Medicinal Chemistry Research (2019) 28:2067–2078
https://doi.org/10.1007/s00044-019-02436-z

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel α-aminophosphonates under microwave irradiation, biological evaluation as antiproliferative agents and apoptosis inducers

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Received: 13 July 2019 / Accepted: 29 August 2019 / Published online: 10 September 2019

Abstract

The synthesis of two series of α-aminophosphonates was achieved by Microwave Irradiation (MW), using the one-pot Kabachnik–Fields reaction. Based on a green chemistry approach, the reactions were carried out using ethanol as the only solvent and without any catalyst, and short reaction times (20–40 min), in variable yields. Both series were tested to determine their cell proliferation inhibition activity in MDA-MB-231, MCF-7 and MCF-10A cell lines. Ethyl 4-(((diphenoxyphosphoryl)(4-(diphenylamino)phenyl)methyl)amino) benzoate **4e** and diphenyl (((4-(((S)-2-hydroxy-1-phenylethyl)carbamoyl)phenyl)amino)(4-hydroxyphenyl)methyl)phosphonate **6b**, showed cell proliferation inhibition activity only in the cancer cell line MCF-7 and no effect on the normal cell line MFC-10A, both compounds caused cell death by inducing apoptosis.

Keywords Aminophosphonates · Green synthesis · Kabachnik–Fields · Cell proliferation inhibition activity · Apoptosis

Introduction

Cancer is considered the second leading cause of mortality worldwide, World Health Organization reported ~14 million new cancer cases, among those, 8.8 million cancer patients died, the number of cases is expected to rise from 14 to 22 million at the year 2030 (Deshmukh et al. 2018). On the other hand, α-aminophosphonates have shown varied biological activities such as enzyme inhibitors, potent antibiotics, antivirals, antifungal agents, and potent antitumor agents (Rezaei et al. 2009, 2011; Mungara et al. 2012; Kraicheva et al. 2012; Abdel-Megeed et al. 2012; Venkata Ramana et al. 2012; Maddina et al. 2014; Subba Reddy et al. 2014; Hudson and Lee 2014; Li et al. 2015; Mirzaei et al. 2015; Gundluru et al. 2016; Fang et al. 2016; Reddy et al. 2016).

Due to the high number of cancer cases and the versatile biological activity of α-aminophosphonates in medicinal chemistry; the synthesis and application of these compounds have attracted considerable interest (Bálint et al. 2018). Among chemotherapy agents, there are not many options of α-aminophosphonates derivatives but fotemustine, which is a chlorethyl nitrosourea derivative, with antitumor activity in disseminated melanoma and primary brain tumors. Fotemustine has been recently used in some trials, for instance a phase II trial showed its activity in central nervous system lymphoma (CNSL) treatment while administrated with teniposide and dexamethasone (Wu et al. 2018). In a phase I/II trial, fotemustine showed efficacy in

Supplementary information The online version of this article (https://doi.org/10.1007/s00044-019-02436-z) contains supplementary material, which is available to authorized users.

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Glioblastoma multiforme (GBM) (Marinelli et al. 2018), and this drug also has shown an effect in four glioblastoma cell lines (A172, T98G, R1, and T2) producing apoptosis (Kiseleva et al. 2018).

In addition, the development of improved methods to synthesize new bioactive α-aminophosphonates has attracted considerable attention. Several multistep synthetic approaches for the synthesis of α-aminophosphonates have been reported in the literature, including the alkylation of nucleophilic Schiff bases, the nucleophilic addition of phosphites to imines (Mirzaei et al. 2015), the conversion of 1-hydroxyphosphonates to the corresponding α-aminophosphonates (Mirzaei et al. 2015; Rádai et al. 2016) and ultrasound three-component coupling reactions (Xia and Lu 2007). Among these methods, the Kabachnik–Fields reaction which involves a one-pot three-component coupling of aldehydes (or ketones), amines, and either a dialkyl or diarylphosphite; it is a useful alternative for the synthesis of this type of carbon–phosphorus bonds (Keglevich and Bálint 2012; Sampath et al. 2016). Considering microwave (MW) irradiation as one of the most effective methods in Organic synthesis compared with conventional heating (hot plates), the one-pot Kabachnik–Fields under MW irradiation has been considered as a convenient method for the synthesis of α-aminophosphonates (Venkata Ramana et al. 2012).

