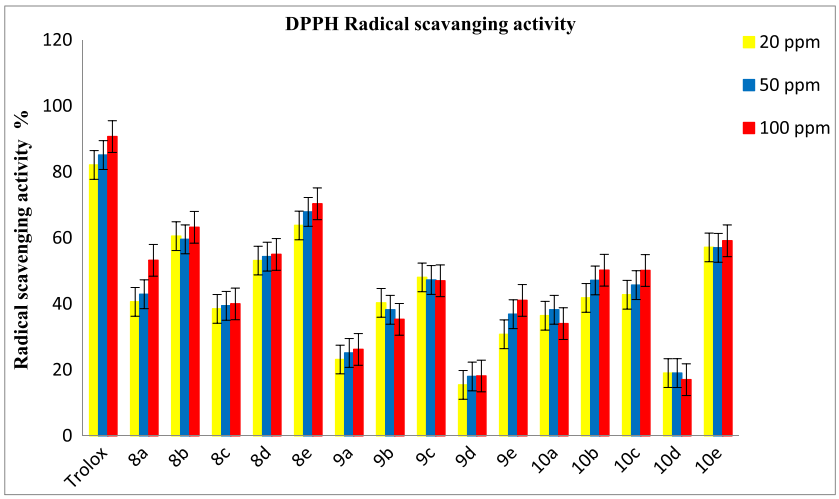


Figure 3. Inhibition percentage of DPPH radical by novel synthesized benzimidazoles compared with Trolox. [Color figure can be viewed at wileyonlinelibrary.com]

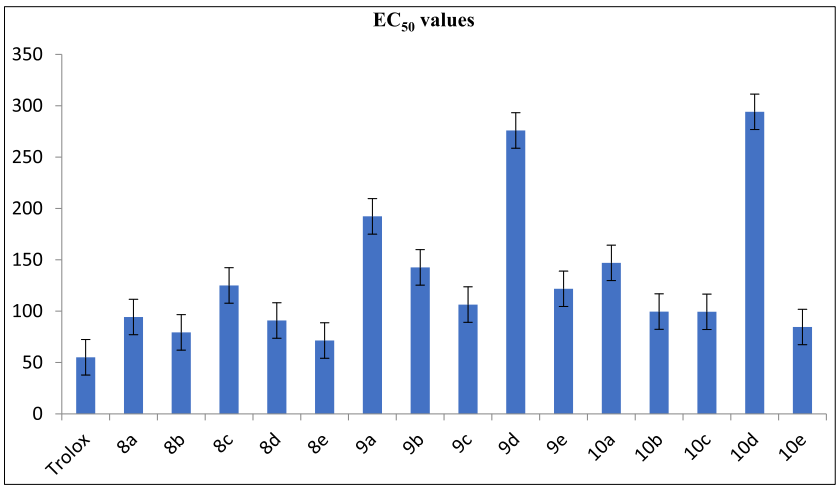


Figure 4. EC_{50}: concentration of novel synthesized benzimidazoles that give half-maximal response compared with Trolox. [Color figure can be viewed at wileyonlinelibrary.com]

half-maximal inhibitory concentration (IC_{50}) values that measured the potency of the compound in inhibiting a specific biological function relative to Doxorubicin (25.66 µg/mL) were evaluated and plotted in Figure 6. The decrease of the IC_{50} value of the tested compound indicated its higher efficiency against tumor cell.

As shown in Figures 5 and 6, the tested compounds exhibited broad-spectrum antitumor activity in different concentrations, where increasing the concentration lead to less viability percentage and the IC_{50} values indicated the medium to higher potency of compounds **9c**, **9e**, and **10a–e** comparing with reference compound. The presence of the antipyrinyl and the 4-phenyl function with a nitro group (e.g., **8e**, **10e**, **8a**, and **10a**) increased the antitumor activity because of their high antioxidant and free radical scavenging activities. This structure may lead to radical formation in more than one site, especially on the benzene ring attached to the nitro group (e.g., **10a**), which is a highly electron withdrawing group that enables the benzene ring to convert to a radical form and formed a new covalent bond with another radical as previously reported [40]. The biocompatibility of the nitrile functionality [41] assisted the prevalence of clinical nitrile-containing pharmaceuticals. It is well known that the nitrile group should be activated by adjacent structural elements such as electron withdrawing groups to play the electrophilic role towards free nucleophiles [42]. Subsequent release of cyanide from alkylnitriles bearing an adjacent proton can be oxidized in the liver to cyanohydrins [43]. The potential oxidation

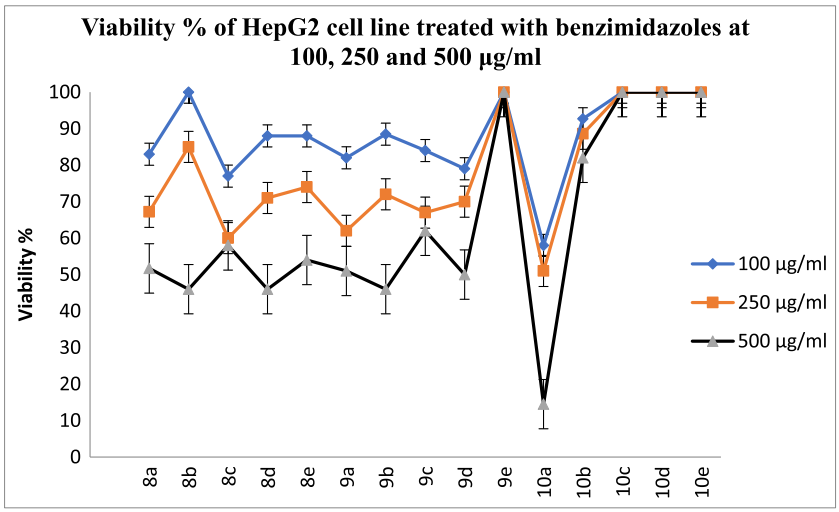


Figure 5. Viability % of HepG2 cell line treated with novel synthesized benzimidazoles at 100, 250, and 500 µg/mL. [Color figure can be viewed at wileyonlinelibrary.com]

