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ARTICLE

Synthesis, structural characterization, and in vitro anti-cancer activities of new phenylacrylonitrile derivatives

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Abstract The present study was designed to both synthesize phenylacrylonitrile compounds (**1a–k**) and their anti-tumor activities on human breast cancer cell line (MCF-7) were determined. The structures of all the compounds were defined by melting point, elemental analysis, FT-IR, ^{1}H, ^{13}C, ^{13}C-APT, and HETCOR-NMR spectroscopy. Anti-tumor activities of these compounds on cell viability were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay against MCF-7. The MCF-7 cell lines were treated with 1, 5, 25, 50, and 100 μM concentrations of phenylacrylonitrile compounds for 24 h. At the end of the experiments, **1a**, **1b**, **1c**, **1g**, and **1h** compounds reduced cell viability ($p < 0.01$). Additionally, the anti-cancer activities of these compounds on MCF-7 were investigated by comparing IC_{50} values. In conclusion, while some of the synthesized phenylacrylonitrile compounds (**1a**, **1b**, **1c**, **1g**, and **1h**) have anti-tumor activity, other phenylacrylonitrile compounds (**1d**, **1e**, **1f**, **1k**, and **1h**) have no effect on human breast cell lines.

Keywords Anti-tumor activity · Breast cancer · MCF-7 · NMR-spectroscopy phenylacrylonitrile

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Introduction

Cancer is associated with a collection of the related diseases. Cancer is the uncontrolled growth of abnormal cells in the body. In all types of cancer, some of the body's cells begin to divide without stopping and expand to the related tissues. There are many factors and types of cancer. Breast cancer is a potentially life-threatening and one of the most common types of cancer among women worldwide. Although the treatment and diagnosis of breast cancer have improvement over the decades, it still causes high mortality among women worldwide (Grayson 2012; Maxmen 2012). Tamoxifen is commonly used for adjuvant therapy of breast cancer and is an important drug for prevention of breast cancer in women (Tormey et al. 1996). The mechanism of action of tamoxifen is still unclear, although its antiproliferative effect may be via a receptor-mediated cytostatic activity, a non-specific activity, or a receptor-mediated cytotoxic activity. In this regard, a major challenge is to develop effective anti-cancer agents (Nayfield 1995; Rutqvist et al. 1995).

More recently, the synthesized organic and inorganic compounds such as chalcone bearing cyclotriphosphazene derivatives (Görgülü et al. 2015), transition-metal complexes of a binaphthyl-linked bipyridine ligand (Beynek et al. 2009), androstane D-homo lactone derivatives (Djurendić et al. 2012), thiazolidin-4-ones compounds (Isloor et al. 2013), propanedinitrile analogues (Soung et al. 2009), benzochalcone derivative (Song et al. 2013) and acenaphthopyrazine derivatives (Xing et al. 2012) were studied for its effects on the human breast cancer.

Substituted acrylonitrile derivatives possess a wide range of various physical and biological properties. They have wide range of applications as antiproliferative activity (Carta et al. 2002, 2004), anti-microbial agents (Alam et al.

2013), anti-bacterial agents (Saczewski et al. 2008), and fluorescence properties (Percino et al. 2011). Moreover, aryl-acrylonitriles were utilized in the area of organic materials to achieve high-electron affinity compounds, which can be used to produce LEDs (light emitting diodes) with air stable electrodes (Maruyama et al. 1998; Gomez et al. 1999; Segura et al. 1999). The limited studies have been reported about cytotoxic properties of phenylacrylonitrile compounds (Segura et al. 1999; Saczewski et al. 2004; Tarleton et al. 2012, 2013).

The aim of this study was both to synthesize new phenylacrylonitrile compounds and to evaluate to their possible anti-carcinogenic properties on MCF-7 cell lines. For this purpose, the firstly compounds **1a–k** were synthesized according to the Knoevenagel condensations protocol (Buu-Hoi et al. 1969; Basaran et al. 2008). The structures of these compounds were determined by various spectroscopic techniques and then anti-tumor activities of compounds on cell viability were investigated. Our results indicate that phenylacrylonitrile derivatives displayed potential anti-tumor activity against human breast cancer cell lines (MCF-7).

Materials and methods

All the chemicals were purchased from Sigma-Aldrich (USA) and Merck (Germany). Solvents and other liquids used in the experimental works were dried by conventional methods. FT-IR spectra were recorded on a Perkin Elmer (USA) FT-IR spectrometer. Elemental analysis was carried out by a LECO 932 CHNS-O apparatus. Thermal analysis of the compounds were investigated by differential scanning calorimetry (DSC) using a SHIMADZU DSC thermobalance (10 °C/min). All the NMR spectra were obtained using a Bruker (USA) DPX-400 spectrometer. 1D (^{1}H and ^{13}C) chemical shifts were obtained using tetramethylsilane as an internal standard. For the NMR studies, the chloroform-d was used as solvent for the compounds **1a-k**.

Chemistry

All phenylacrylonitrile compounds were prepared and synthesized according to a method reported in the literature (Buu-Hoi et al. 1969; Basaran et al. 2008). General method for the reactions of phenylacrylonitrile is stated as below:

A solution of 2,4,5-trimethoxybenzaldehyde (**1**) (15.0 mmol) and phenylacetonitrile (**a–k**) (16.5 mmol) in ethyl alcohol (50 mL) was heated to 70 °C and then NaOH solution (25 %) was added dropwise to the reaction mixture until the initiation of turbidity. After cooling to room temperature, the solution was quenched with ice, washed with hot-water by filtration. The filtrated product was recrystallized from ethanol.