The Kabachnik–Fields reaction method has been carried out in one-pot procedures using different catalysts such as $BiCl_3$, $TaCl_5$, $InCl_3$, $SbCl_3$ (Sampath et al. 2016), phosphosulfonic acid (Gundluru et al. 2016), Amberyst-15 (Subba Reddy et al. 2014), $FeCl_3$, $CuCl_2$ (Rezaei et al. 2011), $LiClO_4$ (Kenawy et al. 2015), $Mg(ClO_4)_2$ (Bhagat and Chakraborti 2007), TiO_2 (Hosseini-Sarvari 2008), VCl_3, $TiCl_4$, and $ZrOCl_2.8H_2O$; however, most of these catalysts are toxic and are regarded as pollutants, not to mention the use of environmentally unfriendly organic solvents (Mirzaei et al. 2015).

In consequence of the recent concerns related to the disposal of toxic reagents and solvents, it is essential to develop efficient and eco-friendly methods for the synthesis of bioactive α-aminophosphonates using green chemistry methods (Sampath et al. 2016; Tiwari et al. 2018). Also, there are only a few examples that have been reported using the synthetic method of MW-assisted and catalyst-free Kabachnik–Fields condensations (Bálint et al. 2018).

In this study, we report the synthesis of two series of new α-aminophosphonates (ester and amide derivatives) using the one-pot three-component Kabachnik–Fields reaction by MW irradiation and a green chemistry approach. The synthesis of ester derivatives was achieved with noticeable results; compounds were characterized by ^{1}H, ^{13}C, ^{31}P NMR, and high-resolution mass spectrometry. The structures of the derivatives have been proposed based on their potential application as anticancer agents due to the versatile biological activity of α-aminophosphonates in medicinal chemistry. Compounds **4e** and **6c** showed cell proliferation inhibition activity only in the cancer cell line MCF-7 and no cytotoxic effect on the normal cell line MFC-10A; furthermore, both compounds caused cell death by inducing apoptosis.

Materials and methods

Chemistry

All used reactants were high purity grade (Sigma-Aldrich) and were used without further purification. Flash purification was carried out with Isolera Biotage LC (cartridge SNAP 10 g, detection mode UV1 254 nm + UV2 280 nm). The ^{1}H NMR spectra were recorded on Varian-NMR System at 400 MHz in $CDCl_3$ or DMSO-D_6, ^{13}C NMR 101 MHz, and ^{31}P NMR 162 MHz and on a Bruker Ascend 600 system, 600 MHz in DMSO-D_6, ^{13}C NMR 151 MHz, and ^{31}P NMR 243 MHz, with TMS as internal standard (chemical shifts are expressed in ppm) and coupling constant (J) is reported in Hertz (Hz); Mass spectrometric measurements were performed on an HPLC-MS Agilent technologies 1260 infinity instrument.

General procedure for the synthesis of α-aminophosphonates ester derivatives (4a–4i)

The corresponding aldehyde (**1**) (1 eq), ethyl 4-aminobenzoate (**2**) (1 eq), and diphenylphosphite (**3**) (1.5 eq) were dissolved in ethanol (3 mL). The reaction mixture was exposed to microwave irradiation using a microwave synthesis reactor Monowave 300 Anton-Paar, at the corresponding temperature (60, 80, or 90 °C) for 20, 30, or 40 min as it corresponds, the progress of the reaction was monitored by TLC. The solvent was evaporated, the crude material was purified by column chromatography using silica gel 60 (230–400 mesh) as the stationary phase and different ratios of hexane–ethyl acetate were used as mobile phase system.

Ethyl 4-(((4-chlorophenyl)(diphenoxyphosphoryl)methyl) amino) benzoate, 4a

(1.52 g, 82%); white solid; mp 116–118 °C; $R_f = 0.49$ (7:3 hexane–ethyl acetate). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.68–7.79 (m, 4H, ArH, H-8), 7.67 (dd, $J = 5.3$, 10.3 Hz, 1H, NH), 7.49 (d, $J = 8.3$, 2H, ArH, H-6), 7.21 (m, 10 H, ArH, H-5), 6.95 (d, $J = 8.7$ Hz, 2H, ArH, H-4), 5.88 (dd,

$J = 10.2$, 25.4 Hz, 1H, CH-P), 4.21 (q, $J = 7.0$ Hz, 2H, CH_2-CH_3), and 1.26 (t, $J = 7.0$ Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.1 (C=O), 151.5 (C-2), 150.4 (C-25), 150.2 (C-31), 134.7 (C-13), 133.3 (C-20), 131.1 (C-4, C-6), 130.9 (C-19, C-21), 130.3 (C-27, C-29, C-33, C-35), 128.9 (C-18, C-22), 125.8 (C-28, C-34), 120.9 (C-26, C-30), 120.7 (C-32, C-36), 118.7 (C-5), 113.2 (C-1, C-3), 60.2 (C-10), 53.6 (d, $J = 156.3$ Hz, C-P), and 14.8 (C-11). ^{31}P NMR (162 MHz, DMSO-d_6): δ 15.24 (d, $J = 25.1$ Hz). HRMS (ESI$^+$) *m/z* calcd for $C_{28}H_{26}ClNO_5P$ $[M + H]^+$ 522.1159; found 522.1094.

