Research Article

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# Synthesis and evaluation of new benzimidazole derivatives with hydrazone moiety as anticancer agents

# Hidrazon yapısı içeren yeni benzimidazol türevlerinin sentezi ve antikanser ajan olarak değerlendirilmesi

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## Abstract

**Objectives:** Cancer is one of the leading causes of death throughout the world. Current therapy options suffer from the major limitations of side effects and drug resistance. Thus, continuing search for newer and safer anticancer drugs remains critically important. From this point of view, in the present study benzimidazole-hydrazone derivatives were synthesized by aiming at the identification of new chemical entities as potent anticancer agents.
**Material and methods:** A series of 12 new compounds of 4-(5(6)-substituted-1*H*-benzimidazol-2-yl)-*N*′thiophen/furan-2-yl-methylene) benzohydrazide derivatives were synthesized. The structures of the obtained compounds were elucidated using by IR, $^{1}$H NMR, $^{13}$C NMR, mass spectroscopy and elemental analyses. In vitro cytotoxic activity of the compounds against A549, MCF-7 and NIH/3T3 cell lines was evaluated by MTT assay.
**Results:** Among the tested compounds, compound **3e** showed higher cytotoxicity against MCF-7 human breast cancer cells when compared with cisplatin. Also, it has lower cytotoxicty against healthy cell line, NIH/3T3.
**Conclusions:** It was determined that compound **3e** showed inhibition towards MCF-7. Considering the substituent effect on cytotoxic activity, compound **3e** bearing 2-methylthiophene has attracted attention with its higher anticancer activities.

**Keywords:** Benzimidazole; Hydrazone; Cytotoxic activity.

## Özet

**Amaç:** Kanser, dünyadaki ölümlerin önde gelen nedenlerinden biridir. Günümüzdeki tedavi seçenekleri yan etki ve ilaç direnci gibi önemli kısıtlılıklara sahiptir. Bu nedenle daha yeni ve güvenli antikanser ilaçlar için devam eden araştırmalarının kritik önemi vardır. Bu noktadan hareketle, bu çalışmada yeni kimyasal yapıların güçlü antikanser yapılar olarak tanımlanmasını amaçlayarak benzimidazol-hidrazon türevlerini sentezledik.
**Gereç ve Yöntem:** 12 yeni bileşikten oluşan 4-(5(6)-Sübstitüe-1*H*-benzimidazol-2-il)-*N*′(tiyofen/furan-2-il-metilen) benzohidrazid serisi sentezlenmiştir. Elde edilen bileşiklerin yapıları FT-IR, $^{1}$H-NMR, $^{13}$C-NMR, kütle spektroskopisi verileri ve elementel analiz kullanılarak aydınlatılmıştır. Bileşiklerin in vitro sitotoksik aktivitesi MTT metodu kullanılarak değerlendirilmiştir.
**Bulgular:** Test edilen bileşikler arasında bileşik **3e**, sisplatin ile karşılaştırıldığında MCF-7 insan meme kanser hücrelerine karşı sitotoksik aktivite göstermiştir.

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**Sonuç:** Bileşik **3e**'nin MCF-7'ye karşı selektif sitotoksiste gösterdiği tespit edilmiştir. Kimyasal yapıdaki değişken grupların sitotoksik aktivite üzerine etkileri değerlendirildiğinde, 2-metiltiyofen yapısı içeren bileşik **3e**, gösterdiği yüksek aktivite ile dikkat çekmektedir.

**Anahtar kelimeler:** Benzimidazol; Hidrazon; Sitotoksik aktivite.

## Introduction

Cancer is the most leading cause of death characterized by uncontrolled growth of cells resulting invasion of surrounding tissue and often spreading to other parts of the body. In spite of considerable development in the understanding of molecular mechanisms of the pathogenesis of cancer, no particular treatment is available yet to cure the disease completely. Chemotherapy is the most effective tool against the neoplastic diseases and the majority of the clinically used anticancer molecules are of synthetic origin. These drugs act by various molecular mechanisms which may involve inhibition of initiation, promotion, progression and metastasis of cancerous cells. However, they can also damage normal cells leading to toxicity during this process. Due to side effects of the these drugs and the development of drug resistance in cancer cells, there is an urgent need to design and synthesize potent and highly selective molecules to improve the current anticancer treatment options with least or no toxicity to normal cells [1–6].

Benzoheterocycles always exhibit a wide range of biological activities and they are also extremely versatile scaffold units serving as important synthons for construction of various heterocyclic derivatives in the field of drug design. Among these heterocyclic molecules, benzimidazole skeleton is regarded as a promising class of building blocks for pharmaceuticals [7, 8]. Due to the structural similarity of the benzimidazole nuclei with some of the naturally occurring moieties such as purines and vitamin B12, benzimidazole derivatives easily interact with biomolecules of living organisms. Furthermore, benzimidazole consists of two aromatic N-heterocycles that can bind to enzymes or receptors via hydrogen bonds coordinated with metal ions or hydrophobic interactions. Several promising antitumor agents were found to contain the benzimidazole ring system [9–17].