*2-(2,4,5-Trimethoxyphenyl)-1-(3-methylphenyl)acrylonitrile (**1a**)*

2,4,5-trimethoxybenzaldehyde (**1**) (1.60 g, 5.4 mmol) and 3-methylbenzylcyanide (**a**) (0.67 g, 5.4 mmol) were used. Green solid; Yield: 90 % (1.5 g), m.p. 113–114 °C. FT-IR (KBr, cm^{-1}) ν: 3043 and 3014 (Ar–CH), ν: 2199 (C≡N), ν: 1613, 1578 and 1512 (C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.44 (3H, s, H^{19}), 3.98 (3H, s, H^{8}), 3.97 (3H, s, H^{9}), 3.91 (3H, s, H^{7}), 6.55 (1H, s, H^{2}), 7.20 (1H, d, H^{16}), 7.35 (1H, t, H^{17}), 7.50-7.48 (2H, m, H^{14} and H^{18}), 7.96 (2H, s, H^{5} and H^{10}). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 21.5 C^{19}, 56.5 C^{8}, 56.4 C^{9}, 56.1 C^{7}, 96.4 $C^{2,}$ 108.0 $C^{11,}$ 110.4 C^{5}, 114.6 C^{6}, 119.2 C^{12}, 122.9 C^{18}, 126.5 C^{14}, 128.8 C^{17}, 129.3 C^{16}, 135.2 C^{13}, 136.3 C^{15}, 138.6 C^{10}, 143.0 C^{4}, 152.1 C^{3}, 153.7 C^{1}. Anal. Calcd. for $C_{19}H_{19}NO_3$ (MW: 309.36): C, 73.77 %; H, 6.19 %; N, 4.53 %. Found: C, 73.83 %; H, 6.13 %; N, 4.55 %.

*2-(2,4,5-Trimethoxyphenyl)-1-(4-methylphenyl)acrylonitrile (**1b**)*

2,4,5-trimethoxybenzaldehyde (**1**) and 4-methylbenzylcyanide (**b**) (0.67 g, 5.4 mmol) were used. Yellow solid; Yield: 88 % (1.47 g), m.p. 142–143 °C. FT-IR (KBr, cm^{-1}) ν: 3050, 3000 (Ar–CH), ν: 2201 (C≡N), ν: 1610, 1585 and 1518 (C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.41 (3H, s, H^{17}), 3.98 (3H, s, H^{8}), 3.97 (3H, s, H^{9}), 3.91 (3H, s, H^{7}), 6.55 (1H, s, H^{2}), 7.26 (2H, d, H^{15}), 7.59 (2H, t, H^{14}), 7.95 (2H, s, H^{5} and H^{10}). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 21.2 C^{17}, 56.5 C^{8}, 56.4 C^{9}, 56.1 C^{7}, 96.4 C^{2}, 107.9 C^{11}, 110.4 C^{5}, 114.6 C^{6}, 119.2 C^{12}, 125.7 C^{14}, 129.6 C^{15}, 132.5 C^{13}, 135.5 C^{16}, 138.5 C^{10}, 143.0 C^{4}, 152.0 $C^{3,}$ 153.6 C^{1}. Anal. Calcd. for $C_{19}H_{19}NO_3$ (MW: 309.36): C, 73.77 %; H, 6.19 %; N, 4.53 %. Found: C, 73.69 %; H, 6.11 %; N, 4.48 %.

*2-(2,4,5-Trimethoxyphenyl)-1-(3-(trifluoromethyl)phenyl) acrylonitrile (**1c**)*

2,4,5-trimethoxybenzaldehyde (**1**) (1.60 g, 5.4 mmol) and 3-(trifluoromethyl) phenylacetonitrile (**c**) (1.00 g, 5.4 mmol) were used. Green solid; Yield: 80 % (1.57 g), m.p. 169–170 °C. FT-IR (KBr, cm^{-1}) ν: 3050 and 3000 (Ar–CH), ν: 2201 (C≡N), ν: 1612, 1581, 1527 (C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.98 (3H, s, H^{8}), 3.97 (3H, s, H^{9}), 3.94 (3H, s, H^{7}), 6.56 (1H, s, H^{2}), 7.56-7.62 (2H, m, H^{16} and H^{17}), 7.86–7.90 (2H, m, H^{14} and H^{18}), 7.96 (1H, s, H^{5}), 8.03 (1H, s, H^{10}). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 56.1 C^{8}, 56.4 C^{9}, 56.4 C^{7}, 96.1 C^{2}, 106.1 C^{11}, 110.2 C^{5}, 113.9

C^6, 118.7 C^{12}, 122.4 C^{14}, 125.0 C^{16}, 129.1 C^{18}, 129.5 C^{17}, 131.2 C^{15}, 132.6 C^{19}, 136.3 C^{13}, 137.9 C^{10}, 143.1 C^4, 152.8 C^3, 154.1 C^1. Anal. Calcd. for $C_{19}H_{16}F_3NO_3$ (MW: 363.33): C, 62.81 %; H, 4.44 %; N; 3.86 %. Found: C, 62.77 %; H, 4.39 %; N, 3.89 %.

*2-(2,4,5-Trimethoxyphenyl)-1-(4-(trifluoromethyl) phenyl)acrylonitrile (**1d**)*

2,4,5-trimethoxybenzaldehyde (**1**) (1.60 g, 5.4 mmol) and 4-(trifluoromethyl) phenylacetonitrile (**d**) (1.00 g, 5.4 mmol) were used. Yellow solid; Yield: 78 % (1.53 g), m.p. 137-138 °C. FT-IR (KBr, cm^{-1}) ν: 3050, 3000 (Ar–CH), ν: 2203 (C≡N), ν: 1616, 1584 and 1526 (C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.98 (3H, s, H^8), 3.93 (3H, s, H^9), 3.94 (3H, s, H^7), 6.56 (1H, s, H^2), 7.71 (2H, d, H^{14}), 7.79 (2H, d, H^{15}), 8.00 (1H, s, H^5), 8.03 (1H, s, H^{10}). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 56.4 C^8, 56.4 C^9, 56.1 C^7, 96.1 C^2, 105.9 C^{11}, 110.2 C^5, 113.4 C^6, 118.7 C^{12}, 125.9 C^{13}, 126.0 C^{14}, 130.1 C^{17}, 130.3 C^{16}, 138.2 C^{10}, 138.8 C^{15}, 143.1 C^4, 152.9 C^3, 154.2 C^1. Anal. Calcd. for $C_{19}H_{16}F_3NO_3$ (MW: 363.33): C, 62.81 %; H, 4.44 %; N, 3.86 %. Found: C, 62.85 %; H, 4.39 %; N, 3.81 %.