Ethyl 4-(((diphenoxyphosphoryl)(4-hydroxyphenyl)methyl) amino) benzoate, 4b

(1.50 g, 72%); dark orange solid; mp 154–156 °C; $R_f = 0.52$ (1:1 hexane–ethyl acetate). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.57 (s, 1H, OH), 7.71 (d, $J = 9.0$ Hz, 2H, ArH, H-7), 7.56 (d, $J = 4.6$ Hz, 1H, NH), 6.85–7.60 (m, 14H, ArH, H-5), 6.80 (d, $J = 8.8$ Hz, 2H, ArH, H-4), 5.64 (dd, $J = 10.0$, 24.1 Hz, 1H, CH-P), 4.21 (q, $J = 7.0$ Hz, 2H, CH_2-CH_3), and 1.26 (t, $J = 7.1$ Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.2 (C=O), 157.8 (C-20), 151.8 (C-2), 150.5 (C-26), 150.4 (C-32), 131.1 (C-4, C-6), 130.4 (C-13), 130.3 (C-28, C-30) 130.2(C-34, C-36), 125.7 (C-18) 125.6 (C-22), 125.3 (C-29, C-35), 121.0 (C-27, C-31), 120.8 (C-33, C-37), 118.3 (C-5), 115.6 (C-19, C-21), 113.1 (C-1, C-3), 60.2 (C-10), 53.8 (d, $J = 157.6$ Hz, C-P), and 14.8 (C-11). ^{31}P NMR (162 MHz, DMSO-d_6): δ 16.30 (d, $J =$ 24.0 Hz). HRMS (ESI$^+$) *m/z*, calcd for $C_{28}H_{27}NO_6P$ $[M + H]^+$ 504.1498; found 504.1485.

Ethyl 4-(((diphenoxyphosphoryl)(4-methoxyphenyl)methyl) amino)benzoate, 4c

(1.66 g, 87%); white solid; mp 147–149 °C; $R_f = 0.31$ (7:3 hexane–ethyl acetate). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.71 (d, $J = 8.6$ Hz, 2H, ArH, H-9) 7.65 (d, $J = 8.6$ Hz, 2H, ArH, H-8), 7.59 (dd, $J = 4.9$, 10.2 Hz, 1H, NH), 6.99–7.39 (m, 10H, ArH, H-7), 6.97 (d, $J = 8.5$ Hz, 2H, ArH, H-6), 6.91 (d, $J = 8.1$ Hz, 2H, ArH, H-5), 5.72 (dd, $J = 10.1$, 24.4 Hz, 1H, CH-P), 4.21 (q, $J = 7.0$ Hz, 2H, CH_2), 3.74 (s, 3H, OCH_3), and 1.26 (t, $J = 7.1$ Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.2 (C=O), 159.6 (C-20), 151.7 (C-2), 150.5 (C-24), 150.3 (C-30), 131.1 (C-13), 130.3 (C-4, C-6), 130.2 (C-26, C-28, C-32 C-34), 127.2 (C-18, C-22), 125.7 (C-27, C-33), 121.0 (C-25, C-29), 120.8 (C-31, C-35), 118.4 (C-5), 114.3 (C-19, C-21), 113.1 (C-1, C-3), 60.2 (C-10), 55.6 (C-37), 53.7 (d, $J = 157.2$ Hz, C-P), and 14.8 (C-11). ^{31}P NMR (162 MHz, DMSO-d_6): δ 16.08 (d, $J = 24.2$ Hz). HRMS (ESI$^+$) *m/z*, calcd for $C_{29}H_{29}NO_6P$ $[M + H]^+$ 518.1654; found 518.1636.

