



Synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(substituted-phenyl)acrylonitriles: in vitro anticancer activity against MCF-7, PC-3 and A2780 cancer cell lines

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Received: 5 February 2016 / Accepted: 26 April 2016 / Published online: 7 May 2016
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Abstract A series of 2-(2,3,4-trimethoxyphenyl)-1-(substituted-phenyl)acrylonitrile (**2–9**) were designed and synthesized to develop new cancer drugs. The structures of synthesized compounds **2–9** were described by using melting point, mass (MALDI-TOF-MS), FT-IR, elemental analysis, ¹H, ¹³C, ¹³C-APT and 2D NMR spectroscopy. The in vitro anticancer activities of **2–9** against human breast cancer (MCF-7), human prostate cancer (PC-3) and human ovarian cancer cells (A2780) were investigated by [3-(4,5-dimethylthiazol)-2-yl]-2,5-diphenyl-2H-tetrazolium bromide] (MTT) assay method. Additionally, the LogIC₅₀ values of these compounds on A2780, MCF-7 and PC-3 cell lines were calculated by using inhibition % values by the GraphPad Prism 6 program on a computer. The results indicated that these compounds have high anticancer activity against MCF-7, PC-3 and A2780 cell lines (especially A2780 cell lines, $p < 0.05$).

Keywords Phenylacrylonitrile derivatives · Human ovarian cancer cell · MCF-7 · Anticancer evaluation · PC-3

Introduction

Cancer, also known as a malignant neoplasm or tumor, is a group of diseases involving abnormal cell growth in any part of the body. Irregular cell growth can begin almost anywhere in the body of humans, which consists of trillions of cells. Various cancer types have been identified such as stomach, breast, prostate and ovarian. Breast and ovarian cancers are the most common malignancy among

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women in many countries; on the other hand, prostate cancer is the most widespread malignancy and age-related cause of cancer deaths among males worldwide [1–4]. Several treatment methods have been developed for such cancers like chemotherapy and radiation therapy. Although these therapy methods are the most valid to cope with cancer, they possess many drawbacks such as a decrease in susceptibility to infection and production of blood cells, skin, mouth and gum problems, inflammation of the lining of the digestive tract, hair loss, etc. Cancer drugs which are used in chemotherapy are distributed evenly within the body of a patient and cannot distinguish the cancer cells from healthy ones which produces several side effects [5, 6]. Drug resistance in the treatment of cancer is a cause that hinders achievement. Thus, augmentations of the effectiveness of existing drugs or designing novel drugs are being tried. For this reason, the development of potential anticancer drugs is important. Recently, to reduce the negative effects of chemotherapeutic drugs and to increase productivity, the development of new anticancer drugs such as acrylonitrile analogues [7–10], coumarin derivatives [11–13], phosphazene compounds [14–16], chalcone compounds [17–19], some pyrazoline derivatives [20, 21], and metal complexes [22–24] has been reported.

The anticancer and antibacterial properties of heteroaryl-acrylonitrile analogues have been reported in the literature [7–10]. But no studies were found about the anticancer activities of 2-(2,3,4-trimethoxyphenyl)-1-(substituted-phenyl)acrylonitrile derivatives.

In the present study, we aimed to design and synthesize phenylacrylonitrile compounds **2–9** in order to determine the anticancer activities against three different types of human cancer cells (MCF-7, PC-3, A2780). For this reason, 2-(2,3,4-trimethoxyphenyl)-1-(substituted-phenyl)acrylonitrile compounds **2–9** were obtained by using the Knoevenagel condensations protocol [25–27]. The structures of these compounds **2–9** were described by using melting point, mass (MALDI-TOF-MS), FT-IR, elemental analysis, ^1H , ^{13}C , ^{13}C -APT and 2D NMR spectroscopy. And their possible anticancer properties were investigated against onCF-7, PC-3 and A2780 cell lines by using the MTT assay method. The logIC_{50} values were determined. Our results indicate that these phenylacrylonitrile compounds displayed strong anticancer activity towards MCF-7, PC-3 and A2780 cell lines.

Experimental

Reagents and equipment

2,3,4-Trimethoxybenzaldehyde, 3-methylphenylacetone nitrile, 4-methylphenylacetone nitrile, 3-(trifluoromethyl)phenylacetone nitrile, 4-(trifluoromethyl)phenylacetone nitrile, 3,4-(methylenedioxy)phenylacetone nitrile, 3,5-bis(trifluoromethyl)phenylacetone nitrile, 3-chlorophenylacetone nitrile, 4-chlorophenylacetone nitrile, ethyl alcohol and NaOH were purchased from Sigma-Aldrich (USA). The PC-3, MCF-7 and A2780 cancer cell lines were supplied from the American Type Culture Collection. Trypsin, calf serum, streptomycin and penicillin were supplied by Hyclone (Waltham, USA).

FT-IR and mass results were recorded on a Perkin Elmer spectrum one FT-IR and on a Bruker Daltonics microflex mass spectrometer, respectively. Positive ion and linear mode MALDI TOF-MS spectra of phenylacrylonitrile derivatives were recorded on a MALDI matrix [in 1,8,9-anthracenetriol (20 mg/mL Tetrahydrofuran)] using a nitrogen laser accumulating 50 laser shots. 1D and 2D NMR analysis were recorded using a Bruker DPX-400 spectrometer at ambient temperature with SiMe₄ as an internal standard. CDCl₃-d was used as solvent for the NMR studies. Elemental analysis was carried out by a CHNS-932 (LECO) apparatus. The melting points of **2–9** were determined using a SHIMADZU DSC-50 thermobalance (10 °C/min).

General methods for 2-(2,3,4-trimethoxyphenyl)-1-(substituted-phenyl)acrylonitrile derivatives (**2–9**)

Absolute ethyl alcohol (100 mL), substituted benzylcyanide (5.4 mmol), and 2,3,4-trimethoxybenzaldehyde (5.4 mmol) were added to three-necked reaction flask with a mechanical stirrer. The reaction mixture was stirred for 0.5 h at 70 °C. Then, a sodium hydroxide (20 %) solution was added dropwise until a precipitate formed. The reaction was cooled to room temperature and the mixture was poured into ice-water. The residue was filtered and washed with hot water to pH 7. The crude product was dried under vacuum and then recrystallized in ethanol [25–27].

Synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(3-methylphenyl)acrylonitrile (**2**)

1.06 g (5.4 mmol) 2,3,4-trimethoxybenzaldehyde (**1**), 0.67 g (5.4 mmol) 3-methylbenzylcyanide, yellow crystalline solid, m.p. 79–80 °C, yield: 90 %. FT-IR (KBr) cm⁻¹ = 3059, 3094 ν_{C-H(Ar)}, 2831, 2935 ν_{C-H(Aliph.)}, 2214 ν_{C≡N}, 1498, 1583, 1601 ν_{C=C}. ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 2.45 (s, 3H, H¹⁹), 3.92 (s, 3H, H⁹), 3.96 (s, 3H, H⁸), 3.97 (s, 3H, H⁷), 6.83 (s, 1H, H⁴), 7.21 (d, 1H, H¹⁶), 7.38 (t, 1H, H¹⁷), 7.50–7.48 (m, 2H, H¹⁴ and H¹⁸), 7.86 (s, 1H, H¹⁰), 8.05 (d, 1H, H⁵). ¹³C NMR (400 MHz, CDCl₃): δ (ppm) = 21.5 (C¹⁹), 56.1 (C⁹), 60.9 (C⁸), 61.8 (C⁷), 107.4 (C⁴), 109.1 (C¹¹), 118.6 (C¹²), 121.0 (C⁶), 123.0 (C¹⁸), 123.4 (C⁵), 126.69 (C¹⁴), 128.8 (C¹⁷), 129.6 (C¹⁶), 134.8 (C¹³), 136.5 (C¹⁵), 138.8 (C¹⁰), 141.9 (C²), 153.1 (C¹), 155.8 (C³). MALDI-MS: *m/z* calc. 309.36; found: 309.66 [M]⁺. Anal. calcd. for C₁₉H₁₉NO₃: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.46; H, 5.9; N, 4.19 %.

Synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(4-methylphenyl)acrylonitrile (**3**)

1.06 g (5.4 mmol) 2,3,4-trimethoxybenzaldehyde (**1**), 0.67 g (5.4 mmol) 4-methylbenzylcyanide, yellow crystalline solid, m.p. 153–154 °C, yield: 88 %. FT-IR (KBr) cm⁻¹ = 3010, 3038 ν_{C-H(Ar)}, 2835, 2935 ν_{C-H(Aliph.)}, 2212 ν_{C≡N}, 1461, 1510, 1587 ν_{C=C}. ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 2.42 (s, 3H, H¹⁷), 3.92 (s, 3H, H⁹), 3.95 (s, 3H, H⁸), 3.96 (s, 3H, H⁷), 6.82 (s, 1H, H⁴), 7.28 (d, 2H, H¹⁵), 7.61 (t, 2H, H¹⁴), 7.83 (s, 1H, H¹⁰), 8.05 (d, 1H, H⁵). ¹³C NMR (400 MHz, CDCl₃): δ (ppm) = 21.2 (C¹⁹), 56.1 (C⁹), 60.9 (C⁸), 61.8 (C⁷), 107.4 (C⁴), 109.9 (C¹¹), 118.6 (C¹²), 121.0 (C⁶), 123.3 (C⁵), 125.8 (C¹⁴, C¹⁸), 129.6 (C¹⁵, C¹⁷), 132.1 (C¹³), 135.17

(C¹⁰), 138.9 (C¹⁶), 141.9 (C²), 153.1 (C¹), 155.7 (C³). MALDI-MS: m/z calc. 309.36; found: 310.27 [M + H]⁺. Anal. calcd. for C₁₉H₁₉NO₃: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.53; H, 6.02; N, 4.32 %.

Synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(3-(trifluoromethyl)phenyl)acrylonitrile (4)

1.06 g (5.4 mmol) 2,3,4-trimethoxybenzaldehyde (**1**), 1.0 g (5.4 mmol) 3-(trifluoromethyl)benzylcyanide, yellow crystalline solid, m.p. 72–73 °C, yield: 40 %. FT-IR (KBr) cm⁻¹ = 3010, 3038 $\nu_{C-H(Ar)}$, 2835, 2935 $\nu_{C-H(Aliph.)}$, 2212 $\nu_{C\equiv N}$, 1461, 1496, 1582, 1600 $\nu_{C=C}$. ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 3.92 (s, 3H, H⁹), 3.97 (s, 3H, H⁸), 4.0 (s, 3H, H⁷), 6.84 (d, 1H, H⁴), 7.62 (t, 1H, H¹⁶), 7.67 (m, 1H, H¹⁷), 7.89 (d, 1H, H¹⁸), 7.93 (s, 2H, H¹⁴), 8.08 (d, 1H, H⁵). ¹³C NMR (400 MHz, CDCl₃): δ (ppm) = 56.1 (C⁹), 60.9 (C⁸), 61.9 (C⁷), 107.4 (C⁴), 108.3 (C¹¹), 118.1 (C¹²), 120.3 (C⁶), 122.6 (C¹⁴), 123.5 (C⁵), 125.3 (C¹⁶), 129.1 (C¹⁸), 129.5 (C¹⁷), 131.4 (C¹⁵), 131.7 (C¹⁹), 135.9 (C¹³), 138.2 (C¹⁰), 141.9 (C²), 153.4 (C¹), 156.4 (C³). MALDI-MS: m/z calc. 363.33; found: 364.27 [M + H]⁺. Anal. calcd. for C₁₉H₁₆F₃NO₃: C, 62.81; H, 4.44; N, 3.86. Found: C, 62.71; H, 4.26; N, 3.67 %.

Synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(4-(trifluoromethyl)phenyl)acrylonitrile (5)

1.06 g (5.4 mmol) 2,3,4-trimethoxybenzaldehyde (**1**), 1.0 g (5.4 mmol) 4-(trifluoromethyl)benzylcyanide, yellow crystalline solid, m.p. 101–102 °C, yield: 50 %. FT-IR (KBr) cm⁻¹ = 3005, 3030 $\nu_{C-H(Ar)}$, 2940, 2967 $\nu_{C-H(Aliph.)}$, 2209 $\nu_{C\equiv N}$, 1461, 1497, 1588, 1615 $\nu_{C=C}$. ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 3.92 (s, 3H, H⁹), 3.98 (s, 3H, H⁸), 3.99 (s, 3H, H⁷), 6.85 (d, 1H, H⁴), 7.74 (d, 2H, H¹⁴), 7.82 (d, 2H, H¹⁵), 7.97 (s, 1H, H¹⁰), 8.11 (d, 1H, H⁵). ¹³C NMR (400 MHz, CDCl₃): δ (ppm) = 56.1 (C⁹), 60.9 (C⁸), 61.9 (C⁷), 107.5 (C⁴), 108.2 (C¹¹), 118.1 (C¹²), 120.3 (C⁶), 123.5 (C⁵), 125.9 (C¹³), 126.1 (C¹⁴, C¹⁸), 130.4 (C¹⁶), 130.7 (C¹⁹), 138.4 (C¹⁰), 138.5 (C¹⁵, C¹⁷), 141.9 (C²), 153.4 (C¹), 156.5 (C³). MALDI-MS: m/z calc. 363.33; found: 364.27 [M + H]⁺. Anal. calcd. for C₁₉H₁₆F₃NO₃: C, 62.81; H, 4.44; N, 3.86. Found: C, 62.73; H, 4.25; N, 3.75 %.

Synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(3,4-(methylenedioxy)phenyl)acrylonitrile (6)

1.06 g (5.4 mmol) 2,3,4-trimethoxybenzaldehyde (**1**), 0.87 g (5.4 mmol) 3,4-(methylenedioxy)benzylcyanide, pale yellow crystalline solid, m.p. 125–126 °C, yield: 80 %. FT-IR (KBr) cm⁻¹ = 3015, 3043 $\nu_{C-H(Ar)}$, 2895, 2939 $\nu_{C-H(Aliph.)}$, 2211 $\nu_{C\equiv N}$, 1413, 1495, 1590 $\nu_{C=C}$. ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 3.92 (s, 3H, H⁹), 3.95 (s, 3H, H⁸), 3.97 (s, 3H, H⁷), 6.09 (s, 2H, H¹⁹), 6.81 (d, 1H, H⁴), 6.90 (d, 1H, H¹⁷), 7.16 (s, 1H, H¹⁴), 7.22 (s, 1H, H¹⁰), 7.71 (s, 1H, H⁵), 8.01 (d, 1H, H¹⁸). ¹³C NMR (400 MHz, CDCl₃): δ (ppm) = 56.1 (C⁹), 60.9 (C⁸), 56.1 (C⁷), 61.8 (C⁷), 101.6 (C¹⁹), 105.9 (C¹⁴), 107.4 (C⁴), 108.6 (C¹⁷), 109.6 (C¹¹), 118.62 (C¹²), 120.5 (C⁵), 120.9 (C⁶), 123.2 (C¹⁸), 129.2 (C¹³), 135.2 (C¹⁰), 141.9 (C²), 148.3

(C¹⁶), 148.4 (C¹⁵), 153.0 (C¹), 155.7 (C³). MALDI-MS: m/z calc. 339.34; found: 339.41 [M]⁺. Anal. calcd. for C₁₉H₁₇NO₅: C, 67.25; H, 5.05; N, 4.13. Found: C, 67.03; H, 4.98; N, 3.99 %.

Synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(3,5-bis(trifluoromethyl)phenyl)acrylonitrile (7)

1.06 g (5.4 mmol) 2,3,4-trimethoxybenzaldehyde (**1**), 0.87 g (5.4 mmol) 3,5-bis(trifluoromethyl)benzylcyanide, pale yellow crystalline solid, m.p. 124–125 °C, yield: 88 %. FT-IR (KBr) cm⁻¹ = 3000, 3040 $\nu_{C-H(Ar)}$, 2923, 2950 $\nu_{C-H(Aliph.)}$, 2211 $\nu_{C\equiv N}$, 1413, 1465, 1500, 1578 $\nu_{C=C}$. ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 3.92 (s, 3H, H⁹), 3.99 (s, 3H, H⁸), 4.02 (s, 3H, H⁷), 6.85 (d, 1H, H⁴), 7.90 (s, 1H, H¹⁰), 7.99 (d, 1H, H⁵), 7.22 (s, 1H, H¹⁰), 7.71 (s, 1H, H⁵), 8.09 (s, 3H, H¹⁴, H¹⁶, H¹⁸). ¹³C NMR (400 MHz, CDCl₃): δ (ppm) = 56.2 (C⁹), 60.9 (C⁸), 61.9 (C⁷), 106.7 (C⁴), 107.5 (C¹¹), 117.6 (C¹²), 119.8 (C⁶), 121.6 (C²⁰), 122.1 (C¹⁹), 123.7 (C⁵), 124.3 (C¹⁶), 125.8 (C¹⁴, C¹⁸), 132.4 (C¹⁷), 132.7 (C¹⁵), 137.3 (C¹³), 139.8 (C¹⁰), 141.8 (C²), 153.6 (C¹), 157.1 (C³). MALDI-MS: m/z calc. 431.33; found: 431.48 [M]⁺. Anal. calcd. for C₂₀H₁₅F₆NO₃: C, 55.69; H, 3.51; N, 3.25. Found: C, 55.39; H, 3.31; N, 3.07 %.

Synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(3-(chlorophenyl)acrylonitrile (8)

1.06 g (5.4 mmol) 2,3,4-trimethoxybenzaldehyde (**1**), 0.82 g (5.4 mmol) 3-chlorobenzylcyanide, yellow crystalline solid, m.p. 98–99 °C, yield: 85 %. FT-IR (KBr) cm⁻¹ = 3000, 3054 $\nu_{C-H(Ar)}$, 2829, 2938 $\nu_{C-H(Aliph.)}$, 2211 $\nu_{C\equiv N}$, 1497, 1581, 1596, 1626 $\nu_{C=C}$. ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 3.98 (s, 3H, H⁹), 3.96 (s, 3H, H⁸), 3.92 (s, 3H, H⁷), 6.83 (d, 1H, H⁴), 7.38–7.40 (m, 2H, H¹⁵, H¹⁷), 7.58(d, 1H, H¹⁶), 7.68 (s, 1H, H¹⁴), 7.88 (s, 1H, H¹⁰), 8.07 (d, 1H, H⁵). ¹³C NMR (400 MHz, CDCl₃): δ (ppm) = 61.9 (C⁷), 60.9 (C⁸), 56.1 (C⁹), 106.1 (C¹¹), 107.4 (C⁴), 108.3 (C¹¹), 118.2 (C¹²), 120.4 (C⁶), 123.5 (C⁵), 124.1 (C¹⁸), 125.9 (C¹⁴), 128.7 (C¹⁶), 130.2 (C¹⁷), 135.1 (C¹³), 136.7 (C¹⁵), 141.9 (C²), 143.1 (C²), 153.3 (C¹), 156.3 (C³). MALDI-MS: m/z calc. 329.77; found: 329.35 [M]⁺. Anal. calcd. for C₁₈H₁₆ClNO₃: C, 65.56; H, 4.89; N, 4.25. Found: C, 65.49; H, 4.63; N, 4.11 %.

Synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(4-(chlorophenyl)acrylonitrile (9)

1.06 g (5.4 mmol) 2,3,4-trimethoxybenzaldehyde (**1**), 0.82 g (5.4 mmol) 4-chlorobenzylcyanide, yellow crystalline solid, m.p. 125–126 °C, yield: 78 %. FT-IR (KBr) cm⁻¹ = 3065, 3098 $\nu_{C-H(Ar)}$, 2974, 2939 $\nu_{C-H(Aliph.)}$, 2211 $\nu_{C\equiv N}$, 1501, 1518, 1581, 1609 $\nu_{C=C}$. ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 3.91 (s, 3H, H⁹), 3.96 (s, 3H, H⁸), 3.97 (s, 3H, H⁷), 6.82 (d, 1H, H⁴), 7.41 (d, 2H, H¹⁵), 7.63 (d, 2H, H¹⁴), 7.85 (s, 1H, H¹⁰), 8.05 (d, 1H, H⁵). ¹³C NMR (400 MHz, CDCl₃): δ (ppm) = 61.8 (C⁷), 60.9 (C⁸), 56.1 (C⁹), 107.5 (C⁴), 108.6 (C¹¹), 118.2 (C¹²), 120.6 (C⁶), 123.4 (C⁵), 127.1 (C¹⁵, C¹⁷), 129.2 (C¹⁴, C¹⁸), 133.5 (C¹³), 134.7 (C¹⁶), 136.9 (C¹⁰), 141.9 (C²), 153.3 (C¹), 156.2 (C³). MALDI-MS: m/z calc. 329.77; found:

329.34 [M]⁺. Anal. calcd. for C₁₈H₁₆ClNO₃: C, 65.56; H, 4.89; N, 4.25. Found: C, 65.39; H, 4.69; N, 4.08 %.

In vitro antitumor evaluation

PC-3, MCF-7 and A2780 cell lines were preserved in DMEM (Dulbecco's modified Eagle's medium) culture medium supplemented with 4500 mg/L glucose (10 % heat-inactivated fetal bovine serum, L-glutamine (4 mM), 100 U/mL penicillin–streptomycin) and addition of 10 mM non-essential amino acids for the culture of A2780, PC-3 and MCF-7 cells. The anticancer activities of compounds **2–9** against A2780, MCF-7 and PC-3 cell lines were examined by the MTT assay method, which provides a simple way to detect living and growing cells without using radioactivity. Briefly, 15 × 10³ A2780, PC-3 and MCF-7 cell lines were plated in triplicate in 96-well tissue culture plates, and treated with dimethyl sulfoxide (for negative control or control group) and at 1, 5, 25, 50 and 100 μM concentrations of compounds **2–9** in dimethyl sulfoxide. Then, A2780, PC-3 and MCF-7 cell lines were kept for 24 h at 37 °C in a 5 % CO₂ moistened incubator. After 24 h, MTT (0.005 g/mL in phosphate buffer saline) was added to the cell culture and incubated for 3 h. The formazan crystals formed during the interaction of active mitochondria with MTT were dissolved in 100 mL (0.04 N) isopropyl alcohol and readings were recorded on a microplate reader using a 570-nm filter. The relative cell viability (%) was expressed as a percentage relative to the untreated control cells. Each value represented an average of 10 measurements. All cellular results were obtained against negative control cells [28–31].

Quantitative data are presented as mean ± standard deviation (SD). Normal distribution was confirmed using the Kolmogorov–Smirnov test. Quantitative data were analyzed using the 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. The logIC₅₀ values (the half-maximal effective concentration) were determined by using inhibition % values by the GraphPad Prism 6 program on a computer.

Results and discussion

Chemistry

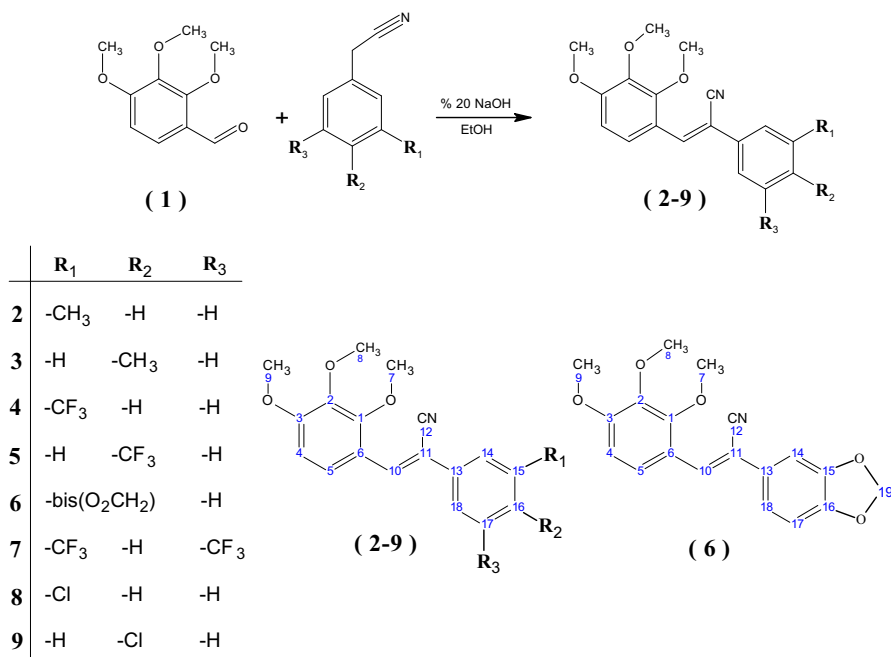
In this work, 2-(2,3,4-trimethoxyphenyl)-1-(substituted-phenyl)acrylonitrile compounds **2–9** were prepared by the interaction of 2,3,4-trimethoxybenzaldehyde (**1**) with substitute benzyl cyanide (3-methylbenzylcyanide, 4-methylbenzylcyanide, 3-(trifluoromethyl)benzylcyanide, 4-(trifluoromethyl)benzylcyanide, 3,4-(methylenedioxy)benzylcyanide, 3,5-bis(trifluoromethyl)benzylcyanide, 3-chlorobenzylcyanide, 4-chlorobenzylcyanide) in the presence of ethyl alcohol and aqueous NaOH at 70 °C [25–27]. The structures of compounds **2–9** have been described by MS, FT-IR, microanalysis, 1D (¹H and ¹³C-APT) and 2D (HETCOR) NMR spectroscopic methods. The locations of characteristic peaks of primary, secondary and tertiary carbon atoms