*2-(2,4,5-Trimethoxyphenyl)-1-(3,4-(methylenedioxy) phenyl)acrylonitrile (**1e**)*

2,4,5-trimethoxybenzaldehyde (**1**) (1.60 g, 5.4 mmol) and 3,4-(methylenedioxy) benzylcyanide (**e**) (0.87 g, 5.4 mmol) were used. Yellow solid; Yield: 85 % (1.55 g), m.p. 147–148 °C. FT-IR (KBr, cm^{-1}) ν: 3043, 3007 (Ar–CH), ν: 2214 (C≡N), ν: 1609, 1590, 1501 (C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.98 (3H, s, H^8), 3.97 (3H, s, H^9), 3.93 (3H, s, H^7), 6.05 (2H, s, H^{19}), 6.55 (1H, s, H^2), 6.89 (1H, d, H^{17}), 7.17 (1H,s, H^{14}), 7.20 (1H, d, H^{18}), 7.83 (1H, s, H^{10}), 7.92 (1H, s, H^5). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 56.5 C^8, 56.4 C^9, 56.1 C^7, 96.4 C^2, 101.5 C^{19}, 105.9 C^{14}, 107.6 C^{11}, 108.5 C^{17}, 110.3 C^5, 114.5 C^6, 119.1 C^{12}, 120.3 C^{18}, 129.7 C^{13}, 135.0 C^{10}, 143.0 C^4, 148.0 C^{15}, 148.3 C^{16}, 152.0 C^3, 153.5 C^1. Anal. Calcd. for $C_{19}H_{17}NO_5$ (MW: 339.34): C, 67.25 %; H, 5.05 %; N, 4.13 %. Found: C, 67.33 %; H, 5.12 %; N, 4.18 %.

*2-(2,4,5-Trimethoxyphenyl)-1-(3,5-bis(trifluoromethyl) phenyl)acrylonitrile (**1f**)*

2,4,5-trimethoxybenzaldehyde (**1**) (1.60 g, 5.4 mmol) and 3,5-bis(trifluoromethyl) benzylcyanide (**f**) (1.37 g, 5.4 mmol) were used. Yellow solid; Yield: 72 % (1.67 g), m.p. 185–186 °C. FT-IR (KBr, cm^{-1}) ν: 3065, 3014 (Ar–CH), ν: 2210 (C≡N), ν: 1613, 1576, 1506 (C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.01 (3H, s, H^8), 3.96 (3H, s, H^9), 3.98 (3H, s, H^7), 6.56 (1H, s, H^2), 7.87 (1H, s, H^5), 7.97 (1H, s, H^{16}), 8.09 (3H, s, H^{10}, H^{14} and H^{18}). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 56.4 C^8, 56.3 C^9, 56.1 C^7, 96.0 C^2, 104.4 C^{11}, 110.1 C^5, 113.4 C^6, 118.2 C^{12}, 121.7 C^{16}, 124.4 C^{18}, 125.7 C^{14}, 131.9-132.9 C^{15}, C^{17}, C^{19} and C^{20}, 137.8 C^{13}, 139.3 C^{10}, 143.1 C^4, 153.5 C^3, 154.6 C^1. Anal. Calcd. for $C_{20}H_{17}F_6NO_3$ (MW: 431.33): C, 55.69 %; H, 3.51 %; N, 3.25 %. Found: C, 55.73 %; H, 3.47 %; N, 3.20 %.

*2-(2,4,5-Trimethoxyphenyl)-1-(4-nitrophenyl)acrylonitrile (**1g**)*

2,4,5-trimethoxybenzaldehyde (**1g**) (1.60 g, 5.4 mmol) and 4-nitrobenzylcyanide (**g**) (0.87 g, 5.4 mmol) were used. Orange solid; Yield: 50 % (0.92 g), m.p. 207–208 °C. FT-IR (KBr, cm^{-1}) ν: 3072, 3000 (Ar–CH), ν: 2202 (C≡N), ν: 1614, 1598, 1574 (C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.01 (3H, s, H^8), 3.98 (3H, s, H^9), 3.95 (3H, s, H^7), 6.56 (1H, s, H^2), 7.86 (2H, d, H^{15}), 8.02 (1H, s, H^5), 8.17 (1H, s, H^{10}), 8.32 (2H, d, H^{14}). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 56.1 C^8, 56.4 C^9, 56.4 C^7, 96.0 C^2, 104.8 C^{11}, 110.0 C^5, 113.6 C^6, 118.4 C^{12}, 124.2 C^{15}, 126.2 C^{14}, 139.3 C^{10}, 141.7 C^{13}, 143.2 C^4, 147.2 C^{16}, 153.6 C^3, 154.7 C^1. Anal. Calcd. for $C_{18}H_{16}N_2O_5$ (MW: 340.33): C, 63.52 %; H, 4.74 %; N, 8.23 %. Found: C, 63.57 %; H, 4.70 %; N, 8.26 %.

*2-(2,4,5-Trimethoxyphenyl)-1-(3-chlorophenyl)acrylonitrile (**1h**)*

2,4,5-trimethoxybenzaldehyde (**1h**) (1.60 g, 5.4 mmol) and 3-chlorobenzylcyanide (**h**) (0.82 g, 5.4 mmol) were used. Green solid; Yield: 90 % (1.60 g), m.p. 164–165 °C. FT-IR (KBr, cm^{-1}) ν: 3050, 3009 (Ar–CH), ν: 2196 (C≡N), ν: 1612, 1580, 1510 (C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.99 (3H, s, H^8), 3.97 (3H, s, H^9), 3.93 (3H, s, H^7), 6.54 (1H, s, H^2), 7.33-7.40 (2H, m, H^{16} and H^{17}), 7.58 (1H, d, H^{18}), 7.67 (1H, s, H^5), 7.95–7.99 (2H, s, H^{14}, H^{10}). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 56.1 C^8, 56.4 C^9, 56.8 C^7, 96.1 C^2, 106.1 C^{11}, 110.1 C^5, 113.9 C^6, 118.8 C^{12}, 124.0 C^{14}, 125.7 C^{16}, 128.4 C^{18}, 130.1 C^{17}, 134.9 C^{15}, 137.1 C^{13}, 137.4 C^{10}, 143.0 C^4, 152.6 C^3, 154.1 C^1. Anal. Calcd. for $C_{18}H_{16}ClNO_3$ (MW: 329.78): C, 65.56 %; H, 4.89 %; N, 4.25 %. Found: C, 65.61 %; H, 4.82 %; N, 4.30 %.