Ethyl 4-(((diphenoxyphosphoryl)(phenyl)methyl)amino) benzoate, 4d

(1.71 g, 92%); white solid; mp 142–143 °C; $R_f = 0.42$ (7:3 hexane–ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (2H, d, $J = 8.7$ Hz, ArH, H-6), 6.71–7.64 (15H, m, ArH, H-5), 6.60 (2H, d, $J = 8.8$ Hz, ArH, H-4), 5.20 (1H, dd, $J =$ 5.2, 24.7 Hz, CH-P), 4.29 (2H, q, $J = 7.1$ Hz, CH_2), and 1.32 (3H, t, $J = 7.1$ Hz, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.6 (C=O), 150.2 (C-2), 150.1 (C-24), 149.8 (C-30), 134.2 (C-13), 131.4 (C-4, C,6), 129.8 (C-26, C-28), 129.7 (C-32, C-34), 128.9 (C-19, C-21), 128.6 (C-20), 128.2 (C-18, C-22), 125.6 (C-27), 125.4 (C-33), 120.6 (C-25, C-29), 120.4 (C-5), 120.2 (C-31, C-35), 112.8 (C-1, C-3), 60.3 (C-10), 55.4 (d, $J = 154.5$ Hz, C-P), and 14.4 (C-11). ^{31}P NMR (162 MHz, $CDCl_3$): δ 14.58 (d, $J =$ 24.3 Hz). HRMS (ESI$^+$) *m/z*, calcd for $C_{28}H_{27}NO_5P$ $[M + H]^+$ 488.1549; found 488.1553.

Ethyl 4-(((diphenoxyphosphoryl)(4-(diphenylamino)phenyl) methyl)amino) benzoate, 4e

(0.70 g, 58%); brown solid; mp 144–146 °C; $R_f = 0.50$ (7:3 hexane–ethyl acetate). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.71 (d, $J = 8.8$ Hz, 2H, ArH, H-7), 7.65 (d, $J = 4.6$ Hz, 1H, NH), 6.92–7.65 (m, 24H, ArH, H-5), 6.86 (d, $J = 8.2$ Hz, 2H, ArH, H-4), 5.74 (dd, $J = 10.1$, 24.4 Hz, 1H, CH-P), 4.21 (q, $J = 7.1$ Hz, 2H, CH_2), and 1.27 (t, $J = 7.1$ Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.2 (C=O), 151.7 (C-2), 150.5 (C-20), 150.4 (C-25), 147.7 (C-31), 147.5 (C-37, C-38), 131.2 (C-4, C-6), 130.3 (C-13), 130.2 (C-27, C-29, C-33, C-35), 130.0 (C-40, C-42, C-45, C-47), 129.2 (C-39, C-43, C-44, C-48), 125.8 (C-18, C-22, C-41, C-46), 124.4 (C-28, C-34), 123.6 (C-26, C-30), 121.0 (C-32, C-36), 120.6 (C-5), 118.4 (C-1, C-3), 113.0 (C-19, C-21), 60.2 (C-10), 53.8 (d, $J = 156.0$ Hz, C-P), and 14.8 (C-11). ^{31}P NMR (162 MHz, DMSO-d_6): δ 15.92 (d, $J =$ 24.5 Hz). HRMS (ESI$^+$) *m/z*, calcd for $C_{40}H_{34}N_2O_5P$ $[M - H]^+$ 653.2205; found 653.2128.

Ethyl 4-(((diphenoxyphosphoryl)(4-morpholinophenyl) methyl) amino)benzoate, 4f

(0.89 g, 59%); dark brown solid; mp 98–100 °C; $R_f = 0.59$ (4:6 hexane–ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.83 (dd, $J = 1.4$, 8.8 Hz, 2H, ArH, H-9), 7.03–7.48 (m, 10 H, ArH, H-8), 6.83–6.90 (m, 4H, ArH, H-7), 6.65 (dd, $J = 1.4$, 8.8 Hz, 2H, ArH, H-6), 5.14 (d, $J = 24.2$ Hz, 1H, CH-P), 4.29 (q, $J = 7.1$ Hz, 2H, CH_2), 3.85 (t, $J = 4.8$ Hz, 4H, CH_2-CH_2-O), 3.14 (dd, $J = 3.7$, 6.1 Hz, 4H, CH_2-CH_2-N), and 1.34 (t, $J = 7.1$ Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$, MeOD): δ 166.8 (C=O), 150.1 (C-2), 150.0 (C-25, C-31), 149.9 (C-20), 131.4 (C-4, C-6), 129.8 (C-27, C-29),

129.6 (C-33, C-35), 129.0 (C-13), 125.5 (C-18, C-22), 125.4 (C-28, C-34), 120.6 (C-26, C-30), 120.3 (C-32, C-36), 120.1 (C-5), 115.8 (C-1, C-3), 112.8 (C-19, C-21), 66.7 (C-39, C-40), 60.4 (C-10), 54.6 (d, $J = 156.4$ Hz, C-P), 49.0 (C-37), 47.2 (C-38), and 14.3 (C-11). ^{31}P NMR (243 MHz, $CDCl_3$): δ 14.96 (d, $J = 23.9$ Hz). HRMS (ESI^+) *m/z*, calcd for $C_{32}H_{34}N_2O_6P$ $[M + H]^+$ 573.2076; found 573.1992.