were determined by using ^{13}C -APT NMR technique. The HETCOR NMR technique was used for determination of $-\text{C}-\text{H}$ carbon atoms. The process of the reactions and structures is shown in Scheme 1.

The differential scanning calorimetry (DSC) spectra of compounds **2–9** showed a single melting point (Fig. 1). Additionally, the molecular ion peaks of compounds **2–9** were obtained by MALDI TOF-MS analysis. The mass analyses of phenylacrylonitriles were determined by the MALDI TOF-MS technique. The molecular ion peak of each compound is given in the “Experimental” section. As an example, the mass spectrum of compound **6** is given in Fig. 2.

The characteristic peaks in the 1D NMR and FT-IR spectra of **2–9** are given in the “Experimental” section. The aldehyde carbonyl stretching vibrations were not observed in the FT-IR spectra of **2–9**. The $-\text{C}\equiv\text{N}$ peaks were shown between 2209 and 2214 cm^{-1} and aliphatic $-\text{C}=\text{C}$ stretching vibrations were observed in the range from 1578 to 1626 cm^{-1} .

The aldehyde carbonyl protons were not observed in the ^1H -NMR spectrum of phenyl acrylonitrile derivatives **2–9**. The ratio of the protons integral height in the spectra of the compounds **2–9** supports the proposed structures. The methoxy protons for compounds **2–9** (7, 8 and 9 numbered protons in the Scheme 1) were observed in the range from 3.92 to 4.02 ppm. The methyl protons for **2** and **3** in the ^1H -NMR spectra were shown at 2.45 and 2.42, respectively. The methylene protons and carbon peak at HETCOR NMR spectrum of **6** were observed at 6.09 and 101.61 ppm, respectively. The $-\text{C}\equiv\text{N}$ carbon peaks for compounds **2–9** were



Scheme 1 General presentations of compounds **2–9**

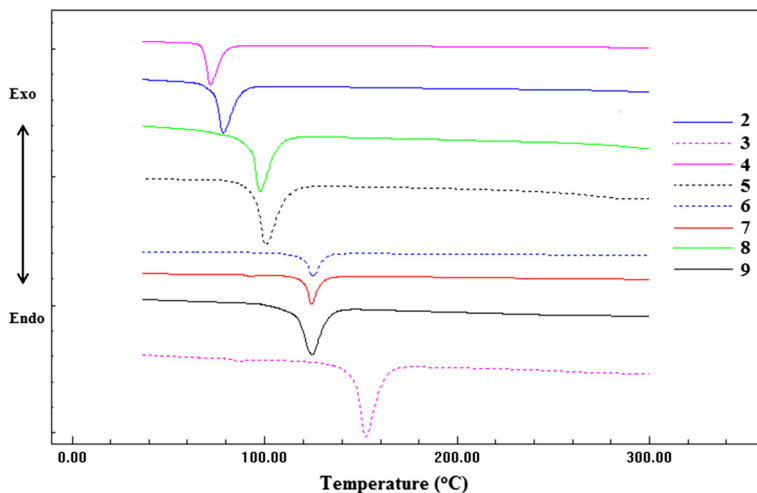


Fig. 1 The comparative melting points of compounds 2–9. These melting points were obtained by differential scanning calorimetry using a SHIMADZU DSC thermo balance (10 °C/min)

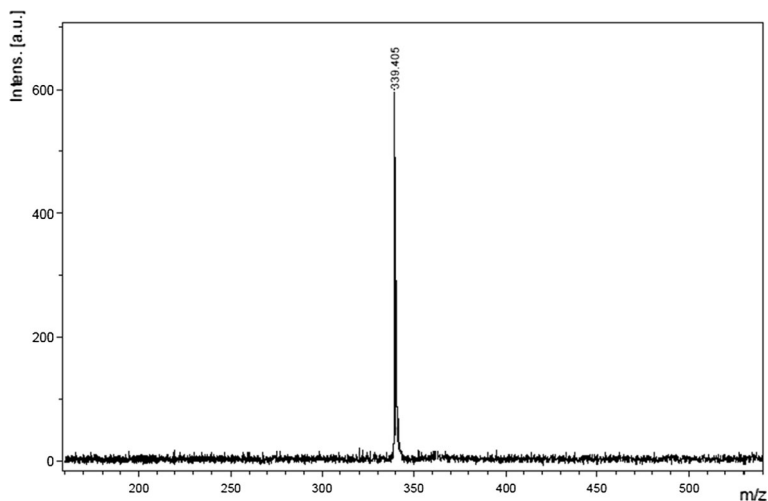


Fig. 2 MALDI TOF-MS spectrum of compound 6

observed between 117.6 and 118.6 ppm. The ^1H , ^{13}C and HETCOR NMR spectra of **6** isaredepicted in Figs. 3, 4 and 5, respectively.