*2-(2,4,5-Trimethoxyphenyl)-1-(4-chlorophenyl)acrylonitrile (**1k**)*

2,4,5-trimethoxybenzaldehyde (**1k**) (1.60 g, 5.4 mmol) and 4-chlorobenzylcyanide (**k**) (0.82 g, 5.4 mmol) were used. Yellow solid; Yield: 80 % (1.42 g), m.p. 140–141 °C. FT-IR (KBr, cm^{-1}) ν: 3065, 2998 (Ar–CH), ν: 2207 (C≡N), ν: 1609, 1581,1518 (C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.99 (3H, s, H^9), 3.97 (3H, s, H^8), 3.92 (3H, s, H^7), 6.55 (1H, s, H^2), 7.43 (2H, d, H^{14}), 7.63–7.61 (2H, s, H^5 and

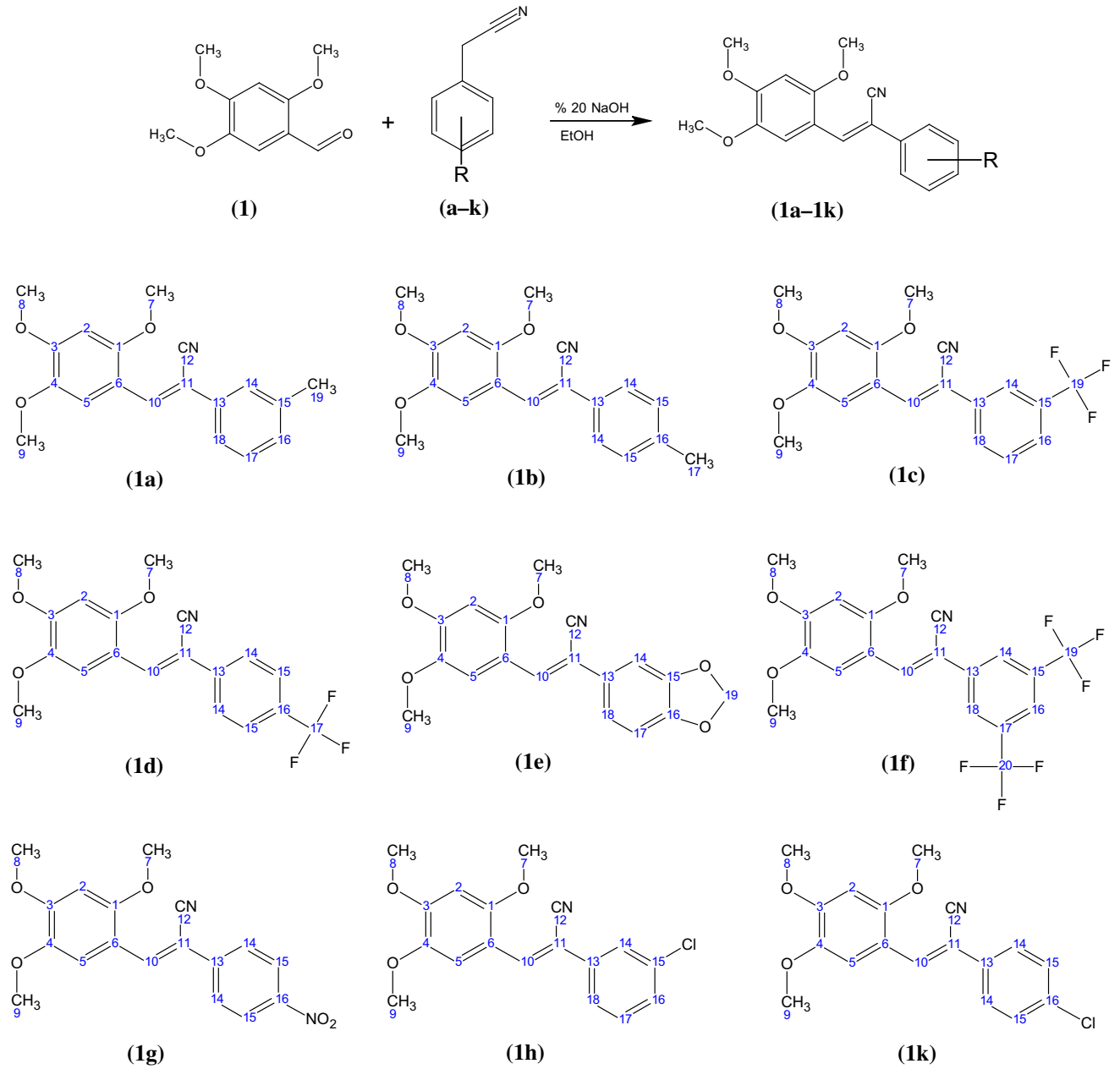

Fig. 1 Synthesis and structures of 2-(2,4,5-trimethoxyphenyl)-1-(substituted-phenyl)acrylonitrile compounds (**1a–k**)

H^{10}), 7.96 (2H, d, H^{15}). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 56.1 C^9, 56.4 C^8, 56.4 C^7, 96.1 C^2, 106.4 C^{11}, 110.1 C^5, 114.1 C^6, 118.9 C^{12}, 127.0 C^{14}, 129.1 C^{15}, 133.8 C^{13}, 134.3 C^{16}, 136.7 C^{10}, 143.0 C^4, 152.5 C^3, 153.9 C^1. Anal. Calcd. for $C_{18}H_{16}ClNO_3$ (MW: 329.78): C, 65.56 %; H, 4.89 %; N, 4.25 %. Found: C, 65.52 %; H, 4.82 %; N, 4.31 %.