Ethyl 4-(((diphenoxyphosphoryl)(1-methyl-1H-pyrazol-4-yl) methyl)amino) benzoate, 4g

(0.89 g, 97%); pale-yellow solid; mp 122–124 °C; $R_f = 0.45$ (3:7 hexane–ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (d, $J = 8.7$ Hz, 2H, ArH, H-9), 7.60 (s, 1H, ArH, H-8), 7.46–7.51 (m, 1H, ArH, H-7), 6.95–7.32 (m, 10H, ArH, H-6), 6.66 (d, $J = 8.8$ Hz, 2H, ArH, H-5), 5.25 (d, $J = 22.1$ Hz, 1H, CH-P), 4.31 (q, $J = 7.1$ Hz, 2H, CH_2), 3.74 (s, 3H, CH_3-N), and 1.35 (t, $J = 7.1$ Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.6 (C=O), 150.2 (C-2), 150.1 (C-19), 149.7 (C-25), 138.7 (C-34), 131.5 (C-4, C-6), 129.9 (C-21, C-23), 129.8 (C-27, C-29), 129.5 (C-31), 125.5 (C-22, C-28), 120.6 (C-20, C-24), 120.5 (C-13), 120.4 (C-26, C-30), 114.9 (C-5), 112.7 (C-1, C-3), 60.4 (C-10), 47.0 (d, $J = 163.0$ Hz, C-P), 39.1 (C-35), and 14.4 (C-11). ^{31}P NMR (243 MHz, $CDCl_3$): δ 14.46 (d, $J = 21.9$ Hz). HRMS (ESI^+) *m/z*, calcd for $C_{26}H_{27}N_3O_5P$ $[M + H]^+$ 492.1610; found 492.1612.

Ethyl 4-((1-(diphenoxyphosphoryl)-2-methylpropyl)amino) benzoate, 4h

(2.32 g, 74%); white solid; mp 103–105 °C; $R_f = 0.39$ (7:3 hexane–ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, $J = 8.7$ Hz, 2H, ArH, H-9), 6.84–7.38 (m, 10 H, ArH, H-8), 6.65 (d, $J = 8.7$ Hz, 2H, ArH, H-7), 4.64 (dd, $J = 3.5$, 10.7 Hz, 1H, NH), 4.33 (q, $J = 7.1$ Hz, 2H, CH_2), 4.13 (ddd, $J = 4.2$, 10.7, 19.0 Hz, 1H, CH-P), 2.44–2.56 (m, 1H, CH–CH), 1.37 (t, $J = 7.1$ Hz, 3H, CH_3-CH_2), and 1.16 (dd, $J = 6.8$, 12.5 Hz, 6H, CH_3-CH). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.7 (C=O), 151.1 (C-2), 150.2 (C-19), 150.0 (C-25), 131.6 (C-4, C-6), 129.9 (C-21, C-23), 129.6 (C-27, C-29), 125.4 (C-22), 125.2 (C-28), 120.5 (C-20, C-24), 120.4 (C-26, C-30), 119.8 (C-5), 112.2 (C-1, C-3), 60.4 (C-10), 55.6 (d, J = 151.9 Hz, C-P), 30.1 (C-13), 20.7 (C-31), 17.9 (C-32), and 14.5 (C-11). ^{31}P NMR (162 MHz, $CDCl_3$): δ 17.54 (dd, $J = 19.2$, 8.3 Hz). HRMS (ESI^+) *m/z*, calcd for $C_{25}H_{29}NO_5P$ $[M + H]^+$ 454.1705; found 454.1658.