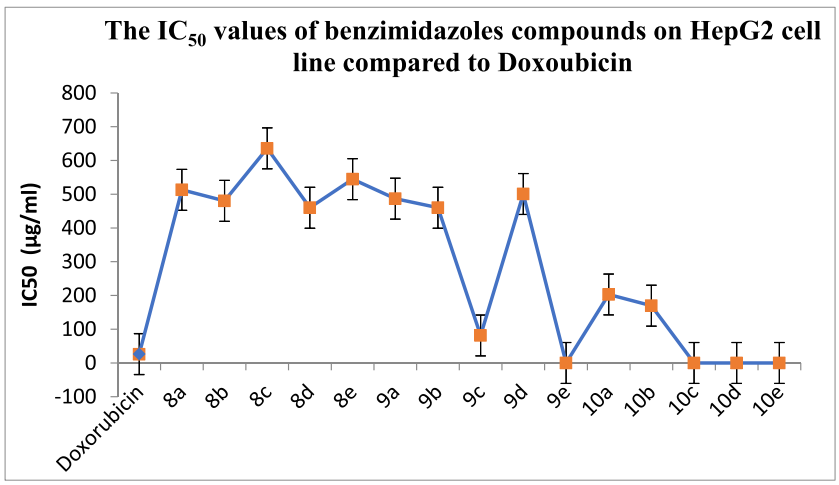


Figure 6. IC_{50}: half-maximal inhibitory concentration values relative to Doxorubicin. [Color figure can be viewed at wileyonlinelibrary.com]

and cyanide ejection likely explains the success of several alkenenitrile-containing pharmaceuticals in this study.

CONCLUSIONS

The newly synthesized benzimidazole derivatives **8a–e** to **10a–e** were prepared and fully characterized. They exhibited variable *in vitro* antibacterial activities against *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria) comparing with Ampicillin and Kanamycin as standard antibacterial agent related to their chemical structure. The compounds **8a**, **8b**, **8d**, **8e**, **10b**, **10c**, and **10e** showed more inhibition effect against DPPH (the lowest EC_{50} values) compared with Trolox (EC_{50} = 55.13) and other tested compounds. They were screened for their antitumor activities against human liver cancer cell line (HepG2) using NR technique to evaluate cytotoxic effects of gradient concentrations (100 to 500 µg/mL) of test compounds on human liver (HepG2) cell line and in terms of cell viability and IC_{50} values. The tested compounds exhibited acceptable efficiency against human liver (HepG2) cell line. The increase in efficiency among synthesized compounds may be due to the presence of electron withdrawing substituent, pyrazole, benzimidazole and antipyrinyl rings in one compound and fewer rings in others.

EXPERIMENTAL

Materials for chemical reactions. All chemicals and reagents used were analytical grade or chemically pure and supplied by Sigma Aldrich Co., Darmstadt, Germany.

Micro and spectroscopic analysis. All the synthesized compounds were micro-analyzed satisfactorily for C, H, and N by the PerkinElmer 2400 Analyzer, series II (PerkinElmer Co., Shelton, UK); their results were found to be in good agreement (±0.3%) with the calculated values. All of the corrected melting points are in degree centigrade and measured using a Stuart SMP20 melting point apparatus (Bibby Scientific Limited, Staffordshire, UK). The IR spectra were recorded on a PerkinElmer Alpha platinum-ATR spectrometer, the ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Mercury VXR-300 spectrometer (Varian Inc., Palo Alto, CA, USA), and the chemical shifts were related to that of the solvent DMSO-d_6 using TMS as an internal standard. The EI-MS analysis was carried out using TRACE GC Ultra Gas Chromatographs–mass spectrometry, coupled with THERMO mass spectrometer detector–ISQ Single Quadrupole Mass Spectrometer (THERMO Scientific Corp., Waltham, MA, USA), and was obtained by electron ionization at 70 eV, using a spectral range of *m*/*z* 50–1000. All of the microanalyses and spectral analyses were performed by the Micro Analytical Centers of Taif University–Saudi Arabia [IR spectra and high-resolution mass spectrometry (HRMS)], Cairo University (^{1}H NMR and ^{13}C NMR), and National Research Center–Egypt (mass spectra).

Biological analysis. All used kits for biochemical parameters were supplied by Diagnostic Company, Maidenhead, UK. All chemicals used were supplied by Gibco®Life Technologies, Waltham, MA, USA. The biological tests were performed by the Microbiology Units, Agricultural Research Center and Soil, Water & Environment Research Institute, Giza, Egypt. Human transformed cell lines from liver (HepG2) were obtained from Egyptian company for vaccine and serum (VACSERA, Cairo, Egypt). The statistical package for XLFIT5 software (IDBS, London, England) was used for the statistical analyses.

Neutral red reagent. A quantity of 0.33 g of NR desorb solution (1% glacial acetic acid and 50% ethanol made in water) was added to 100 mL H_2O_2 filter sterilized and stored at room temperature, protected from light. On the day of use, the reagent was diluted in media to obtain a final concentration of 33 μg/mL.

General procedure for the synthesis of 2-(bromomethyl)-1*H*-benzimidazole (1). The 2-bromomethyl benzimidazole (**1**) was prepared according to the literature procedure [23–25], where *o*-phenylenediamine (2.16 g, 20 mmol) was dissolved in 30–40 mL of 4*M* HCl by heating. The bromoacetic acid (4.16 g, 30 mmol) was added thoroughly, and then the reaction mixture was boiled under reflux for 5 h. The mixture was neutralized with ammonia, and the solid product was filtered, washed with water, and dried. The obtained product was recrystallized from acetone to give red crystals. Yield 86%, mp 140–141°C (60°C [44], 59–61°C [45]).

Synthesis of 2-(hydrazineylmethyl)-1*H*-benzimidazole (2). A mixture of 2-(bromomethyl)-1*H*-benzimidazole (**1**) (2.11 g, 10 mmol) and hydrazine hydrate 80% (1.25 mL, 25 mmol) was heated under reflux for 2 h. The reaction mixture allowed cooling at room temperature, and the formed solid product was then recrystallized from ethanol/water to obtain the 2-(hydrazineylmethyl)-1*H*-benzimidazole (**2**) as yellow solid. Yield 62%, mp 175–178°C (197–198°C [46], 114°C [47]).