Hydrazones, which are containing a highly reactive group (CO-NH-N=CH), are important synthons for several transformations and have gained importance due to their broad spectrum of biological activities. Among them, aryl hydrazones have attracted attention due to their therapeutic activity and applications in materials research. Their linkage provides a suitable system for pH-dependent release of drugs from drug-conjugates. Several hydrazone derivatives have been shown to exhibit antiproliferative activities and act as cytotoxic agents with the ability to prevent cell progression in cancerous cells through different mechanisms [18–25].

With the aim of obtaining novel anticancer agents, herein we report the synthesis of series of benzimidazole-hydrazones followed by anticancer evaluation of newly synthesized products.

## Materials and methods

### Chemicals

All of the chemicals used in the study were purchased from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA) and Merck (Merck KGaA, Darmstadt, Germany), and used without further chemical or biological purification. Microwave syntheses were realized by using a Monowave 300 high-performance microwave reactor (Anton-Paar, Graz, Austria). Melting points (°C, uncorrected) of the synthesized compounds were determined by using Electrothermal melting point apparatus (Electrothermal, 9100 Digital, Burlington, NJ, USA). $^{1}$H NMR and $^{13}$C NMR spectras were recorded with a Bruker 500 MHz and 125 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA), in DMSO-$d_6$ solvent including TMS as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Coupling constants ($J$) are reported as Hertz. The elemental compositions were recorded on a Leco CHNS-932 analyser (Leco Corporation, Saint Joseph, MI, USA). The IR spectra were obtained on a Shimadzu, IR Prestige-21 (Shimadzu, Tokyo, Japan); Mass spectra electrospray ionization were determinated on a Schimadzu, 8040 LC-MS-MS spectrophotometer (Shimadzu, Tokyo, Japan). The completion of reactions and purities of compounds were checked by TLC on silica gel 60 F254 (Merck KGaA, Darmstadt, Germany).

### Synthesis of the compounds

#### Synthesis of methyl-4-(5(6)-substituted-1*H*-benzimidazol-2-yl)benzoate derivatives (1a, 1b)

Methyl-4-formylbenzoate (4.8 g, 0.03 mol) and sodium disulfite (5.7 g, 0.03 mol) were dissolved in DMF (5 mL). The mixture was added into a vial (30 mL) of microwave

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synthesis reactor (Anton-Paar Monowave 300) and heated under conditions of 240°C and 10 bar for 5 min. After this time, 5-substitue-1,2-phenylenediamine was added into the reaction mixture and was heated under the same reaction conditions. After cooling, the mixture was poured into iced-water, precipitated product was washed with water, dried and recrystallized from ethanol. Yield: 82%. m.p. 227°C.

### Synthesis of 4-(5(6)-substituted-1*H*-benzimidazol-2-yl) benzoic acid hydrazide derivatives (2a, 2b)

In a vial (30 mL) of microwave synthesis reactor (Anton-Paar Monowave 300), a mixture of methyl-4-(5(6)substituted-1*H*-benzimidazol-2-yl)benzoate (**1**) (0.02 mol) and excess of hydrazine hydrate (5 mL) was reacted in ethanol. The reaction mixture was kept under the conditions of 240°C and 10 bar for 10 min. After cooling, the mixture was poured into iced-water, precipitated product was washed with water, dried and recrystallized from ethanol. Yield: 81%. m.p. 283°C.

## General synthesis of compounds (3a–3l)

Corresponding 4-(5(6)-substituted-1*H*-benzimidazol-2-yl) benzoic acid hydrazide derivative (**2a** or **2b**) (0.001 mol) and appropriate aldehyde derivative (0.001 mol) and catalytic amount of glacial acetic acid were refluxed in ethanol for 2 h. The precipitated residue was filtered, dried and recrystallized from butanol.

### 4-(5(6)-Chloro-1*H*-benzimidazol-2-yl)-*N*′-(furan-2-yl-methylene)benzohydrazide (3a)

Yield: 83%. M.p. 243.1°C. Rf: 0.57 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3182 (N-H), 1662 (C=O) 1624–1421 (C=C and C=N), 854 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 6.66 (1H, s, Furan-CH), 6.96 (1H, d, $J$ = 2.25 Hz, Furan-CH), 7.26 (1H, dd, $J$ = 8.35 and $J$ = 2.25 Hz, $C_6H_5$-H), 7.65 (2H, s, $C_6H_5$-H), 7.88 (1H, s, furan-CH), 8.08 (2H, d, $J$ = 8.00 Hz, $C_6H_4$-H), 8.31 (2H, d, $J$ = 8.00 $C_6H_4$-H), 8.37 (1H, s, N-CH), 11.93 (1H, s, NH-CO), 13.29 (1H, s, Benzimidazole-NH). $^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 112.73 (CH), 114.18 (CH), 118.75 (CH), 119.21 (CH), 124.13 (CH), 127.01 (CH), 128.81 (C), 130.68 (CH), 133.01 (C), 134.87 (C), 136.72 (C), 138.27 (CH), 138.74 (C), 141.11 (CH), 145.79 (C), 149.88 (C), 162.81 (C). ES-MS (m/z): 365.05 [% 100, M + 1], 367.05 [% 31.50, M + 3], 368.10 [% 6.55, M + 4]. Anal. calcd. For $C_{19}H_{13}ClN_4O_2$, C, 62.56; H, 3.59; N, 15.36. Found: C, 62.48; H, 3.58; N, 15.39.