Ethyl 4-((1-(diphenoxyphosphoryl)-2-ethylbutyl)amino) benzoate, 4i

(2.17 g, 90%); white crystalline solid; mp 121–123 °C; $R_f = 0.48$ (7:3 hexane–ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (d, $J = 8.7$ Hz, 2H, ArH, H-10), 6.84–7.38 (m, 10 H, H-9), 6.67 (d, $J = 8.7$ Hz, 2H, ArH, H-8), 4.53 (dd, $J = 2.7$, 10.8 Hz, 1H, CH–CH_2), 4.41 (dd, $J = 3.6$, 10.9 Hz, 1H, CH-P), 4.35 (q, $J = 7.2$ Hz, 2H, –CH_2–O), 1.88–2.10 (m, 2H, CH_2–CH), 1.45–1.74 (m, 2H, –CH_2–CH), 1.39 (t, $J = 7.1$ Hz, 3H, CH_3–CH_2–O), 0.99 (t, $J = 7.3$ Hz, 6H, CH_3–CH_2–CH). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.6 (C=O), 150.8 (C-2), 150.3 (C-19), 150.2 (C-25), 131.6 (C-4, C-6), 129.8 (C-21, C-23), 129.6 (C-27, C-29), 125.3 (C-22), 125.1 (C-28), 120.4 (C-20, C-24), 120.3 (C-26, C-30), 120.0 (C-5), 112.1 (C-1, C-3), 60.3 (C-10), 52.3 (d, $J = 153.5$ Hz, C-P), 43.3 (C-13), 22.8 (C-34), 22.3 (C-31), 14.4 (C-11), 12.0 (C-33), and 11.9 (C-32). ^{31}P NMR (162 MHz, $CDCl_3$): δ 18.22 (dd, $J = 18.6$, 10.7 Hz). HRMS (ESI^+) *m/z*, calcd for $C_{27}H_{33}NO_5P$ $[M + H]^+$ 482.2018; found 482.2020.

General procedure for the synthesis of (S)-4-amino-N-(2-hydroxy-1-phenylethyl)benzamide (5)

The amide **5** was synthesized and then used as a starting material in a normal Kabachnik-Field reaction for the synthesis of the second serial of α-aminophosphonates (amide derivatives). The synthesis was done using the Caldwell method (Caldwell et al. 2013): ethyl 4-aminobenzoate (1 eq) and (*S*)-phenylglycinol (1 eq) and K_3PO_4 (0.3 eq) were reacted at 70 °C for 24 h, using isopropanol as solvent, the purification was done in flash chromatography with hexane–ethyl acetate-methanol (5:4:1) as mobile phase to yield the amide (75%)**;** white solid; mp 174–176 °C; $R_f = 0.36$ (5:4:1 hexane–ethyl acetate-methanol). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.18 (d, $J = 8.1$ Hz, 1H, NH), 7.65 (d, $J = 8.6$ Hz, 2H, ArH, H-8), 7.20–7.39 (m, 5H, ArH, H-7), 6.56 (d, $J = 8.6$ Hz, 2H, H-6), 5.61 (s, 2H, NH_2), 5.04 (q, $J = 7.8$ Hz, 1H, CH–Ar), 4.88 (t, $J = 5.9$ Hz, 1H, OH), and 3.61–3.73 (m, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO): δ 166.5 (C=O), 152.1 (C-2), 142.4 (C-14), 129.4 (C-4, C-6), 128.4 (C-6, C-18), 127.5 (C-15, C-19), 127.1 (C-17), 121.8 (C-5), 112.9 (C-1, C-3), 65.1 (C-12), and 56.0 (C-11). HRMS (ESI^+) *m/z*, calcd for $C_{15}H_{16}N_2O_2$ $[M + H]^+$ 257.1285; found 257.1288.

General procedure for the synthesis of α-aminophosphonates amide derivatives (6a–6i)

The corresponding aldehyde (**1**) (1.5 eq), (*S*)-4-amino-N-(2-hydroxy-1-phenylethyl) benzamide (**5**) (1 eq), and diphenylphosphite (**3**) (3 eq) were dissolved in ethanol (0.5 mL). The reaction mixture was exposed to MW irradiation at the corresponding temperature (60 or 70 °C), for 20, 30, or 40 min as it corresponds, the progress of the reaction was monitored by TLC. The solvent was evaporated, and the crude material was purified by (1) column chromatography

on silica gel 60 (230–400 mesh) using hexane: ethyl acetate: methanol (5:4:1) as a mobile phase or (2) recrystallization from ethanol to afford the analytically pure compound.