General procedure for the synthesis of aryldiazenyl derivatives (5a–e to 7a–e). The corresponding aryl diazonium chloride was prepared by adding cold sodium nitrite solution (0.69 g in 15 mL H_2O) to a cold suspension of different aryl and hetaryl amines (10 mmol) in concentrated HCl (4 mL) with stirring. To a cold solution of 10 mmol the functionalized active methylene compounds ethyl chloroacetate, ethyl cyanoacetate, and malononitrile (**5**, 1.30 g; **6**, 1.13 g; and **7**, 0.66 g; respectively) in ethanol (20 mL) and sodium acetate (1.6 g, 20 mmol), a cold aqueous solution from the corresponding aryl diazonium chloride was added dropwise with stirring at 0–5°C for 2 h. The solid product obtained was filtered, washed with water followed by cold ethanol, and then dried. The obtained products were recrystallized from ethanol to give target aryldiazenyl reaction intermediates (**5a**–**e** to **7a**–**e**).

Ethyl-2-(2-(4-nitrophenyl)hydrazineylidene)-3-oxobutanoate (5a). Yield 87%, brown solid, mp 158–160°C (145–146°C [26], 120°C [27]).

Ethyl-3-oxo-2-(2-(p-tolyl)hydrazineylidene)butanoate (5b). Yield 76%, reddish brown solid, mp 89–90°C (78°C [48], 66°C [49]).

Ethyl-2-(2-(4-acetylphenyl)hydrazineylidene)-3-oxobutanoate (5c). Yield 58%, brown solid, mp 134–136°C (126°C [28]).

Ethyl-2-(2-(3,4-dichlorophenyl)hydrazineylidene)-3-oxobutanoate (5d). Yield 70%, reddish brown solid, mp 96–98°C. IR: ν_{max} = 3156–2931 (=CH aromatic), 2986 (NH–hydrazo), 1698 (CO ester), 1627 (CO acetyl), 1582 (C=N), 1462 (CH_2) and 1366 (CH_3) cm^{-1}. ^{1}H NMR: δ/ppm = 1.30 (q, 2H, CH_2 aliphatic), 2.27 (s, 3H, CH_3), 3.65 (t, 3H, CH_3), 6.74–7.41 (m, 3H, Ar–H), 12.60 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 12.8, 28.2, 61.4, 115.6, 118.1, 123.4, 128.6, 129.1, 131.7, 142.4, 168.4, 196.4. MS (*m*/*z*, %): 304.12 (M + 1, 32.20). HRMS calc. for $C_{12}H_{12}Cl_2N_2$: 302.0225, found: 302.0221. *Anal.* Calcd. for $C_{12}H_{12}Cl_2N_2$ (303.14): C, 47.55; H, 3.99; N, 9.24. Found: C, 47.16; H, 3.74; N, 9.11 [50].

Ethyl 2-((1,5-dimethyl-3-oxo-2-phenylpyrazolidin-4-yl) diazenyl)-3-oxobutanoate (5e). Yield 61%, reddish brown solid, mp 191°C (179–181°C [51], 169°C [52]).

Ethyl-2-cyano-2-(2-(4-nitrophenyl)hydrazineylidene)acetate (6a). Yield 89%, reddish brown solid, mp 177–179°C (173–174°C [53]).

Ethyl-2-cyano-2-(2-(p-tolyl)hydrazineylidene)acetate (6b). Yield 60%, yellow solid, mp 120–122°C (105°C [29]).

Ethyl-2-(2-(4-acetylphenyl)hydrazineylidene)-2-cyanoacetate (6c). Yield 67%, brown solid, mp 148–149°C (110–11°C, acetic acid [54]).

Ethyl-2-cyano-2-(2-(3,4-dichlorophenyl)hydrazineylidene) acetate (6d). Yield 60%, yellow solid, mp 171–172°C. IR: ν_{max} = 3210–2980 (=CH aromatic, –CH aliphatic), 3070 (NH–hydrazo), 2212 (CN), 1739 (CO ester), 1591 (C=N), 1450 (CH_2) and 1408 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 1.35 (q, 2H, CH_2 aliphatic), 3.87 (t, 3H, CH_3), 6.58–7.67 (m, 3H, Ar–H), 13.60 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 12.9, 61.8, 110.6, 115.3, 116.3, 118.1, 123.4, 129.2, 131.7, 142.5, 161.7. MS (*m*/*z*, %): 287.31 (M + 1, 28.11). HRMS calc. for $C_{11}H_9Cl_{12}N_3O_2$: 285.0072, found: 285.0069. *Anal.* Calcd. for $C_{11}H_9Cl_{12}N_3O_2$ (286.11): C, 46.18; H, 3.17; N, 14.69. Found: C, 45.87; H, 2.84; N, 14.44 [30].

Ethyl-2-cyano-2-(2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazineylidene)acetate (6e). Yield 64%, red solid, mp 167–168°C (162–164°C [55]).

N-(4-Nitrophenyl)carbonohydrazonoyl dicyanide (7a). Yield 85%, yellow solid, mp 206–208°C (214°C [29]).

N-p-Tolylcarbonohydrazonoyl dicyanide (7b). Yield 61%, yellow solid, mp 189–190°C (158°C [29]).

N-(4-Acetylphenyl)carbonohydrazonoyl dicyanide (7c). Yield 65%, brown solid, mp 190–192°C. IR: ν_{max} = 3216–2956 (=CH aromatic), 3050 (NH–hydrazo), 2217 (CN), 1671 (CO acetyl), 1595 (C=N) and 1409 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 3.85 (s, 3H, CH_3), 7.16–7.84 (m, 4H, Ar–H), 12.85 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 26.4, 81.4, 111.6 (2C), 115.2 (2C), 129.9 (2C), 131.2, 147.2, 197.8. MS (*m*/*z*, %): 213.10 (M + 1, 57.13). HRMS calc. for $C_{11}H_8N_4O$: 212.0698, found: 212.0694. *Anal.* Calcd. for $C_{11}H_8N_4O$ (212.21): C, 62.26; H, 3.80; N, 26.40. Found: C, 61.91; H, 3.56; N, 26.30.

N-(3,4-Dichlorophenyl)carbonohydrazonoyl dicyanide (7d). Yield 68%, yellow solid, mp 198–199°C (193–196°C [56]).