### 4-(5(6)-Chloro-1*H*-benzimidazol-2-yl)-*N*′-(5-methyl-furan-2-yl-methylene)benzohydrazide (3b)

Yield: 73%. M.p. 271.5°C. Rf: 0.55 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3186 (N-H), 1662 (C=O) 1620–1421 (C=C and C=N), 854 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 2.37 (3H, s, -$CH_3$), 6.29 (1H, d, $J$ = 2.4 Hz, Furan-CH), 6.86 (2H, d, $J$ = 3.10 Hz furan-CH), 7.27 (1H, dd, $J$ = 8.45 Hz and $J$ = 1.25 Hz, $C_6H_5$-H), 7.70–7.65 (2H, m, $C_6H_5$-H), 8.09 (2H, d, $J$ = 8.30 Hz, $C_6H_4$-H), 8.28 (H, s, N-CH), 8.32 (2H, d, $J$ = 8.25 Hz $C_6H_4$-H), 11.88 (H, s, NH-CO), 13.33 (1H, s, Benzimidazole-NH).$^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 13.99 ($CH_3$), 109.12 (CH), 116.13 (CH), 123.28 (CH), 126.99 (CH), 132.92 (CH), 134.92 (CH), 138.09 (C), 140.68 (CH), 142.18 (C), 142.73 (C), 143.57 (C), 144.54 (CH), 145.81 (C), 148.32 (C), 152.11 (C), 155.19 (C), 162.70 (C). ES-MS (m/z): 379.10 [% 100, M + 1], 380.10 [% 15.88, M + 2], 381.05 [% 27.24, M + 3]. Anal. calcd. For $C_{20}H_{15}ClN_4O_2$, C, 63.41; H, 3.99; N, 14.79. Found: C, 63.28; H, 3.98; N, 14.76.

### 4-(5(6)-Chloro-1*H*-benzimidazol-2-yl)-*N*′-(5-nitro-furan-2-yl-methylene)benzohydrazide (3c)

Yield: 78%. M.p. 301.4°C. Rf: 0.62 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3221 (N-H), 1662 (C=O) 1641–1479 (C=C and C=N), 854 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 7.28 (1H, d, $J$ = 8.55 Hz, $C_6H_4$-H), 7.32 (1H, d, $J$ = 2.60 Hz, Furan-CH), 7.70–7.66 (2H, m, $C_6H_5$-H), 7.82 (1H, d, $J$ = 3.50 Hz, Furan CH), 8.12 (2H, d, $J$ = 7.80 Hz, $C_6H_5$-H), 8.33 (2H, d, $J$ = 8.10 Hz, $C_6H_4$-H), 8.43 (H, s, N-CH), 12.40 (H, s, NH-CO), 13.32 (1H, s, Benzimidazole-NH).$^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 114.63 (CH), 115.49 (CH), 118.29 (CH), 120.77 (CH), 122.80 (CH), 126.62 (CH), 127.62 (C), 128.52 (CH), 130.54 (C), 132.92 (C), 133.04 (C), 135.74 (CH), 139.78 (C), 145.72 (C), 151.66 (C), 151.95 (C), 163.54 (C). ES-MS (m/z): 410.05 [% 100, M + 1], 411.05 [% 19.84, M + 2], 412.00 [% 32.67, M + 3], 413.05 [% 6.74, M + 4]. Anal. calcd. For $C_{19}H_{12}ClN_5O_4$, C, 55.69; H, 2.95; N, 17.09. Found: C, 55.76; H, 2.94; N, 17.12.