In vitro antitumor activity

The anticancer properties of 2-(2,3,4-trimethoxyphenyl)-1-(substituted-phenyl)acrylonitrile derivatives were assessed in vitro using human prostate cancer cells (PC-3),

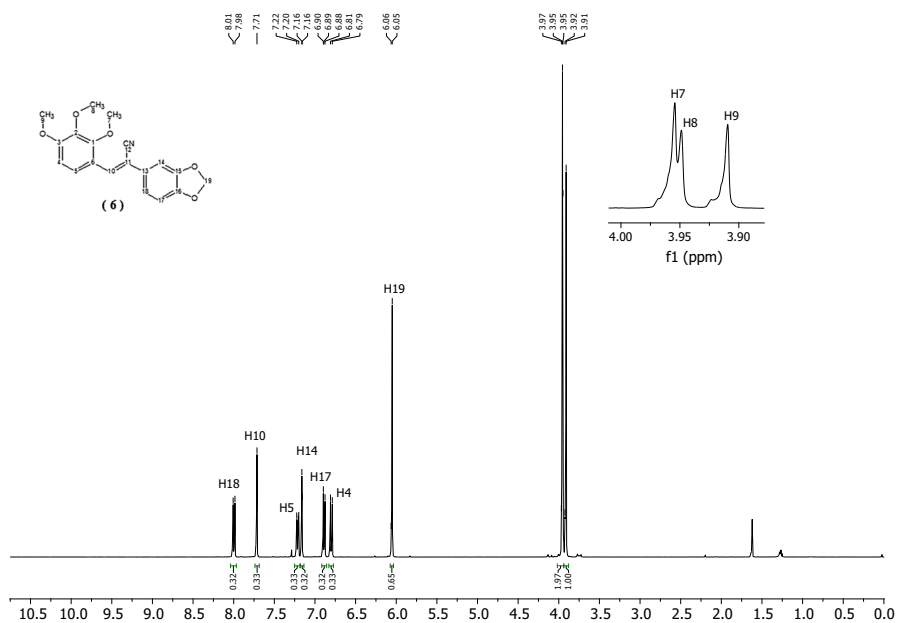


Fig. 3 $^1\text{H-NMR}$ spectrum of compound **6**. Compound **6** dissolved in deuterated chloroform (chloroform-d) and obtained by using a Bruker (USA) DPX-400 spectrometer

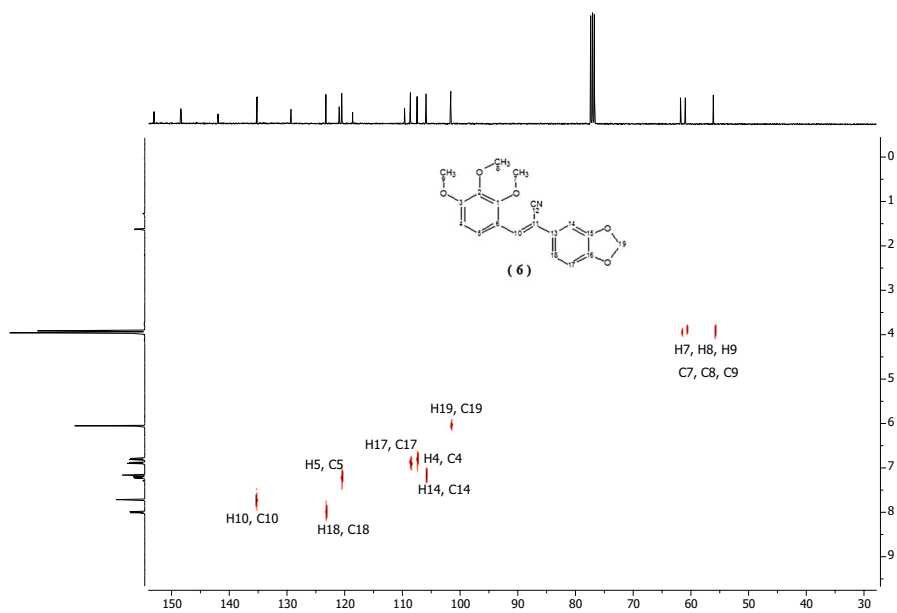


Fig. 4 HETCOR (2D, $^1\text{H-}^{13}\text{C}$ coupling) NMR spectrum of compound **6**. Compound **6** dissolved in deuterated chloroform (chloroform-d) and obtained by using a Bruker (USA) DPX-400 spectrometer

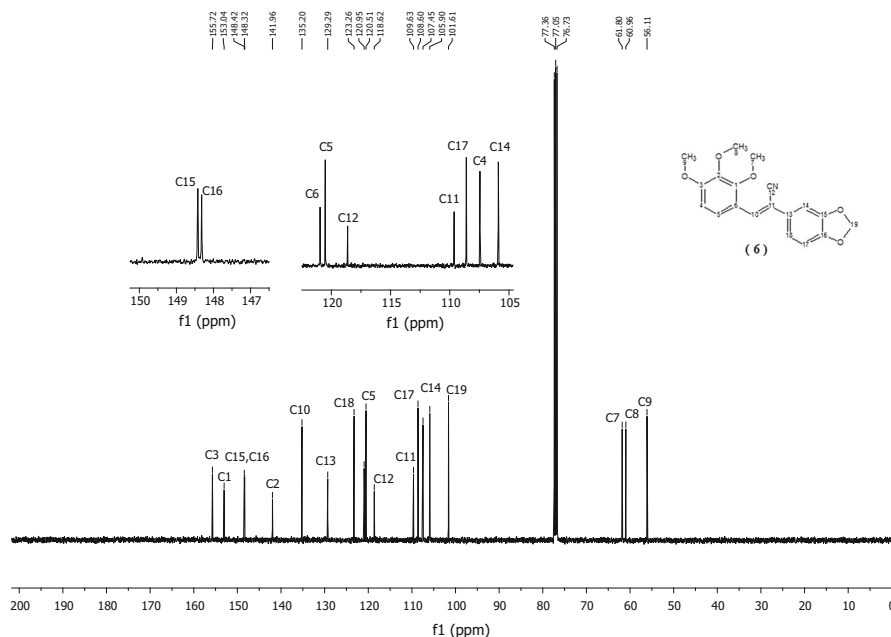


Fig. 5 ^{13}C -NMR spectrum of compound **6**. Compound **6** dissolved in deuterated chloroform (chloroform- d) and obtained by using a Bruker (USA) DPX-400 spectrometer

human ovarian cancer cells (A2780) and human breast cancer cells (MCF-7) at 1, 5, 25, 50 and 100 μM doses. Figure 6 shows the effects of compounds **2–9** on cell viability measured at 24 h after exposure.