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)carbonohydrazonoyl dicyanide (7e). Yield 62%, yellow solid, mp >250°C. IR: ν_{max} = 3227–2941 (=CH aromatic), 3105 (NH–hydrazo), 2224 (CN), 1634 (CO pyrazolone), 1594 (C=N) and 1405 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 3.14 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 7.15–7.82 (m, 5H, Ar–H), 11.47 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 12.5, 34.7, 81.8, 113.2 (2C), 116.2, 122.7, 124.2 (2C), 129.5 (2C), 131.8, 133.1, 160.2. MS (*m*/*z*, %): 280.28 (M^+, 97.0). HRMS calc. for $C_{11}H_8N_4O$: 280.1073, found: 280.1070. *Anal.* Calcd. for $C_{14}H_{12}N_6O$ (280.29): C, 59.99; H, 4.32; N, 29.98. Found: C, 59.84; H, 4.21; N, 29.80 [57].

General procedure for the synthesis of 2-((1*H*-benzimidazol-2-yl)methyl)-4-(2-arylhydrazineylidene)-3,5-disubstituted pyrazolyl derivatives (8a–e to 10a–e). An equimolar ratio of 2-(hydrazineylmethyl)-1*H*-benzimidazole **2** (1.62 g, 10 mmol) and aryldiazenyl derivatives **5a**–**e** to **7a**–**e** (10 mmol) was refluxed in the presence of sodium ethoxide (0.23 g Na/20 mL EtOH, 10 mmol) and ethanol for 6 h. The reaction mixture was then poured onto ice-cold water containing a few drops of dilute hydrochloric acid for neutralization. The solid product obtained was filtered off, washed with cold water, dried, and recrystallized from proper solvent.

2-((1H-Benzimidazol-2-yl)methyl)-5-methyl-4-(2-(4-nitrophenyl)hydrazineylidene)-2,4-dihydro-3H-pyrazol-3-one (8a). Yield 59%, brown solid, mp 204–206°C (EtOH/DMF). IR: ν_{max} = 3264–2848 (NH, =CH aromatic, –CH aliphatic), 3101 (NH–hydrazo), 1677 (CO pyrazolone), 1594 (C=N), 1449 (CH_2) and 1410 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 1.22 (s, 2H, CH_2 aliphatic), 2.16 (s, 3H, CH_3 pyrazolone ring), 6.58–8.31 (m, 8H, Ar–H), 12.24 (s, 1H, NH–benzimidazole ring), 13.45 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 12.5, 51.1, 113.4 (2C), 115.9 (2C), 122.5 (2C), 124.2 (2C), 128.2, 137.7, 139.3 (2C), 141.1, 148.6, 149.7, 169.5. MS (*m*/*z*, %): 378.34 (M + 1, 3.11). *Anal.* Calcd. for $C_{18}H_{15}N_7O_3$ (377.36): C, 57.29; H, 4.01; N, 25.98. Found: C, 57.13; H, 4.15; N, 25.79.

2-((1H-Benzimidazol-2-yl)methyl)-5-methyl-4-(2-(p-tolyl) hydrazineylidene)-2,4-dihydro-3H-pyrazol-3-one (8b). Yield 57%, brownish yellow solid, mp >250°C (acetic acid). IR: ν_{max} = 3278–2850 (NH, =CH aromatic, –CH aliphatic), 3164 (NH–hydrazo), 1691 (CO pyrazolone), 1636 (C=N), 1460 (CH_2) and 1407 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 1.24 (s, 2H, CH_2 aliphatic), 2.27 (s, 3H, CH_3 pyrazolone ring), 2.50 (s, 3H, CH_3), 6.58–8.31 (m, 8H, Ar–H), 12.24 (s, 1H, NH–benzimidazole ring), 13.45 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 11.3, 21.1, 51.4, 115.7 (2C), 116.3 (2C), 122.6 (2C), 129.1 (2C), 128.2, 137.6, 139.2 (2C), 140.4, 141.1, 148.6, 169.5. MS (*m*/*z*, %): 345.4 (M − 1, 44.52). *Anal.* Calcd. for $C_{19}H_{18}N_6O$ (346.39): C, 65.88; H, 5.24; N, 24.26. Found: C, 65.71; H, 5.11; N, 24.19.

2-((1H-Benzimidazol-2-yl)methyl)-4-(2-(4-acetylphenyl) hydrazineylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (8c). Yield 63%, brown solid, mp 119–120°C (EtOH/DMF). IR: ν_{max} = 3576–2992 (NH, NH_2, =CH aromatic, –CH aliphatic), 2918 (NH–hydrazo), 1735 (CO pyrazolone), 1668 (CO acetyl), 1594 (C=N), 1463 (CH_2) and 1420 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 1.24 (s, 2H, CH_2 aliphatic), 2.27 (s, 3H, CH_3 pyrazolone ring), 2.50 (s, 3H, CH_3), 6.58–8.31 (m, 8H, Ar–H), 12.24 (s, 1H, NH–benzimidazole ring), 13.45 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 11.4, 26.1, 51.4, 115.7 (4C), 123.2 (2C), 128.5 (2C), 129.2, 131.3, 138.9 (2C), 141.3, 147.2, 148.4, 169.5, 196.8. MS (*m*/*z*, %): 375.34 (M + 1, 12.2). *Anal.* Calcd. for $C_{20}H_{18}N_6O_2$ (374.40): C, 64.16; H, 4.85; N, 22.45. Found: C, 64.01; H, 4.58; N, 22.13.

2-((1H-Benzimidazol-2-yl)methyl)-4-(2-(3,4-dichlorophenyl) hydrazineylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one

(8d). Yield 59%, dark brown solid, mp 85–86°C (EtOH/DMF). IR: ν_{max} = 3264–2854 (NH, =CH aromatic, –CH aliphatic), 3058 (NH–hydrazo), 1660 (CO pyrazolone), 1590 (C=N), 1463(CH_2) and 1388 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 1.30 (s, 2H, CH_2 aliphatic), 2.27 (s, 3H, CH_3 pyrazolone ring), 6.58–8.31 (m, 7H, Ar–H), 12.45 (s, 1H, NH–benzimidazole ring), 13.60 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 11.5, 51.4, 115.4, 115.6, 118.1, 123.2 (2C), 123.4, 128.6, 129.1, 131.7, 138.9 (2C), 141.3, 142.4, 148.4, 169.5, 196.4. MS (*m*/*z*, %): 401.92 (M + 1, 16.15). *Anal.* Calcd. for $C_{18}H_{14}Cl_2N_6O$ (401.25): C, 53.88; H, 3.52; N, 20.94. Found: C, 53.67; H, 3.40; N, 20.81.