### 4-(5(6)-Chloro-1*H*-benzimidazol-2-yl)-*N*′-(thiophene-2-yl-methylene)benzohydrazide (3d)

Yield: 81%. M.p. 273.8°C. Rf: 0.59 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3186 (N-H), 1658 (C=O) 1620–1419 (C=C and C=N), 854 (1,4-disubstituted

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benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 7.17 (1H, t, $J$ = 4.00 Hz thiophene-CH), 7.27 (1H, d, $J$ = 8.40, $C_6H_4$-H), 7.51 (1H, d, $J$ = 3.00 thiophene-CH), 7.70–7.66 (2H, m, $C_6H_5$-H), 7.70 (1H, d, $J$ = 4.80 Hz, thiophene-CH), 8.11 (2H, d, $J$ = 8.10 Hz, $C_6H_5$-H), 8.32 (2H, d, $J$ = 8.15 Hz, $C_6H_4$-H), 8.70 (H, s, N-CH), 11.96 (H, s, NH-CO), 13.31 (1H, s, benzimidazole-NH). $^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 123.25 (CH), 123.89 (CH), 127.01 (CH), 128.38 (CH), 128.80 (CH), 129.60 (C), 131.61 (CH), 132.98 (CH), 134.90 (CH), 135.88 (CH), 138.91 (C), 139.53 (C), 142.06 (C), 142.40 (C), 143.64 (C), 152.10 (C), 162.76 (C). Es-Ms (m/z): 381 [% 100, M + 1], 382.05 [% 23.73, M + 2], 383.05 [% 40.77, M + 3], 384.05 [% 8.39, M + 4]. Anal. calcd. For $C_{19}H_{13}ClN_4OS$, C, 59.92; H, 3.44; N, 14.71; S, 8.42. Found: C, 59.25; H, 3.43; N, 14.66; S, 8.41.

### 4-(5(6)-Chloro-1*H*-benzimidazol-2-yl)-*N*′-(5-methyl-thiophene-2-yl-methylene)benzohydrazide (3e)

Yield: 86%. M.p. 270.8°C. Rf: 0.57 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3305 (N-H), 1639 (C=O) 1589–1438 (C=C and C=N), 850 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 2.35 (3H, s, -$CH_3$), 6.99 (1H, d, $J$ = 5.00 Hz, thiophene -CH), 7.26 (1H, dd, $J$ = 8.40 Hz and $J$ = 1.55 Hz, thiophene -CH), 7.59 (1H, d, $J$ = 7.95, $C_6H_5$-H), 7.70–7.65 (2H, m, $C_6H_5$-H), 8.09 (2H, d, $J$ = 8.25 Hz, $C_6H_4$-H), 8.31 (2H, d, $J$ = 8.25 Hz $C_6H_4$-H), 8.77 (H, s, N-CH), 11.83 (1H, s, NH-CO), 13.27 (1H, s, benzimidazole-NH).$^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 13.49 ($CH_3$), 124.21 (CH), 126.45 (CH), 128.04 (CH), 128.13 (CH), 128.20 (CH), 130.81 (C), 131.07 (CH), 132.34 (CH), 132.39 (CH), 133.00 (C), 134.37 (C), 137.69 (C), 138.58 (C), 140.09 (C), 142.22 (C). 151.57 (C), 161.89 (C). ES-MS (m/z): 395.05 [% 100, M + 1], 396.10 [% 22.50, M + 2], 397.05 [% 38.69, M + 3], 398.10 [% 8.03, M + 4]. Anal. calcd. For $C_{20}H_{15}ClN_4OS$, C, 60.83; H, 3.83; N, 14.19; S, 8.12. Found: C, 63.61; H, 3.82; N, 14.16; S, 8.13.

### 4-(5(6)-Chloro-1*H*-benzimidazol-2-yl)-*N*′-(5-nitro-thiophene-2-yl-methylene)benzohydrazide (3f)

Yield: 80%. M.p. 314.4°C. Rf: 0.65 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3215 (N-H), 1660 (C=O) 1629–1436 (C=C and C=N), 854 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 7.59 (1H, dd, $J$ = 8.50 Hz and $J$ = 2.0 Hz, $C_6H_5$-H), 7.59 (1H, d, $J$ = 3.80 Hz, thiophene -CH), 7.66–7.64 (2H, m, $C_6H_5$-H), 7.69 (H, s, N-CH), 8.10 (2H, d, $J$ = 6.75 Hz, $C_6H_4$-H), 8.13 (1H, d, $J$ = 4.20 Hz, thiophene -CH), 8.31 (2H, d, $J$ = 8.30 Hz, $C_6H_4$-H). 8.72 (H, s, N-CH), 11.89 (1H, s, NH-CO), 13.30 (1H, s, benzimidazole-NH).$^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 123.31 (CH), 123.36 (CH), 125.76 (CH), 127.06 (CH), 127.88 (CH), 128.12 (CH), 128.99(C), 130.23 (CH), 130.98 (CH), 132.47 (C), 133.35 (C), 134.38 (C), 141.90 (C), 147.11 (C), 152.05 (C), 162.53 (C). ES-MS (m/z): 426 [% 100, M + 1], 427 [% 18.47, M + 2], 428 [% 36.09, M + 3], 428.95 [% 6.34, M + 4]. Anal. calcd. For $C_{19}H_{12}ClN_5O_3S$, C, 53.59; H, 2.84; N, 16.45; S, 7.53. Found: C, 53.40; H, 2.83; N, 16.47; S, 7.55.