In vitro cytotoxicity assay

Human breast cancer cell lines (MCF-7) were maintained in Dulbecco's modified Eagle's medium culture medium supplemented with 4 mM L-glutamine, 4500 mg/L glucose (10 % heat-inactivated fetal bovine serum, 100 U/mL penicillin–streptomycin), and with addition of 10 mM non-essential amino acids for culture of breast cancer cells. The cells were maintained at 37 °C in 5 % CO_2 humidified incubator. The cytotoxicity of phenylacrylonitrile compounds was determined in human breast cancer cell line (MCF-7) by using [3-(4,5-dimethylthiazol)-2-yl]-2,5-diphenyl-2H-tetrazolium bromide] (MTT) assay method (Mosamann et al. 1986; Singh and Singh 2002). Briefly, 15×10^3 breast cancer cells were plated in triplicate in 96-well flat bottom tissue culture plates, and treated with different concentrations of phenylacrylonitrile compounds

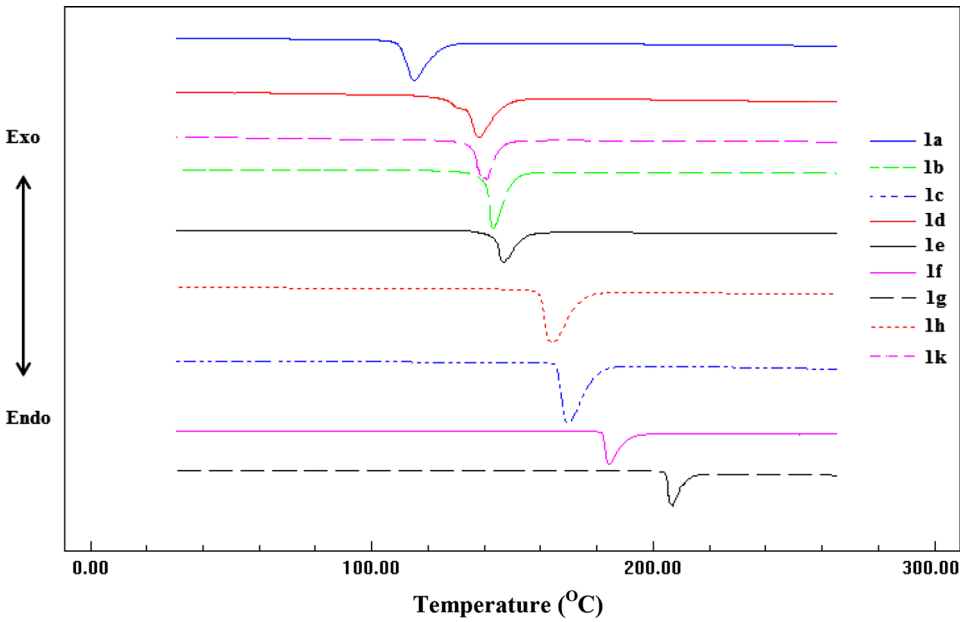

Fig. 2 The comparative melting points of compounds **1a–k**. These melting points were obtained by differential scanning calorimetry using a SHIMADZU DSC thermobalance (10 °C/min)

(1, 5, 25, 50, 100 μM) and vehicle. The culture plate cells were incubated for 24 h at 37 °C in 5 % CO_2 humidified incubator. The cells were incubated with MTT (0.005 g/mL in phosphate-buffered saline) for 3 h and readings were taken in a microtiter plate reader (Biotek Synergy) using a 550-nm fitler (Tekin et al. 2014). Each data represented an average of 10 measurements. Phenylacrylonitrile compounds (**1a–k**) were dissolved in dimethylsulfoxide (DMSO) such that the final DMSO concentration was never higher than 0.2 % (Yilmaz et al. 2006). There was no effect of 0.2 % DMSO on any of the parameters measured.

Statistical analysis

Quantitative data were presented as mean ± standard deviation. Normal distribution was confirmed using Kolmogorov–Smirnov test. Quantitative data were analyzed using Kruskal–Wallis H test following Mann–Whitney U test with Bonferroni adjustment as a post hoc test. All p values <0.05 were considered significant. All analyses were done by IBM SPSS Statistics 22.0 for Windows. IC_{50} values were determined by using inhibition % values by a GraphPad Prism 6 program on a computer.

Result and discussion

Chemistry

All aryl-acrylonitriles **1a–k** were prepared by the Knoevenagel condensation of acetonitriles **a–k** with corresponding 2,4,5-trimethoxybenzaldehyde as depicted in Fig. 1. Thus, the phenylacrylonitriles **1a–k** were obtained upon treatment of the ethanolic solution of phenylacetonitrile (**a–k**) and 2,4,5-trimethoxybenzaldehyde (**1**) with a few drops of 20 % aqueous sodium hydroxide (NaOH) at reflux. The structures of the compounds were elucidated by FT-IR, ^{1}H, ^{13}C, ^{13}C-APT, and 2D (HETCOR) NMR spectroscopy. Phenylacrylonitrile derivatives were mostly obtained in high yields. General appearance of the all reactions and structures of the compounds **1a–k** are shown in Fig. 1.

The melting points of phenylacrylonitriles **1a–k** were identified with DSC (differential scanning calorimetry). In the DSC spectra of **1a–k** were displayed a single melting point (Fig. 2).

The characteristic peaks in the infrared spectra of the aryl-acrylonitrile have been assigned as in experimental section. The products gave medium strength bands in the 2196–2214 cm^{-1} range which can be attributed to the characteristic stretching vibration of the nitrile (C≡N) group. The products also displayed bands in the 1501–1616 and 3072–3000 cm^{-1} ranges which are appointed to C–H and C=C group stretching frequencies, respectively. The NMR data of compounds (**1a–k**) were given in experimental section. Example spectra (^{1}H, ^{13}C, and ^{13}C-APT and 2D HETCOR-NMR spectra of **1e**) are presented in Figs. 3, 4, and 5. The 1D NMR data also confirm the structures of (**1a–k**) (Fig. 1).

In the ^{1}H-NMR spectra, the aromatic protons for all the products (**1a–k**) between 6.55 and 8.09 ppm with the $–OCH_3$ protons between 3.91 and 4.05 ppm appear. The methyl protons were observed at 2.44 and 2.41 ppm for **1a** and **1b**, respectively. The methylenedioxy protons which were numbered as **19** in **1e** compound were