2-((1H-Benzimidazol-2-yl)methyl)-4-(2-(1,5-dimethyl-3-oxo-2-phenylpyrazolidin-4-yl)hydrazineylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (8e). Yield 58%, dark brown solid, mp 131–132°C (EtOH/DMF). IR: ν_{max} = 3264–2854 (NH, =CH aromatic, –CH aliphatic), 2922 (NH–hydrazo), 1653 (CO pyrazolone), 1602 (C=N), 1443 (CH_2) and 1379 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 1.63 (s, 2H, CH_2 aliphatic), 2.16 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 2.89 (s, 3H, CH_3), 7.14–7.95 (m, 10H, Ar–H), 12.32 (s, 1H, NH–benzimidazole ring). ^{13}C NMR: δ/ppm = 11.5, 15.1, 43.3, 51.4, 53.1, 61.7, 115.4 (2C), 123.2 (2C), 128.9, 128.6 (2C), 128.1, 131.8 (2C), 134.5, 138.9 (2C), 141.4, 148.3, 169.4, 169.7. MS (*m*/*z*, %): 442.29 (M − 2, 15.12). *Anal.* Calcd. for $C_{23}H_{24}N_8O_2$ (444.50): C, 62.15; H, 5.44; N, 25.21. Found: C, 61.91; H, 5.23; N, 25.08.

2-(2-((1H-Benzimidazol-2-yl)methyl)hydrazineyl)-N-(4-nitrophenyl)-2-oxoacetohydrazonoyl cyanide (9a). Yield 57%, dark brown solid, mp 116–117°C (EtOH/DMF). IR: ν_{max} = 3263–2853 (NH, =CH aromatic, –CH aliphatic), 3103 (NH–hydrazo), 2220 (CN), 1656 (CO amide), 1593 (C=N), 1447(CH_2) and 1326, 1507 (NO_2) cm^{-1}. ^1H NMR: δ/ppm = 2.27 (s, 2H, CH_2 aliphatic), 6.65–8.31 (m, 8H, Ar–H; 2H hydrazide), 12.32 (s, 1H, NH–benzimidazole ring), 13.65 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 51.4, 106.1, 110.6, 113.2 (2C), 115.3 (2C), 123.2 (2C), 124.9 (2C), 137.6 (2C), 138.9 (2C), 141.4, 149.0. MS (*m*/*z*, %): 378.78 (M^+, 1.37). *Anal.* Calcd. for $C_{17}H_{14}N_8O_3$ (378.35): C, 53.97; H, 3.73; N, 29.62. Found: C, 53.68; H, 3.60; N, 29.51.

2-(2-((1H-Benzimidazol-2-yl)methyl)hydrazineyl)-2-oxo-N-(p-tolyl)acetohydrazonoyl cyanide (9b). Yield 59%, brown solid, mp 98–99°C (benzene). IR: ν_{max} = 3220–2982 (NH, =CH aromatic, –CH aliphatic), 2918 (NH–hydrazo), 2215 (CN), 1678 (CO amide), 1597 (C=N), 1471 (CH_2) and 1378 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 2.51 (s, 2H, CH_2 aliphatic), 3.30 (s, 3H, CH_3), 7.19–7.41 (m, 8H, Ar–H; 2H hydrazide), 12.16 (s, 1H, NH–benzimidazole ring), 12.95 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 21.3, 51.4, 106.2, 110.6, 115.2 (2C), 116.3 (2C), 123.2 (2C), 129.9 (2C), 131.2, 138.9 (2C), 140.6, 141.4, 162.9. MS (*m*/*z*, %): 346.67 (M − 1, 21.19). *Anal.* Calcd. for $C_{18}H_{17}N_7O$ (347.38): C, 62.24; H, 4.93; N, 28.23. Found: C, 62.11; H, 4.79; N, 28.08.

2-(2-((1H-Benzimidazol-2-yl)methyl)hydrazineyl)-N-(4-acetylphenyl)-2-oxoacetohydrazonoyl cyanide (9c). Yield 76%, dark brown solid, mp 134–135°C (EtOH/DMF). IR: ν_{max} = 3263–2920 (NH, =CH aromatic, –CH aliphatic), 3101 (NH–hydrazo), 2217 (CN), 1665 (CO amide), 1594 (C=N) and 1426 (CH_2) cm^{-1}. ^1H NMR: δ/ppm = 2.51 (s, 2H, CH_2 aliphatic), 3.86 (s, 3H, CH_3), 7.16–7.88 (m, 8H, Ar–H; 2H hydrazide), 12.41 (s, 1H, NH–benzimidazole ring), 12.85 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 26.2, 51.4, 106.2, 110.6, 115.2 (4C), 123.2 (2C), 129.9 (2C), 131.2, 138.9 (2C), 141.4, 147.2, 162.9, 196.8. MS (*m*/*z*, %): 376.92 (M + 1, 12.16). *Anal.* Calcd. for $C_{19}H_{17}N_7O_2$ (375.39): C, 60.79; H, 4.56; N, 26.12. Found: C, 60.62; H, 4.44; N, 25.97.

2-(2-((1H-Benzimidazol-2-yl)methyl)hydrazineyl)-N-(3,4-dichlorophenyl)-2-oxoacetohydrazonoyl cyanide (9d). Yield 65%, yellowish brown solid, mp 116–117°C (EtOH/H_2O). IR: ν_{max} = 3212–2920 (NH, =CH aromatic, –CH aliphatic), 2983 (NH–hydrazo), 2220 (CN), 1688 (CO amidic), 1588 (C=N), 1461(CH_2) and 1376 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 3.87 (s, 2H, CH_2 aliphatic), 6.58–8.87 (m, 7H, Ar–H; 2H hydrazide), 12.32 (s, 1H, NH–benzimidazole ring), 13.60 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 51.4, 106.2, 110.6, 115.1 (2C), 116.3, 118.1, 123.4, 123.0 (2C), 129.2, 131.7, 138.9 (2C), 141.4, 142.5, 162.9. MS (*m*/*z*, %): 403.31 (M + 1, 20.01). *Anal.* Calcd. for $C_{17}H_{13}Cl_2N_7O$ (402.24): C, 50.76; H, 3.26; N, 24.38. Found: C, 50.65; H, 3.12; N, 24.19.