### 4-(5(6)-Fluoro-1*H*-benzimidazol-2-yl)-*N*′-(furan-2-yl-methylene)benzohydrazide (3g)

Yield: 61%. M.p. 249.8°C. Rf: 0.60 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3197 (N-H), 1666 (C=O) 1625–1427 (C=C and C=N), 854 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 6.67 (1H, s, Furan-CH), 6.98 (1H, s, Furan-CH), 7.11 (1H, m, $C_6H_3$-H), 7.35–7.73 (2H, m, $C_6H_3$-H), 7.88 (1H, s, $C_6H_3$-H), 8.09 (2H, d, $J$ = 7.70 Hz, $C_6H_4$-H), 8.31 (2H, s, $C_6H_4$-H), 8.37 (H, s, N-CH), 11.92 (H, s, NH-CO), 13.23 (1H, s, Benzimidazole-NH). $^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 112.73 (CH), 114.17 (CH), 120.52 (CH), 120.61 (CH), 126.78 (CH), 126.91 (CH), 128.79 (CH), 130.62 (CH), 132.25 (C), 133.14 (C), 134,62 (C), 138.23 (C), 140.97 (C), 145.77 (CH), 149.89 (C), 156.17 (C), 162.84 (C). ES-MS (m/z): 349.10 [% 100, M + 1], 350.10 [% 30.38, M + 2]. Anal. calcd. For $C_{19}H_{13}FN_4O_2$, C, 65.51; H, 3.76; N, 16.08. Found: C, 65.28; H, 3.75; N, 16.12.

### 4-(5(6)-Fluoro-1*H*-benzimidazol-2-yl)-*N*′-(5-methyl-furan-2-yl-methylene)benzohydrazide (3h)

Yield: 64%. M.p. 253.5°C. Rf: 0.58 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3190 (N-H), 1664 (C=O) 1624–1427 (C=C and C=N), 854 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 2.37 (3H, s, -$CH_3$), 6.29 (1H, s, Furan-CH), 6.85 (1H, s, Furan-CH), 7.11 (1H, s, $C_6H_3$-H), 7.39–7.72 (2H, m, $C_6H_5$-H), 8.08 (2H, d, $J$ = 8.15 Hz, $C_6H_4$-H), 8.27 (1H, s, N-CH), 8.29 (3H, d, $J$ = 8.15 Hz, $C_6H_4$-H), 11.88 (H, s, NH-CO), 13.24 (1H, S, benzimidazole-NH). $^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 13.98 ($CH_3$), 106.54 (CH), 109.11 (CH), 113.62 (CH), 114.80 (CH), 116.07 (CH), 126.83 (CH), 128.89 (CH), 133.11 (CH), 138.13 (C), 141.02 (C), 144.61 (C), 144.61 (C), 148.34 (C), 155.18 (C), 156.75 (C), 162.73 (C). ES-MS (m/z): 363.10 [% 100, M + 1], 364.10 [% 22.25, M + 2]. Anal. calcd. For $C_{20}H_{15}FN_4O_2$, C, 66.29; H, 4.17; N, 15.46. Found: C, 66.42; H, 4.16; N, 15.51.

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**4-(5(6)-Fluoro-1*H*-benzimidazol-2-yl)-*N*′-(5-nitro-furan-2-yl-methylene)benzohydrazide (3ı)**

Yield: 67%. M.p. 300.3°C. Rf: 0.66 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3217 (N-H), 1664 (C=O) 1639–1446 (C=C and C=N), 854 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 77.11 (1H, s, Furan-CH), 7.31 (1H, s, Furan-CH), 7.47 (1H, d, $J$ = 3.50 Hz $C_6H_3$-H), 7.81–7.83 (2H, m, $C_6H_5$-H), 8.10 (2H, d, $J$ = 7.30 Hz, $C_6H_4$-H), 8.32 (2H, d, $J$ = 7.60 Hz, $C_6H_4$-H), 8.48 (1H, s, Hz $C_6H_3$-H) 8.73 (1H, s, N-CH), 12.36 (1H, s, NH-CO), 13.21 (1H, s, benzimidazole-NH). $^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 114.63 (CH), 115.49 (CH), 118.29 (CH), 120.77 (CH), 122.80 (CH), 126.62 (CH), 127.62 (CH), 128.52 (C), 130.54 (C), 132.92 (C), 133.04 (C), 135.74 (CH), 139.78 (C), 145.72 (C), 151.66 (C), 151.95 (C), 163.54 (C). ES-MS (m/z): 394.05 [% 100, M + 1], 395.10 [% 39.90, M + 2], 396.10 [% 5.62, M + 3]. Anal. calcd. For $C_{19}H_{12}FN_5O_4$, C, 58.02; H, 3.07; N, 17.81. Found: C, 57.83; H, 3.08; N, 17.76.