In the MCF-7 cell lines, compounds **2**, **3**, **4**, **8** and **9** significantly reduced % cell viability comparative to the control ($p < 0.05$). This decrease usually showed at the highest concentration tested (100 μM , $p < 0.05$). When the structure activities of the compounds **2–9** were investigated, the *meta* substituted compounds **2**, **4** and **8** against MCF-7 cell lines were generally observed to be more active than the others. Only the *para* substituted compounds **3** and **5** containing chloride and methyl groups showed a similar effect to the *meta* substituted compounds. The calculated logIC_{50} values for compounds are given in Table 1.

All the compounds **2–9** showed reduced % cell viability and were dose-dependent ($p < 0.05$) towards A2780 cell lines ($p < 0.05$). All doses (1, 5, 25, 50 and 100 μM) of the compound **2** have good anticancer activity ($p < 0.01$). At 5, 25, 50 and 100 μM doses of **6**, **7** and **8** were found to be effective against A2780 cell lines ($p < 0.05$). When the structure activities of **2–9** were investigated, all the compounds against A2780 cell lines were observed to be quite active. As a result, compounds **2–9** exhibit significantly improved anticancer activity when compared to the phenylacrylonitrile compounds given in the literature [10, 32–34].

Compound **4** did not show anticancer activity against PC-3 cell lines. At 25, 50 and 100 μM doses of the compounds **2**, **3**, **6** and **7** usually showed the highest anticancer activity ($p < 0.05$). Compound **5** only showed anticancer activity at the

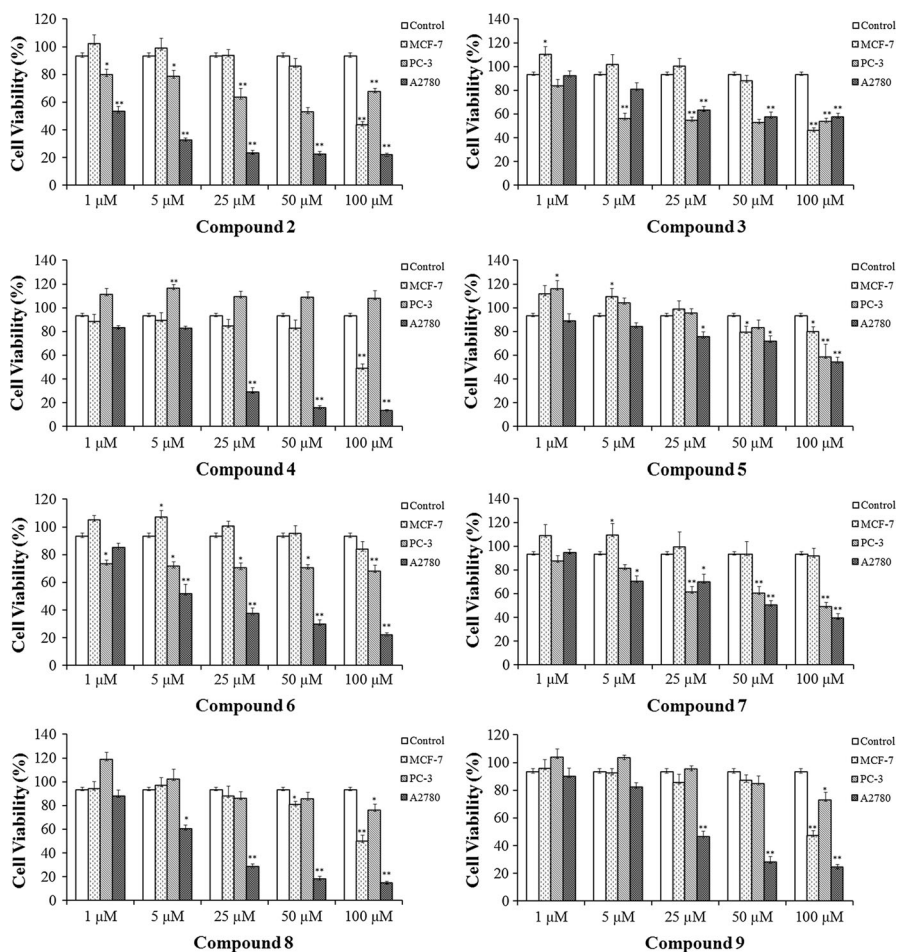


Fig. 6 The relative cell viability (%) of MCF-7, A2780 and PC-3 cells after a 24-h treatment with all the compounds 2–9. The changes on the cell viability (%) caused by compounds 2–9 are compared with the control data. Each data point is an average of 10 viabilities (**p* < 0.01)

Table 1 Evaluation of the cytotoxicity and LogIC₅₀ values (μM), of phenylacrylonitrile analogues 2–9 against a panel of three cancer cell lines

LogIC₅₀ is the half-maximal effective concentration of a drug that reduces cell growth by 50 %

NC not converged

Compound	MCF-7 LogIC ₅₀ (μM)	PC-3 LogIC ₅₀ (μM)	A2780 LogIC ₅₀ (μM)
2	2.15	1.91	0.29
3	2.21	1.68	1.87
4	2.11	NC	1.1
5	2.54	2.28	2.02
6	2.82	2.09	1.05
7	3.02	1.81	1.70
8	2.13	2.46	0.96
9	2.14	2.46	1.35

highest concentration tested (100 μM , $p < 0.01$). When the structure activities of these compounds **2–9** were investigated, all the compounds we generally showed anticancer activity similar to each other on PC-3 cell lines. The changes of the functional groups (methyl, chlorine, etc.) in the structures of **2–9** did not change the anticancer activities on PC-3 cells.

Conclusions

In conclusion, in the present study, we successfully reported the synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(substituted-phenyl)acrylonitrile derivatives **2–9**, and their anticancer properties against three different human cancer cell lines (A2780, PC-3 and MCF-7) were determined by using MTT assay. The structural characterizations of compounds **2–9** were performed by FT-IR, elemental analysis, melting point, mass, 1D and 2D NMR techniques. The results demonstrate that these compounds have anticancer activity against three different human cancer cell lines. Our results showed that A2780 cell lines are more effective than PC-3 and MCF-7 cell lines. Overall, compounds **2–9** may be candidates for anticancer drug development in the future.