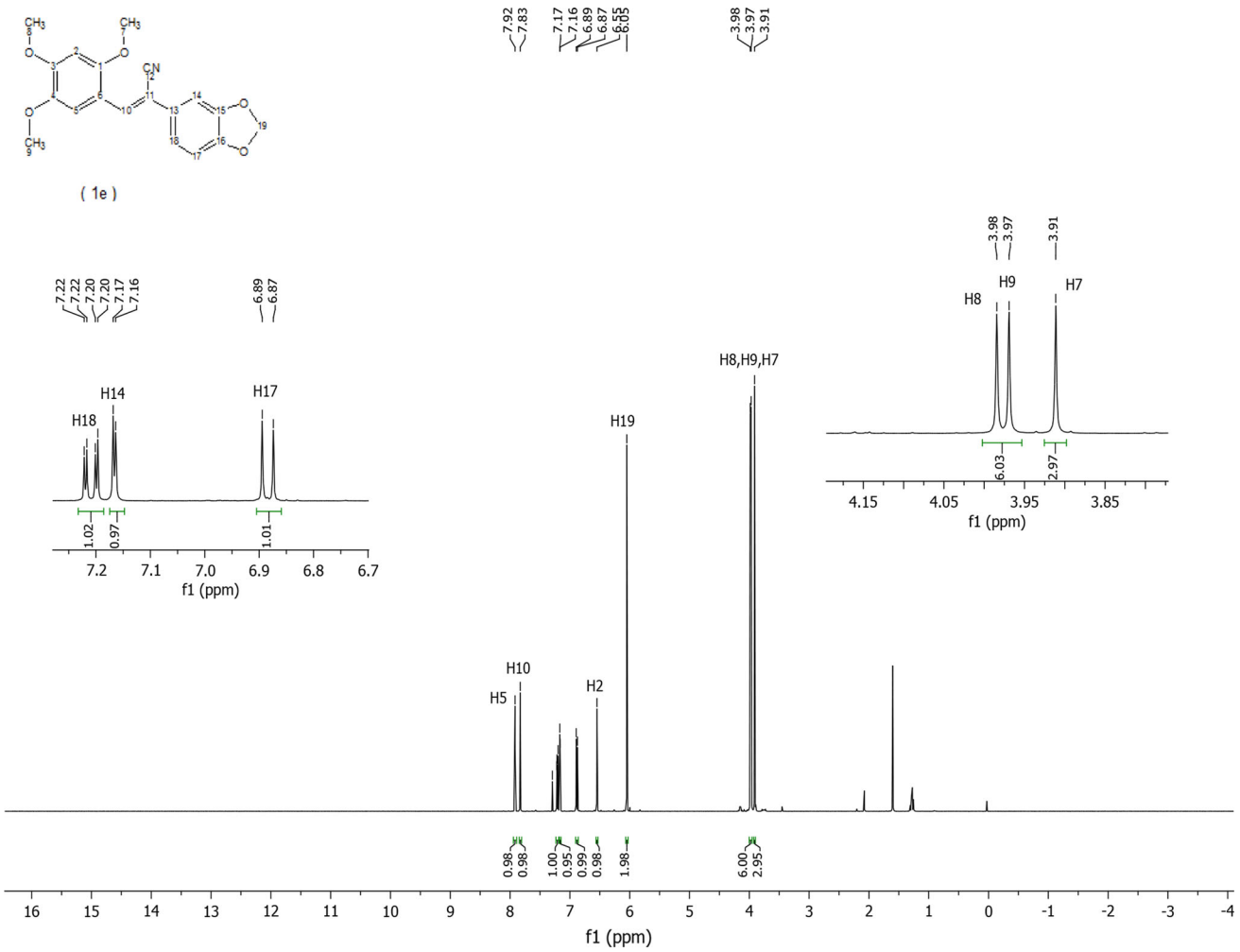

Fig. 3 ^{1}H-NMR spectrum of compound **1e**. Compound **1e** dissolved in deuterated chloroform (chloroform-d) and were obtained by using a Bruker (USA) DPX-400 spectrometer

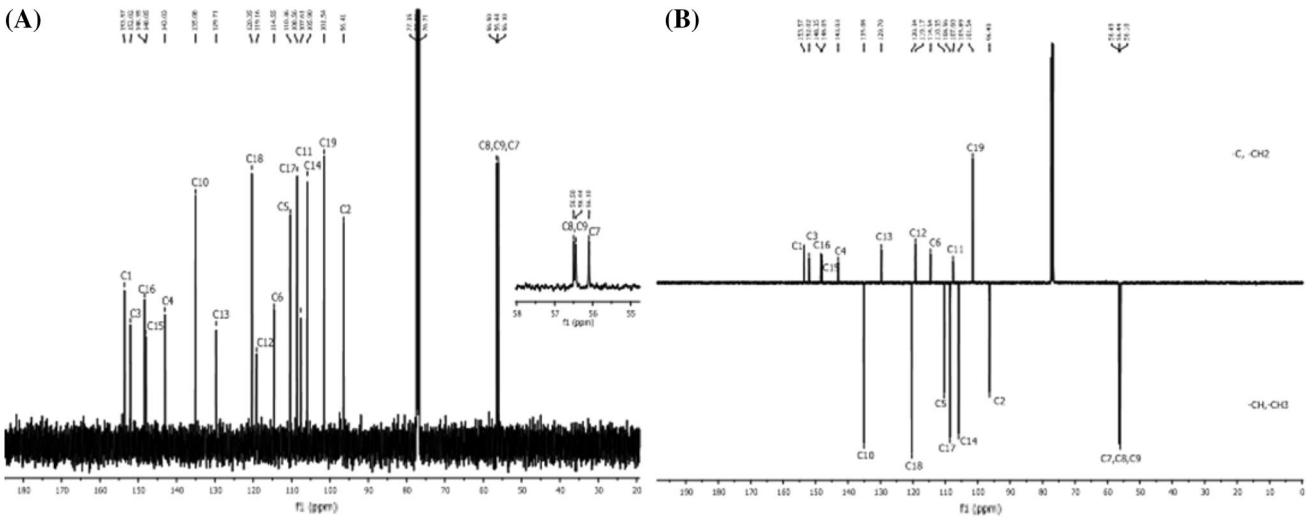

Fig. 4 (**A**) ^{13}C and (**B**) ^{13}C-APT NMR spectra of compound **1e**. Compound **1e** dissolved in deuterated chloroform (chloroform-d) and were obtained by using a Bruker (USA) DPX-400 spectrometer