**4-(5(6)-Fluoro-1*H*-benzimidazol-2-yl)-*N*′-(thiophene-2-yl-methylene)benzohydrazide (3j)**

Yield: 79%. M.p. 302.8°C. Rf: 0.61 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3196 (N-H), 1660 (C=O) 1624–1425 (C=C and C=N), 854 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 7.08–7.16 (2H, m, thiophene -CH), 7.31 (1H, s, thiophene -CH), 7.44–7.69 (4H, m,$C_6H_3$-H), 7.81–7.83 (2H, m, $C_6H_5$-H), 8.08 (2H, d, $J$ = 7.95 Hz, $C_6H_4$-H), 8.30 (2H, d, $J$ = 7.95 Hz, $C_6H_4$-H), 8.70 (1H, s, N-CH), 11.93 (1H, s, NH-CO), 13.22 (1H, s, benzimidazole-NH). $^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 111.12 (CH), 111.23 (CH), 123.43 (CH), 126.85 (CH), 127.53 (CH), 128.36 (CH), 128.78 (CH), 129.56 (CH), 131.55 (CH), 133.02 (C), 133.18 (C), 134.75 (C), 139.54 (C), 143.65 (C), 151.98 (C), 156.47 (C), 162.82 (C). ES-MS (m/z): 365.05 [% 100, M + 1], 366.10 [% 29.60, M + 2], 367.10 [% 9.05, M + 3]. Anal. calcd. For $C_{19}H_{13}FN_4OS$, C, 62.63; H, 3.60; N, 15.38; S, 8.80. Found: C, 62.53; H, 3.59; N, 15.32; S, 8.78.

**4-(5(6)-Fluoro-1*H*-benzimidazol-2-yl)-*N*′-(5-methyl-thiophene-2-yl-methylene)benzohydrazide (3k)**

Yield: 80%. M.p. 284.4°C. Rf: 0.62 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3180 (N-H), 1645 (C=O) 1587–1419 (C=C and C=N), 854 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 2.34 (3H, s, -$CH_3$), 6.98 (1H, d, $J$ = 5.00 Hz, thiophene-CH), 7.10 (1H, t, $J$ = 8.85 $C_6H_3$-H), 7.27–7.43 (2H, m, $C_6H_5$-H), 7.59 (1H, d $J$ = 5.05, tiyofen-CH), 8.08 (2H, d, $J$ = 8.35 Hz, $C_6H_4$-H), 8.30 (2H, d, $J$ = 8.35 Hz, $C_6H_4$-H), 8.76 (H, s, N-CH), 11.82 (1H, s, NH-CO), 13.22 (1H, s, benzimidazole-NH). $^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 14.06 ($CH_3$), 112.29 (CH), 117.34 (CH), 118.21 (CH), 123.41 (CH), 126.86 (CH), 127.48 (CH), 128.61 (CH), 128.68 (CH), 131.39 (C), 132.92 (C), 133.15 (C), 134.77 (C), 140.64 (C), 142.74 (C), 150.20 (C), 156.85 (C), 162.47 (C). ES-MS (m/z): 379.10 [% 100, M + 1], 380.05 [% 32.27, M + 2], 381.05 [% 10.03, M + 3]. Anal. calcd. For $C_{20}H_{15}FN_4OS$, C, 63.48; H, 4.00; N, 14.81; S, 8.47. Found: C, 62.65; H, 3.98; N, 14.76; S, 8.42.

**4-(5(6)-Fluoro-1*H*-benzimidazol-2-yl)-*N*′-(5-nitro-thiophene-2-yl-methylene)benzohydrazide (3l)**

Yield: 75%. M.p. 313.3°C. Rf: 0.67 (1:1, petroleum ether: ethyl acetate). IR $\nu_{max}$ ($cm^{-1}$): 3213 (N-H), 1660 (C=O) 1629–1440 (C=C and C=N), 842 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 7.11 (1H, s, thiophene -CH), 7.31 (1H, s, thiophene -CH), 7.47 (1H, d, $J$ = 3.50 Hz $C_6H_3$-H), 7.81–7.83 (2H, m, $C_6H_5$-H), 8.10 (2H, d, $J$ = 7.30 Hz, $C_6H_4$-H), 8.32 (2H, d, $J$ = 7.60 Hz, $C_6H_4$-H), 8.48 (1H, s, Hz $C_6H_3$-H) 8.73 (1H, s, N-CH), 12.36 (1H, s, NH-CO), 13.21 (1H, s, benzimidazole-NH). $^{13}$C-NMR (125 MHz) (DMSO-$d_6$) δ (ppm): 123.31 (CH), 123.36 (CH), 125.76 (CH), 127.06 (CH), 127.88 (CH), 128.12 (CH), 128.99 (CH), 130.23 (CH), 130.98 (C), 132.47 (C), 133.35 (C), 134.38 (C), 141.90 (C), 147.11 (C), 152.05 (C), 157.12 (C), 162.53 (C). ES-MS (m/z): 410 [% 100, M + 1], 411.05 [% 20.83, M + 2], 412.05 [% 7.07, M + 3]. Anal. calcd. For $C_{19}H_{12}FN_5O_3S$, C, 55.74; H, 2.95; N, 17.11; S, 7.83. Found: C, 55.82; H, 2.96; N, 17.13; S, 7.86.