2-(2-((1H-Benzimidazol-2-yl)methyl)hydrazineyl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-oxoacetohydrazonoyl cyanide (9e). Yield 65%, brown solid, mp 219–220°C (dioxane). IR: ν_{max} = 3212–2854 (NH, =CH aromatic, –CH aliphatic), 2913 (NH–hydrazo), 2175 (CN), 1732 (CO pyrazolone), 1642 (CO amidic), 1602 (C=N), 1461 (CH_2) and 1379 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 2.19 (s, 2H, CH_2 aliphatic), 3.15 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 7.16–8.85 (m, 9H, Ar–H; 2H hydrazide), 12.32 (s, 1H, NH–benzimidazole ring), 12.85 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 12.4, 34.8, 51.4, 106.2, 110.6, 115.1 (2C), 116.1, 122.7, 123.0 (2C), 123.8 (2C), 129.2 (2C), 131.6, 133.7, 138.8 (2C), 141.4, 160.5, 162.9. MS (*m*/*z*, %): 443.87 (M^+, 7.96). *Anal.* Calcd. for $C_{22}H_{21}N_9O_2$ (443.47): C, 59.58; H, 4.77; N, 28.43. Found: C, 59.41; H, 4.58; N, 28.22.

2-(2-((1H-Benzimidazol-2-yl)methyl)hydrazineyl)-2-amino-N-(4-nitrophenyl)acetohydrazonoyl cyanide (10a). Yield 63%, dark brown solid, mp 128–129°C (EtOH). IR: ν_{max} = 3740 (NH_2), 3263–2853 (NH, =CH aromatic, –CH aliphatic), 3103 (NH–hydrazo), 2220 (CN), 1680 (CO amidic), 1589 (C=N), 1447 (CH_2) and 1310, 1502 (NO_2) cm^{-1}. ^1H NMR: δ/ppm = 3.43 (s, 2H, CH_2 aliphatic), 6.59–8.31 (m, 8H, Ar–H; 2H hydrazide), 12.43 (s, 1H, NH–benzimidazole ring), 13.61 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 51.4, 66.4, 110.6, 113.2

(2C), 115.2 (2C), 123.0 (2C), 124.6 (2C), 134.2, 136.8, 138.7 (2C), 141.4, 149.1. MS (*m/z*, %): 378.28 (M + 1, 0.84). *Anal.* Calcd. for $C_{17}H_{15}N_9O_2$ (377.37): C, 54.11; H, 4.01; N, 33.41. Found: C, 53.90; H, 4.15; N, 33.26.

2-(2-((1H-Benzimidazol-2-yl)methyl)hydrazineyl)-2-amino-N-(p-tolyl)acetohydrazonoyl cyanide (10b). Yield 68%, dark brown solid, mp 104–105°C (benzene). IR: ν_{max} = 3739 (NH_2), 3231–3057 (NH, =CH aromatic, –CH aliphatic), 2914 (NH–hydrazo), 2215 (CN), 1605 (C=N), 1453 (CH_2) and 1390 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 2.49 (s, 3H, CH_3), 3.88 (s, 2H, CH_2 aliphatic), 7.20–7.38 (m, 8H, Ar–H; 2H amino), 12.40 (s, 1H, NH–benzimidazole ring), 12.95 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 21.3, 51.4, 66.4, 110.6, 115.2 (2C), 116.1 (2C), 123.0 (2C), 124.6 (2C), 131.2, 136.8, 138.7 (2C), 140.1, 141.5. MS (*m/z*, %): 346.49 (M^+, 1.26). Anal. calcd. for $C_{18}H_{18}N_8$ (346.40): C, 62.41; H, 5.24; N, 32.35. Found: C, 62.30; H, 5.05; N, 32.18.

2-(2-((1H-Benzimidazol-2-yl)methyl)hydrazineyl)-N-(4-acetylphenyl)-2-aminoacetohydrazonoyl cyanide (10c). Yield 52%, reddish brown solid, mp 108–109°C (EtOH). IR: ν_{max} = 3738 (NH_2), 3231–3056 (NH, =CH aromatic, –CH aliphatic), 2914 (NH–hydrazo), 2185 (CN), 1665 (CO acetyl), 1594 (C=N), 1426(CH_2) and 1390 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 2.45 (s, 3H, CH_3), 3.31 (s, 2H, CH_2 aliphatic), 7.39–7.59 (m, 8H, Ar–H; 2H amino), 12.32 (s, 1H, NH–benzimidazole ring). ^{13}C NMR: δ/ppm: 26.3, 51.1, 110.6, 116.1, 115.2 (2C), 116.2 (2C), 123.0 (2C), 129.6 (2C), 131.2, 138.8 (2C), 141.4, 143.5, 152.6, 197.0. MS (*m/z*, %): 375.16 (M + 1, 20.5). *Anal.* Calcd. for $C_{19}H_{18}N_8O$ (374.41): C, 60.95; H, 4.85; N, 29.93. Found: C, 60.71; H, 4.79; N, 29.81.

2-(2-((1H-Benzimidazol-2-yl)methyl)hydrazineylidene)-2-amino-N-(3,4-dichlorophenyl)acetohydrazonoyl cyanide (10d). Yield 65%, brownish yellow solid, mp 138–139°C (dioxane). IR: ν_{max} = 3740 (NH_2), 3227–3172 (NH, =CH aromatic, –CH aliphatic), 3048 (NH–hydrazo), 2224 (CN), 1594 (C=N) and 1455 (CH_2) cm^{-1}. ^1H NMR: δ/ppm = 3.90 (s, 2H, CH_2 aliphatic), 7.15–7.66 (m, 8H, Ar–H; 2H amino), 12.32 (s, 1H, NH–benzimidazole ring), 12.99 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 50.9, 110.5, 115.2 (2C), 115.6, 115.8, 118.2, 123.0 (2C), 123.4, 129.3, 131.2, 138.8 (2C), 141.5 (2C), 142.4, 152.6. MS (*m/z*, %): 401.08 (M^+, 18.4). *Anal.* Calcd. for $C_{17}H_{14}Cl_2N_8$ (401.26): C, 50.89; H, 3.52; N, 27.93. Found: C, 50.72; H, 3.38; N, 27.79.