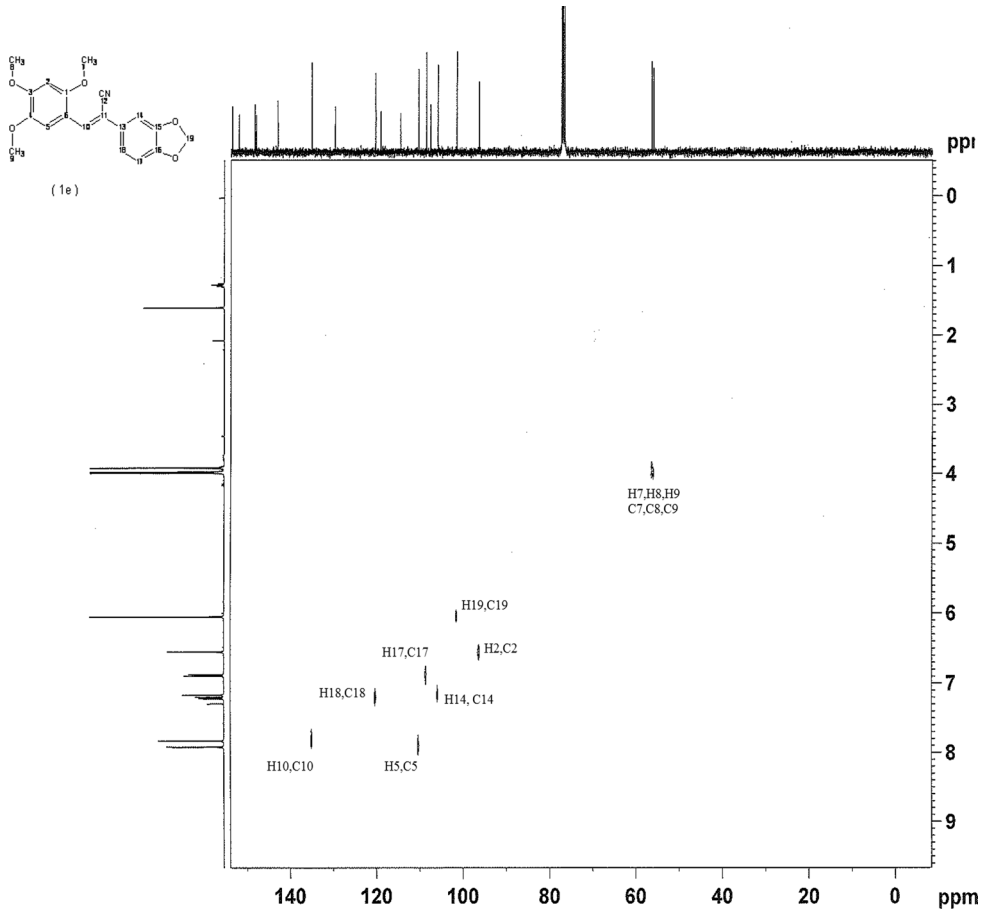

Fig. 5 HETCOR (2D, ^{1}H-^{13}C coupling) NMR spectrum of compound **1e**. Compound **1e** dissolved in deuterated chloroform (chloroform-d) and were obtained by using a Bruker (USA) DPX-400 spectrometer

observed at 6.05 ppm. The aliphatic –CH protons which were numbered as **10** in all compounds (**1a**–**k**) were observed at 7.96, 7.95, 8.03, 8.03, 7.83, 8.09, 8.17, 7.95, and 7.63 ppm, respectively.

The comprehensive ^{13}C-NMR spectral data were given in experimental section. Some important characteristic peaks, for example, the nitrile carbon atoms for **1a**–**k**, were showed at 119.2, 119.2, 118.7, 118.7, 119.1, 118.2, 118.4, 118.8, and 118.9 ppm, respectively. The methyl carbons for the compounds **1a** and **1b** were observed at 21.5 and 21.2 ppm. The $–OCH_3$ carbons (**1a**–**k**) appeared between 56.1 and 56.5 ppm. The aliphatic carbons which were numbered as **10** in **1a**–**k** were observed at 138.6, 138.5, 137.9, 138.2, 135.0, 139.3, 139.3, 137.4, and 136.7 ppm, respectively.

Anti-cancer activity evaluation

The MCF-7 cancer cell lines were preserved in DMEM culture medium supplemented with 4500 mg/L glucose and addition of 10 mM non-essential amino acids for culture of this cancer cells. The cells were preserved in 5 % CO_2 humidified incubator at 37 °C.

MCF-7 cell lines were treated with concentrations of 1, 5, 25, 50, and 100 μM of the phenylacrylonitrile compounds. The cytotoxic properties of compounds **1a**–**k** against MCF-7 cancer cell lines were detected by using MTT assay. Figure 6 shows the effects of the phenylacrylonitrile compounds on cell viability measured at 24 h after exposure.

At 100 μM, concentrations of **1a**, **1b**, **1c**, **1g**, and **1h** from the phenylacrylonitrile compounds significantly reduced the percentage of viability of MCF-7 cells ($p < 0.01$). Phenylacrylonitrile compounds were decreased the cell viability against different human cancer cell lines in the literature articles (Tarleton et al. 2012; Alam et al. 2013). Administration of the lower doses of **1c** (50 μM), **1g** (50 μM), and **1h** (25 and 50 μM) also caused significant decreases in cell viability ($p < 0.01$). The reductions in viability of MCF-7 cells occurred in a dose-dependent manner. The treated doses of tamoxifen significantly and dose-dependently declined cell viability ($p < 0.01$). IC_{50} values of the compounds (**1a**–**k**) were found to be between 10 μM and 15 μM against MCF-7 cell lines. Table 1 shows that IC_{50} values of **1a**–**k** on MCF-7 cell lines.