## Cytotoxicity test

The tetrazolium salt MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide can be used to measure the metabolic activity of viable cells. Tetrazolium salts are reduced to formazan by mitochondrial succinate dehydrogenase, an enzyme which is only active in cells with an intact metabolism and respiratory chain. The formazan is quantified photometrically and correlates with the number of viable cells [26].

A549 (ATCC® CCL-185™, USA) human lung adenocarcinoma epithelial and MCF-7 (ATCC® HTB-22™, USA) human breast adenocarcinoma cell lines were used to test anticancer activity of the compounds. NIH/3T3 (ATCC® CRL-1658™, USA) mouse embryonic fibroblast cell line was also used to test the selective anticancer

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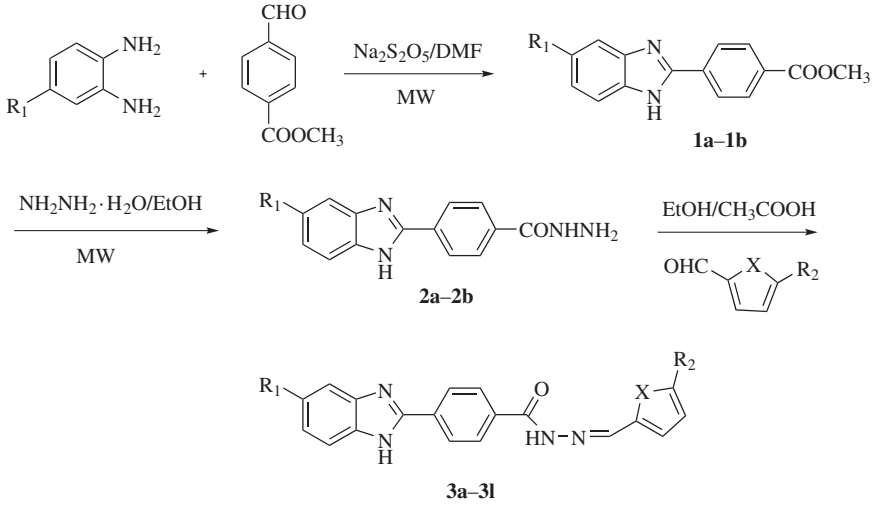
potential of the compounds. Cell lines were incubated in RPMI medium (Hyclone, Thermo Scientific, USA) supplemented with fetal calf serum, 100 IU/mL penicillin and 100 mg/mL streptomycin and 7.5% $NaHCO_3$ at 37°C in a humidified atmosphere of 95% air and 5% $CO_2$. A549 and MCF-7 cells were seeded at $1\times10^4$ cells into each well of 96-well plates. After 24 h of incubating period, the culture mediums were removed and compounds was added to culture medium at 0.000316–1 mM concentrations. After 24 h of incubation, cytotoxicity test was performed using the MTT assay, which measures mitochondrial activity in A549 and MCF-7 cells. MTT solution (5 mg/mL MTT powder in PBS) was prepared and then diluted to a final concentration of 0.5 mg/mL with the culture medium. Hundred microliter of this mixture was added to each cell. After 3 h incubation period at 37°C, 5% $CO_2$, the contents of the wells were removed and DMSO (100 µL) was added to each well. Then, OD of the plate was read at 540 nm. Inhibition % was calculated for each concentration according to the formula below and $IC_{50}$ values were determined by plotting a dose response curve of inhibition % versus compound concentrations tested [27–30]. Cisplatin was used as a positive control.

$$\text{Inhibition \%} = 100 - [(OD_{\text{test compound}} - OD_{\text{blank}} / OD_{\text{solvent control}} - OD_{\text{blank}}) \times 100]$$

## Results and discussion

In the present study, target molecules (**3a**–**3l**) were synthesized in three steps as shown in Figure 1. The starting materials methyl-4-(5(6)-substituted-1*H*-benzimidazol-2-yl)benzoate (**1a, 1b**) and 4-(5(6)-substituted-1*H*-benzimidazol-2-yl)benzoic acid hydrazide (**2a, 2b**) were synthesized in accordance to the method described in the literature [31]. Condensation of the hydrazide with various substituted benzaldehydes gave the corresponding arylidene hydrazides (**3a**–**3l**). Structure elucidations of the final compounds were performed with IR, $^1$H NMR, $^{13}$C NMR, MS spectroscopic methods and elemental analyses. IR data was very informative and provided evidence for the formation of the expected structures. In the IR spectra, significant stretching bands were observed due to N-H, C=O, and C=N and C=C bonds at 3305–3180, 1666–1639 and 1641–1419 $cm^{-1}$, respectively. The stretching absorption belonging to 1,4-disubstituted benzene was determined at 842–854 $cm^{-1}$.