2-(2-((1H-Benzimidazol-2-yl)methyl)hydrazineylidene)-2-amino-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)acetohydrazonoyl cyanide (10e). Yield 66%, brown solid, mp 204–205°C (dioxane). IR: ν_{max} = 3738 (NH_2), 3197–2919 (NH, =CH aromatic, –CH aliphatic), 3103 (NH–hydrazo), 2228 (CN), 1636 (CO pyrazolone), 1595 (C=N), 1450 (CH_2) and 1403 (CH_3) cm^{-1}. ^1H NMR: δ/ppm = 2.20 (s, 2H, CH_2 aliphatic), 3.14 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 7.15–7.82 (m, 8H, Ar–H; 2H amino), 12.41 (s, 1H, NH–benzimidazole ring), 13.52 (s, 1H, NH–hydrazo). ^{13}C NMR: δ/ppm = 12.5, 34.9, 51.1, 110.6, 115.2 (2C), 115.6, 116.2, 122.9, 123.0 (2C), 124.0 (2C), 129.3 (2C), 131.2, 134.1, 138.7 (2C), 141.4, 152.6, 161.2. MS (*m/z*, %): 442.20 (M^+, 100.0). *Anal.* Calcd. for $C_{22}H_{22}N_{10}O$ (442.49): C, 59.72; H, 5.01; N, 31.66. Found: C, 59.58; H, 4.88; N, 31.45.

Antibacterial evaluation. Disc diffusion method was used to evaluate the antibacterial activity of the synthesized compounds [58]. Both Kanamycin (30 μg/mL) and Ampicillin (10 μg/mL) were aseptically used as positive standard reference for Gram-positive strains and Gram-negative strains. The inhibition clear zones (mm) were recorded in all samples and equal zero in no-inhibition results. The method is explained in details in Data S1.

Antitumor evaluation. ***Cell culture.*** Human transformed cell lines from liver (HepG2) were maintained in RPMI-1640 supplemented with 100 μg/mL streptomycin, 100 μg/mL penicillin, and 10% (w/v) heat-inactivated fetal bovine serum in a humidified 5% (v/v) CO_2 atmosphere at 37°C.

Cytotoxicity assay. Neutral red assay was used to evaluate the cytotoxicity effects of prepared compounds [59]. Method is explained in details in Data S1. The percentage of cell death is expressed according to Equation 1:

$$\%\text{Cell death} = \frac{\text{Abs}_{540}\ \text{treated sample}}{\text{Abs}_{540}\ \text{treated sample}} \times 100. \quad (1)$$

The data are based on three independent experiments.

Antioxidant potential. The tested compounds were tested for their antioxidant potential according to the reported technique [60]. The technique is explained in details and provided in Data S1. The antioxidant abilities were expressed as μ*M* Trolox equivalents. Each sample was analyzed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to Equation 2.

$$\text{Inhibition}\% = \frac{[\text{Ab} - \text{Aa}]}{\text{Ab}} \times 100. \quad (2)$$

where Ab is the absorption of the blank sample (t = 0 min) and Aa is the absorption of the tested compounds or standard substance solution (t = 30 min). The EC_{50} value defined as the concentration of antioxidant in the reactive system necessary to decrease the initial DPPH concentration by 50% and was calculated from the data obtained.

Calculation of IC_{50}. The IC_{50} values (50% cell viability maximum inhibition concentration) of the tested and control compound (Doxorubicin) were calculated using XLFIT5 software (IDBS) and expressed in μg mL^{-1} at 95% confidence intervals.

Acknowledgments. This work was funded by the King Abdulaziz City for Science and Technology (KACST)–Saudi Arabia (project no. 1-17-01-022-0006). The authors acknowledge support from the Scientific Research Unit of Taif University, Saudi Arabia.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; and in the decision to publish the results.