Diphenyl ((4-chlorophenyl)((4-(((S)-2-hydroxy-1-phenylethyl)carbamoyl)phenyl) amino)methyl) phosphonate, 6a

(0.13 g, 80%); pale-yellow solid recrystallized from ethanol; mp 148–150 °C; $R_f = 0.48$ (hexane: ethyl acetate: methanol, 5:4:1) ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.26 (dd, $J = 4.5$, 8.1 Hz, 1H, NH–C=O), 7.81–6.88 (m, 24H, ArH), 5.84 (ddd, $J = 2.4$, 10.4, 25.6 Hz, 1H, CH-P), 5.01 (tdd, $J = 1.5$, 5.5, 7.8 Hz, 1H, CH–Ar), 4.86 (dt, $J = 5.9$, 8.8 Hz, 1H, OH), and 3.55–3.76 (m, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6): δ 166.3 (C=O), 150.5 (C-2), 150.2 (C-22), 149.8 (C-28), 142.3 (C-38), 135.0 (C-10), 133.2 (C-17), 130.9 (C-4, C-6), 130.3 (C-24, C-26,C-30, C-32), 129.1 (C-16, C-18), 128.8 (C-41), 128.5 (C-40, C-42), 127.4 (C-15, C-19), 127.1 (C-39, C-43), 125.8 (C-25, C-31), 123.8 (C-5), 121.0 (C-23, C-27), 120.8 (C-29, C-33), 113.0 (C-1, C-3), 65.1 (C-36), 56.1 (C-35), and 53.8 (d, $J = 156.3$ Hz, C-P). ^{31}P NMR (243 MHz, DMSO-d_6): δ 15.59 (d, $J = 26.4$ Hz, P–H). HRMS (ESI$^+$) *m/z*, calcd for $C_{34}H_{30}ClN_2O_5P$ $[M + H]^+$ 613.1654; found 613.1720.

Diphenyl (((4-(((S)-2-hydroxy-1-phenylethyl)carbamoyl) phenyl)amino)(4-hydroxyphenyl)methyl)phosphonate, 6b

(0.04 g, 22%); white solid recrystallized from ethanol; mp 139–141 °C; $R_f = 0.42$ (hexane: ethyl acetate: methanol, 5:4:1) ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.49 (d, $J =$ 2.3 Hz, 1H, Ar-OH), 8.25 (dd, $J = 5.2$, 8.1 Hz, 1H, NH–C=O), 7.77–6.60 (m, 24H, ArH, NH), 5.59 (ddd, $J =$ 2.0, 10.3, 24.4 Hz, 1H, CH-P), 5.02 (td, $J = 5.5$, 7.8 Hz, 1H, CH–Ar), 4.87 (dt, $J = 5.9$, 8.5 Hz, 1H, OH), and 3.55–3.75 (m, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6): δ 166.4 (C=O), 157.7 (C-27), 150.6 (C-32), 150.5 (C-38), 150.2(C-12), 142.3 (C-5), 130.3 (C-14, C-16), 130.2 (C-34, C-36, C-40, C-42), 129.1 (C-7, C-9), 128.5 (C-6, C-10), 127.4 (C-25, C-29), 127.1 (C-8), 125.6 (C-35, C-41), 125.6 (C-20), 123.4 (C-15), 121.1 (C-33, C-37) 120.8 (C-39, C-43), 115.6 (C-11, C-13), 112.9 (C-26, C-28), 65.1 (C-3), 56.1 (C-2), and 53.9 (d, $J = 157.6$ Hz, C-P). ^{31}P NMR (243 MHz, DMSO-d_6) δ 16.66 (d, $J = 24.4$ Hz, P–H). HRMS (ESI$^+$) *m/z*, calcd for $C_{34}H_{31}ClN_2O_6P$ $[M + H]^+$ 595.1992; found 595.2016.

Diphenyl (((4-(((S)-2-hydroxy-1-phenylethyl)carbamoyl) phenyl)amino)(4-methoxyphenyl)methyl)phosphonate, 6c