In the $^1$H-NMR spectra of the compounds, azomethine protons and amine protons were determined at about 8.77–8.27 and 12.40–11.82 ppm, respectively. $^1$H-NMR spectrum showed a broad singlet at 13.33–13.21 ppm due to NH proton of the benzimidazole ring. The aromatic protons belonging to 4-substitutedphenyl gave peaks at 8.12–8.08 and 8.33–8.29 as two doublets. Aromatic protons of




| Comp. | $R_1$ | $R_2$ | X | Comp. | $R_1$ | $R_2$ | X |
|---|---|---|---|---|---|---|---|
| **3a** | Cl | – | O | **3g** | F | – | O |
| **3b** | Cl | $CH_3$ | O | **3h** | F | $CH_3$ | O |
| **3c** | Cl | $NO_2$ | O | **3ı** | F | $NO_2$ | O |
| **3d** | Cl | – | S | **3j** | F | – | S |
| **3e** | Cl | $CH_3$ | S | **3k** | F | $CH_3$ | S |
| **3f** | Cl | $NO_2$ | S | **3l** | F | $NO_2$ | S |

**Figure 1:** Synthesis route to target compounds **3a–3l.**

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benzimidazole were observed at 8.48–6.29 ppm. $^{13}$C-NMR spectrum showed characteristic hydrazone (-CONHN=CH-) signal at 163.54–161.89 due to carbonyl (C=O). Primer, tertiary and quaternary carbons were determined by DEPT experiments. The mass spectra (ES-MS) of the compounds showed [M+1] peaks, in agreement with their molecular formula. The M+2, M+3, M+4 and M+5 peaks were also observed for the chloro substituted compounds (**3a**–**3l**) due to high relative density of chlorine isotopes. All compounds gave satisfactory elemental analyses results, indicating that that all compounds have high peak purity indexes.

According to cancer statistics 2017, lung cancer is the first leading cause of cancer death in both sexes, whereas breast cancer is the second in females [32]. We evaluated the cytotoxic effects of newly synthesized compounds on A549 and MCF-7 cell lines to test their anticancer activity. One of the important criteria for an anticancer agent is to show minimum or no side-effects on healthy cells of the patients receiving chemotherapy. Therefore, the selectivity of the compounds was tested against healthy cell line, NIH/3T3. The $IC_{50}$ values of the compounds are represented in Table 1. Among all compounds, **3b** has the most cytotoxic activity against human lung carcinoma. Also, **3b** did not exert any cytotoxicity against healthy cells according to the MTT assay. Therefore, it can be concluded that compound **3b** has selective anticancer activity against human lung carcimoma. According to $IC_{50}$ values, compound **3e** has the most anticancer effect against human breast carcinoma. Also, it has even lower $IC_{50}$ value than our positive control, cisplatin against MCF-7 cell line. The $IC_{50}$ value of compound **3e** was also higher than its $IC_{50}$ value against NIH/3T3 cell line, showing its selective anticancer potential against MCF-7 cell line.

**Table 1:** $IC_{50}$ (μM) values of compounds **(3a–3l).**

| Compound | A549 | MCF-7 | NIH3T3 |
|---|---|---|---|
| **3a** | >1 | 0.76 | >1 |
| **3b** | 0.316 | >1 | >1 |
| **3c** | >1 | 0.90 | >1 |
| **3d** | >1 | 0.316 | 0.316 |
| **3e** | >1 | 0.0316 | 0.1 |
| **3f** | >1 | >1 | >1 |
| **3g** | >1 | >1 | >1 |
| **3h** | >1 | >1 | >1 |
| **3i** | 0.83 | 0.1 | >1 |
| **3j** | >1 | >1 | >1 |
| **3k** | >1 | >1 | >1 |
| **3l** | >1 | 1 | >1 |
| **Cisplatin** | 0.045 | 0.052 | N.D |

In terms of relationships between chemical structure and cytotoxic activity, adequate information could not be provided for the cytotoxic activity of compounds against A549 cell line because only two of compounds (**3b** and **3i**) displayed an $IC_{50}$ below 1 μM. However, MCF7 cells seemed to be more sensitive to compounds **3a**, **3c**, **3d**, and **3e**. This finding demonstrates that 5-chloro substitution of benzimidazole ring enhances the cytotoxic activity against MCF7 cells comparing 5-fluorosubstitution.

## Conclusions

A total of 12 novel heterocyclic compounds based on the benzimidazole nucleus were prepared, characterized and evaluated for their cytotoxic activity against two human carcinogenic cell lines by the MTT assay. Compound **3e** including 5-methyl-thiophene substituent was the most active compound against the MCF-7 cell line and was identified as a lead moiety.

Therefore, these results imply that the benzimidazole-hydrazone derivatives represent targets for further optimization and development of new anticancer agents to treat breast cancer.

**Conflict of interest statement:** Authors have no conflict of interest regarding this study.

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