REFERENCES AND NOTES

[1] Norrington, F.; Hyde, R.; Williams, S.; Wootton, R. J Med Chem 1975, 18, 604.
[2] Woolley, D. J Biol Chem 1944, 152, 225.
[3] Ansari, K. F.; Lal, C. J Chem Sci 2009, 121, 1017.
[4] Al-Ebaisat, H. S. J Appl Sci Environ 2011, 15, 451.
[5] Chimirri, A.; Grasso, S.; Monforte, P.; Rao, A.; Zappala, M.; Monforte, A.; Pannecouque, C.; Witvrouw, M.; Balzarini, J.; De Clercq, E. Antivir Chem Chemother 1999, 10, 211.
[6] Shinegare, M.; Mane, D.; Shinde, D.; Thore, S.; Bhawsar, S. Asian J Chem 1996, 8, 225.
[7] Kus, C.; Ayhan-Kilcigil, G.; Eke, B. C. Arch Pharm Res 2004, 27, 156.
[8] Kapuriya, K.; Ganure, A.; Davda, S.; Kitawala, M.; Topiya, H. Uni J Pharm 2013, 2, 57.
[9] Tupe, A.; Pawar, P.; Mane, B.; Magar, S. Res J Pharm Biol Chem Sci 2013, 4, 928.
[10] Mariappan, G.; Bhuyan, N.; Kumar, P.; Kumar, D.; Murali, K. Indian J Chem 2011, 50B, 1216.
[11] Kagthara, P.; Upadhyay, T.; Doshi, R.; Parekh, H. Indian J Heterocycl Chem 2000, 10, 9.
[12] Dubey, A.; Sanyal, P. Online Vet J 2010, 5, 63.
[13] Walia, R.; Hedaitullah, M.; Naaz, S. F.; Iqbal, K.; Lamba, H. Int J Res Pharm Chem 2011, 1, 565.
[14] Patil, A.; Ganguly, S.; Surana, S. J Chem Sci 2010, 122, 443.
[15] Kohara, Y.; Kubo, K.; Imamiya, E.; Wada, T.; Inada, Y.; Naka, T. J Med Chem 1996, 39, 5228.
[16] Reddy, B. A. Asian J Pharm Res Health Care 2010 2.
[17] Zhou, C. H.; Wang, Y. Curr Med Chem 2012, 19, 239.
[18] Gaffer, H. E.; Fouda, M. M.; Khalifa, M. E. Molecules 2016, 21, 122.
[19] Khalifa, M.; Abdel-Latif, E.; Gobouri, A. J Heterocyclic Chem 2015, 52, 674.
[20] Khalifa, M. E.; Abdel-Hafez, S. H.; Gobouri, A. A.; Kobeasy, M. I. Phosphorus Sulfur Silicon Relat Elem 2015, 190, 461.
[21] Khalifa, M. E.; Mohamed, M. A.-H.; Alshehri, N. H. Mac J Chem Chem Eng 2015, 34, 309.
[22] Khalifa, M.; Gobouri, A.; Kabli, F.; Altalhi, T.; Almalki, A.; Mohamed, M. Molecules 2018, 23, 3285.
[23] Alasmary, F. A.; Snelling, A. M.; Zain, M. E.; Alafeefy, A. M.; Awaad, A. S.; Karodia, N. Molecules 2015, 20, 15206.
[24] Mariappan, G.; Hazarika, R.; Alam, F.; Karki, R.; Patangia, U.; Nath, S. Arab J Chem 2015, 8, 715.
[25] Srinivas, B.; Priya, V. R.; Babu, G. S.; Rao, J. V.; Malathy, P.; Manohar, K. R.; Prakash, B. C.; Srikanth, L. Der Pharma Chem 2010, 2, 378.
[26] Karcı, F.; Demirçalı, A. Dyes Pigments 2007, 74, 288.
[27] Patel, B. P.; Patel, H. S.; Shah, P. J E-J Chem 2011, 2, 303.
[28] Zsolnai, T. Biochem Pharmacol 1965, 14, 1325.
[29] Zsolnai, T. Biochem Pharmacol 1964, 13, 285.
[30] Zsolnai, T.; Lugosi, G.; Szepesi, I.; Bakonyi, M.; Racz, I.; Nee Hegedus, E. R. Google Patents 1977.
[31] Knorr, L. Ber Dtsch Chem Ges 1883, 16, 2597.
[32] Hiremath, S. P.; Saundane, A. R.; Mruthyunjayaswamy, B. J Heterocyclic Chem 1993, 30, 603.
[33] Ragavan, R. V.; Kumar, K. M.; Vijayakumar, V.; Sarveswari, S.; Ramaiah, S.; Anbarasu, A.; Karthikeyan, S.; Giridharan, P.; Kumari, N. S. Org Med Chem Lett 2013, 3, 6.
[34] Kizer, D. E.; Miller, R. B.; Kurth, M. J. Tetrahedron Lett 1999, 40, 3535.
[35] Cremer, A. Antibiotic sensitivity and assay tests, 1980.
[36] Maris, P. Rev Sci Tech Oie 1995, 14, 47.
[37] Joswick, H.; Corner, T. R.; Silvernale, J.; Gerhardt, P. J Bacteriol 1971, 108, 492.
[38] Nagai, T.; Inoue, R.; Suzuki, N.; Myoda, T.; Nagashima, T. Int J Mol Med 2005, 15, 259.
[39] Sztaricskai, F.; Takács, I. E.; Pusztai, F.; Szabó, G.; Csípõ, I. Arch Pharm 1999, 332, 321.
[40] Shanab, S. M. M.; Shalaby, E. A.; El Fayoumy, E. A. J Biomed Biotechnol 2011, 2011, 1.
[41] Murphy, S. T.; Case, H. L.; Ellsworth, E.; Hagen, S.; Huband, M.; Joannides, T.; Limberakis, C.; Marotti, K. R.; Ottolini, A. M.; Rauckhorst, M. Bioorg Med Chem Lett 2007, 17, 2150.
[42] Oballa, R. M.; Truchon, J.-F.; Bayly, C. I.; Chauret, N.; Day, S.; Crane, S.; Berthelette, C. Bioorg Med Chem Lett 2007, 17, 998.
[43] Tanii, H.; Hashimoto, K. Arch Toxicol 1986, 58, 171.
[44] Patil, A. V.; Bandgar, B. P.; Lee, S.-H. Bull Korean Chem Soc 2010, 31, 1719.
[45] Bhatt, A.; Sanghvi, V.; Parmar, M.; Shah, P.; Karadia, H.; Patel, H. Orient J Chem 2003, 19, 473.
[46] El-Nassan, H. B. Eur J Med Chem 2012, 53, 22.
[47] Zimam, E. H. Am J Drug Deliv 2014, 2, 397.
[48] Rajput, A.; Rajput, S. Int J Pharm Tech Res 2009, 1, 900.
[49] Tirlapur, V. K.; Noubade, K. Indian J Heterocycl Chem 2009, 19, 121.
[50] Chandra, M.; Dwivedi, K.; Kumar, K.; Mehortra, J. J Indian Chem Soc 1974, 51, 524.
[51] Robbins, P. J. J Heterocyclic Chem 1977, 14, 1107.
[52] Singh, C. P.; Ojha, A. C. J Chin Chem Soc 1981, 28, 51.
[53] Panizzi, L. Gazz Chim Ital 1947, 77, 206.
[54] Eid, A. I.; Mikhail, A. N.; Zakaria, I. M. Egypt J Pharm Sci 1987, 28, 413.
[55] Aly, H.; El-Gazzar, M. Drug Res 2012, 62, 105.
[56] Walter, R. In 15 pp; Gmbh, B. M., Ed.; 1965:472052: Germany, 1965.
[57] Abdel-Khalek, A. A. Indian J Chem Sect B 1994, 33, 186.
[58] Baccer, R.; Kirby, M.; Sherris, J.; Turek, M. Am J Clin Pathol 1966, 45, 493.
[59] Fautz, R.; Husein, B.; Hechenberger, C. Mutat Res-Envir Muta 1991, 253, 173.
[60] Brand-Williams, W.; Cuvelier, M. E.; Berset, C. LWT-Food Sci Technol 1995, 28, 25.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.