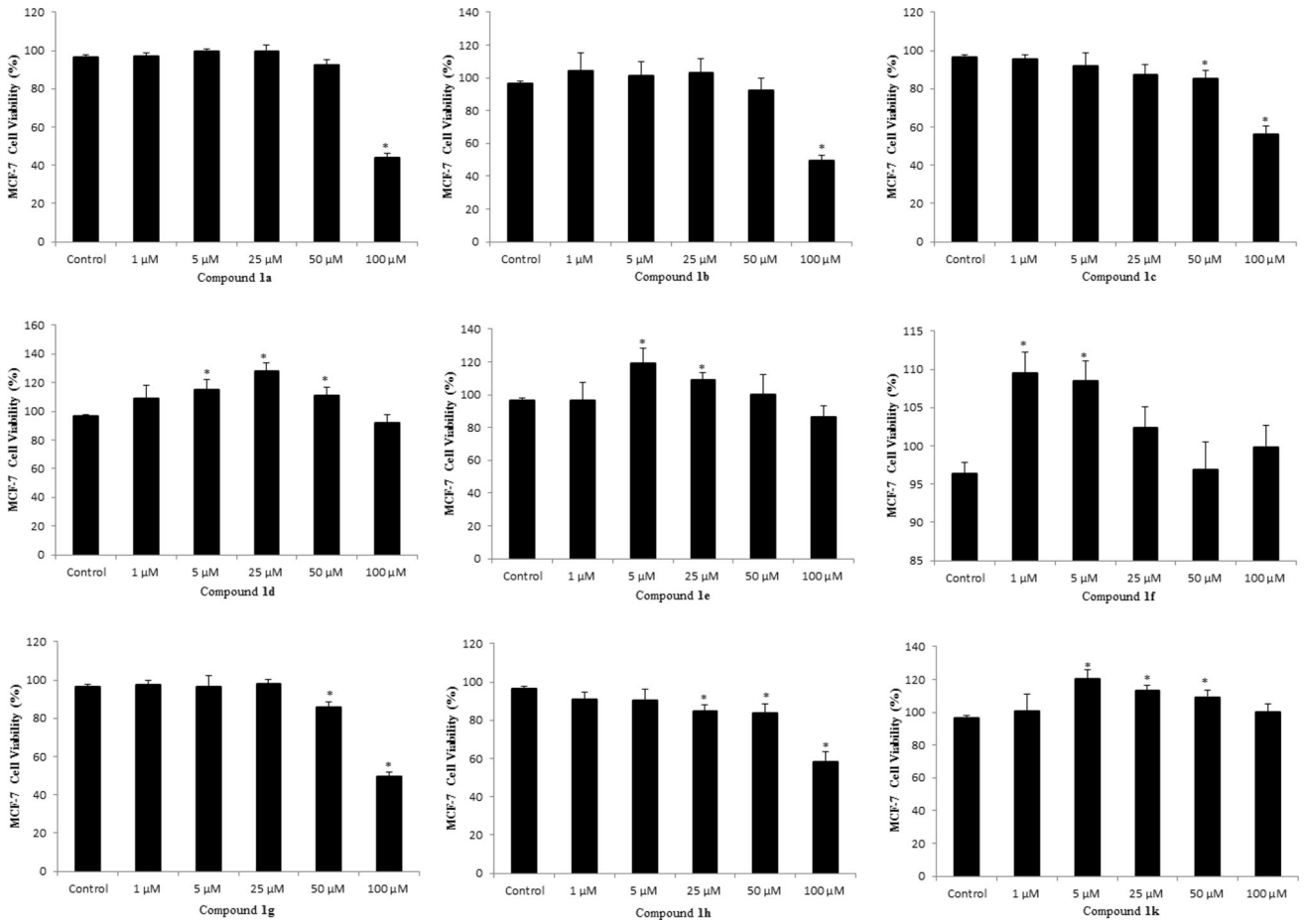

Fig. 6 The cell viability results of MCF-7 cells after a 24-hour treatment with phenylacrylonitrile compounds **1a–k**. The changes on the cell viability (%) caused by phenylacrylonitrile derivatives are compared with the control data. Each data point is an average of 10 viability ($p^* < 0.01$)

Table 1 Evaluation of anti-cancer activities, IC_{50} (μM), of compounds **1a–k** against human breast cancer cell lines. IC-50 is the concentration of drug that reduces cell growth by 50 %

Compounds	1a	1b	1c	1d	1e	1f	1g	1h	1k	Tamoxifen
MCF-7	10.6	10.6	12.7	31.6	29.5	36.3	10.9	14.2	89.7	5.4

Our results show that the new synthesized compounds have anti-tumor activity on MCF-7 cancer cells. The remained phenylacrylonitrile compounds (**1d**, **1e**, **1f**, and **1k**) have no anti-tumor activity on cancer cells.

The structure–activity relationships of phenylacrylonitrile compounds were reported in the literature and according to their results, anti-cancer activities of these compounds are both structure and dose dependent (Saczewski et al. 2008; Tarleton et al. 2011; Alam et al. 2013). For this reason, synthesized compounds (**1a–k**) were examined in terms of structure–activity relationship. It can be said that the compound **1a** is more effective in terms of reducing the cell viability when the electron-donating methyl group is present on the ring compared to the compounds **1c** and **1h** which are substituted with trifluoromethyl and chloro groups, respectively. The result of chloro-substituted compounds **1h** and **1k** on MCF-7 cells were evaluated and it was detected that 25, 50, and 100 μM doses of compound **1h** inhibited cancer cells, but *para*-substituted compound **1k** showed no cytotoxicity effect on MCF-7 cells viability. It is found that the *para*-methyl-substituted compound **1b** inhibited cancer cells compare to other *para*-substituted compounds **1d** and **1k**. According to the results of viability of MCF-7 cells, on the concentration of 50 and 100 μM of compound **1c** which is substituted with $-CF_3$ group on the *meta*-position caused decreasing

effect on cell viability. On the other hand, it was determined that the compound **1d** which substituted with $–CF_3$ group on the *para*-position showed no effect. It is determined that the substitution of one more $–CF_3$ group at 5-position of compound **1f** led to dramatic change in antitumor activity. We believed that this obvious change came from steric effect of di-substituted phenyl ring. Another important point is the electron-withdrawing $–NO_2$ group at compound **1d** displayed inhibition activity compared to other para-substituted $–CF_3$ group. We proposed that this intriguing change took place due to the resonance capability of $–NO_2$ group with phenyl ring.

When the structure activity relation of same R groups were taken into consideration, *meta*-substituted compounds exhibited inhibition of cell viability and can be reduced that *meta*-position is more effective than *para*-position in terms of reducing cell viability.

In conclusion, we designed and synthesized a series of phenylacrylonitrile derivatives, and anti-cancer effects of these compounds were examined against MCF-7 cell lines. The most effective dose is 100 μM for the compounds **1a**, **1b**, **1c**, **1g**, and **1h** which are *meta*-substituted with methyl, trifluoromethyl, and chloro groups, *para*-substituted with methyl and nitro groups on the phenyl ring, respectively. It can be said that cytotoxicity effects of *meta*-substituted phenylacrylonitrile compounds on MCF-7 human breast cancer cells are structured and dose dependent. These results displayed that phenylacrylonitrile derivatives can be considered as potential anti-tumor agents. Thus, the next aim of this study will be to determine against various human cancer cell lines and the water-soluble phenylacrylonitrile derivatives will synthesize to test their in vivo anti-cancer activity.

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