Acknowledgments This research was supported financially by The Scientific and Technological Research Council of Turkey (TUBITAK) (Project Number: 110T652). The authors are grateful to the Research Fund of the TUBITAK for their support.

References

1. E.E. Gudal, I. Durmaz, R.C. Atalay, M. Yarim, J. Enzyme Inhib. Med. Chem. **30**, 649–654 (2015)
2. V.T. Kamble, A.S. Sawant, S.S. Sawant, P.M. Pisal, R.N. Gacche, S.S. Kamble, V.A. Kamble, Arc. Pharm. Chem. Life Sci. **348**, 338–346 (2015)
3. G. Michelle, Nature **485**, S49 (2012)
4. M. Amy, Nature **485**, S50–S51 (2012)
5. R. Airley, *Cancer Chemotherapy* (Wiley, USA, 2009)
6. M.A. Dicato, *Side Effects of Medical Cancer Therapy* (Springer, New York, 2013)
7. F. Saczewski, A. Stencel, A.M. Bienczak, K.A. Langowska, M. Michaelis, W. Werel, R. Halasa, P. Reszka, P.J. Bednarski, Eur. J. Med. Chem. **43**, 1847–1857 (2008)
8. F. Saczewski, P. Reszka, M. Gdaniec, R. Grünert, P.J. Bednarski, J. Med. Chem. **47**, 3438–3449 (2004)
9. M. Tarleton, L. Dyson, J. Gilbert, J.A. Sakoff, A. McCluskey, Bioorg. Med. Chem. **21**, 333–347 (2013)
10. M. Tarleton, J. Gilbert, M.J. Robertson, A. McCluskey, J.A. Sakoff, Med. Chem. Commun. **2**, 31–37 (2011)
11. M.H. Sherif, A.M. Yossef, Res. Chem. Intermed. **41**, 383–390 (2015)
12. M.A. Musa, V.L.D. Badisa, L.M. Latinwo, J. Cooperwood, A. Sinclair, A. Abdullah, Anticancer Res. **31**, 2017–2022 (2011)
13. K.H. Yokohama, A.Y. Chigasaki, H.H. Isehara, S.M. Zama, H.M. Yokohama, United States Patent, 5574062 (1996)
14. Y. Tumer, N. Asmafiliz, Z. Kılıç, T. Hokelek, L.Y. Koc, L. Acık, M.L. Yola, A. Solak, O.Y. Oner, D. Dundar, M. Yavuz, J. Mol. Struct. **1049**, 112–124 (2013)
15. A.O. Görgülü, K. Koran, F. Özen, S. Tekin, S. Sandal, J. Mol. Struct. **1087**, 1–10 (2015)
16. H. Akbas, A. Okumus, Z. Kılıç, T. Hokelek, Y. Süzen, L.Y. Koç, L. Açık, Z.B. Çelik, Eur. J. Med. Chem. **70**, 294–307 (2013)

17. A. Kamal, G. Ramakrishna, P. Raju, A. Viswanath, M.J. Ramaiah, G. Balakishan, P. Bhadra, *Bioorg. Med. Chem. Lett.* **20**, 4865–4869 (2010)
18. C. Jin, Y.J. Liang, H. He, L. Fu, *Biomed. Pharmacother.* **67**, 215–217 (2013)
19. M.F. Mohamed, M.S. Mohamed, S.A. Shouman, M.M. Fathi, I.A. Abdelhamid, *Appl. Biochem. Biotechnol.* **168**, 1153–1162 (2012)
20. M.D. Altintop, A. Özdemir, Z.A. Kaplancikli, G.T. Zitouni, H.E. Temel, G.A. Çiftçi, *Arc. Pharm. Chem. Life Sci.* **346**, 189–199 (2013)
21. B.K. Kaymakçioğlu, N. Beyhan, N. Tabanca, A. Abbas, D.E. Wedge, S.O. Duke, U.R. Bernier, I.A. Khan, *Med. Chem. Res.* **24**, 3632–3644 (2015)
22. W.H. Mahmoud, N.F. Mahmoud, G.G. Mohamed, A.Z. El-Sonbati, A.A. El-Bindary, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **150**, 451–460 (2015)
23. J. Zhao, Y. Guo, J. Hu, H. Yu, S. Zhi, J. Zhang, *Polyhedron* **102**, 163–172 (2015)
24. A.H. Pathan, A.K. Ramesh, R.P. Bakale, G.N. Naik, H.G.R. Kumar, C.S. Frampton, G.M.A. Rao, K.B. Gudasi, *Inorg. Chim. Acta* **430**, 216–224 (2015)
25. I. Basaran, S. Sinan, U. Cakir, M. Bulut, O. Arslan, O. Ozensoy, *J. Enzyme Inhib. Med. Chem.* **23**, 32–36 (2008)
26. N.P. Buu-Hoi, G. Saint-Ruf, B. Lobert, *J. Chem. Soc. C* **16**, 2069–2070 (1969)
27. N.P. Buu-Hoi, B. Ekert, R. Royer, *J. Org. Chem.* **19**, 1548–1552 (1954)
28. T.R. Mosamann, H. Cherwinski, M.V. Bond, M.A. Giedlin, R.L. Coffmann, *J. Immunol.* **136**, 2348–2357 (1986)
29. N.K. Singh, S.B. Singh, *Synth. React. Inorg. Met. Org. Chem.* **32**, 25–47 (2002)
30. S. Tekin, S. Sandal, C. Colak, *Med. Sci.* **3**, 1427–1441 (2014)
31. B. Yilmaz, S. Sandal, C.H. Chen, D.O. Carpenter, *Toxicology* **217**, 184–193 (2006)
32. M. Tarleton, J. Gilbert, J.A. Sakoff, A. McCluskey, *Eur. J. Med. Chem.* **57**, 65–73 (2012)
33. A. Carta, M. Palomba, G. Boatto, B. Busonera, M. Murreddu, R. Loddo, *II Farmaco* **59**, 637–644 (2004)
34. A. Carta, P. Sanna, M. Palomba, L. Vargiu, M. Colla, L.R. Loddo, *Eur. J. Med. Chem.* **37**, 891–900 (2002)