(0.13 g, 80%); white solid recrystallized from ethanol; mp 140–143 °C; $R_f = 0.42$ (hexane: ethyl acetate: methanol, 5:4:1) ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.26 (dd, $J = 8.1$, 5.1 Hz, 1H, NH–C=O), 7.71–6.88 (m, 24H, ArH, NH), 5.68 (ddd, $J = 24.7$, 10.3, 2.3 Hz, 1H, CH-P), 5.02 (td, $J =$ 7.9, 5.4 Hz, 1H, CH–Ar), 4.87 (t, $J = 7.1$ Hz, 1H, OH), 3.74 (d, $J = 1.2$ Hz, 3H, OCH_3), and 3.61–3.71 (m, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6): δ 166.4 (C=O), 159.5 (C-27), 150.6 (C-31), 150.4 (C-37), 150.1 (C-12), 142.3 (C-5), 132.3 (C-14, C-16), 130.4 (C-33, C-35), 130.2 (C-39, C-41), 129.1 (C-7, C-9), 128.4 (C-25, C-29), 127.4 (C-6, C-10), 127.1 (C-8), 125.7 (C-34, C-40), 123.5 (C-20, C-15), 121.1 (C.32, C-36), 120.8 (C-38, C-42), 115.7 (C-11), 115.0 (C-13), 114.2 (C-26), 113.0 (C-28), 65.1 (C-3), 56.1 (C-2), and 55.6 (C-44), 53.8 (d, $J = 157.5$ Hz, C-P).^{31}P NMR (243 MHz, DMSO-d_6) δ 16.45 (d, $J = 24.8$ Hz, P–H). HRMS (ESI$^+$) *m/z*, calcd for $C_{35}H_{33}N_2O_6P$ $[M + H]^+$ 609.2149; found 609.2077.

Diphenyl (((4-(((S)-2-hydroxy-1-phenylethyl)carbamoyl) phenyl)amino)(phenyl) methyl)phosphonate, 6d

(0.16 g, 34%); white solid recrystallized from ethanol; mp 170–172 °C; $R_f = 0.42$ (hexane: ethyl acetate: methanol, 5:4:1) ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.22–8.30 (m, 1H, NH–C=O), 7.85–6.85 (m, 25H, ArH, NH), 5.76 (ddd, $J =$ 25.2, 10.3, 4.7 Hz, 1H, CH-P), 5.02 (qd, $J = 7.8$, 5.2 Hz, 1H, CH–Ar), 4.87 (tt, $J = 7.6$, 5.9 Hz, 1H, OH), and 3.54–3.76 (m, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6): δ 166.3 (C=O), 150.5 (C-31), 150.3 (C-37), 150.0 (C-12), 142.3 (C-5), 135.8 (C-20), 130.3 (C-33, C-35, C-39, C-41), 129.2 (C-14, C-16), 129.1 (C-7, C-9), 128.8 (C-26, C-28), 128.4 (C-25, C-27, C-29), 127.4 (C-6, C-10), 127.1 (C-8), 125.7 (C-34, C-40), 123.6 (C-15), 121.1 (C-32, C-36), 120.8 (C-38, C-42), 113.0 (C-11, C-13), 65.1 (C-3), 56.1 (C-2), and 54.4 (d, $J = 156.0$ Hz, C-P).^{31}P NMR (243 MHz, DMSO-d_6) δ 16.21 (d, $J = 24.8$ Hz, P–H). HRMS (ESI$^+$) *m/z*, calcd for $C_{34}H_{31}N_2O_5P$ $[M + H]^+$ 579.2043; found 579.1975.

Diphenyl ((4-(diphenylamino)phenyl)((4-(((S)-2-hydroxy-1-phenylethyl)carbamoyl) phenyl)amino)methyl) phosphonate, 6e

(0.28 g, 49%); yellow solid recrystallized from ethanol; mp 158–159 °C; $R_f = 0.52$ (hexane: ethyl acetate: methanol, 5:4:1) ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.27 (dd, $J = 8.1$, 4.0 Hz, 1H, NH–C=O), 7.77–6.81 (m, 34H, ArH, NH), 5.71 (ddd, $J = 24.7$, 10.3, 2.6 Hz, 1H, CH-P), 5.03 (td, $J =$ 8.0, 5.4 Hz, 1H, CH–Ar), 4.88 (q, $J = 5.8$ Hz, 1H, OH), and 3.59–3.75 (m, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.3 (C=O), 150.6 (C-32), 150.5 (C-38), 150.1 (C-12), 147.6 (C-27), 147.5 (C-44, C-45), 142.3 (C-5), 130.4 (C-14, C-16), 130.2 (C-34, C-36, C-40, C-42), 130.0 (C-47, C-49, C-52, C-54), 129.5 (C-20), 129.1 (C-7, C-9), 128.4 (C-6, C-

10), 127.4 (C-46, C-50, C-51, C-55), 127.1 (C-25, C-29), 125.8 (C-48), 125.6 (C-53), 124.3 (C-35, C-41), 123.6 (C-26, C-28), 123.4 (C-15), 121.1(C-33, C-37), 120.7 (C-39, C-43), 112.8 (C-11, C-13), 65.1 (C-3), 56.1 (C-2), and 53.90 (d, $J = 156.2$ Hz, C-P).^{31}P NMR (243 MHz, DMSO-d_6) δ 16.28 (d, $J = 24.5$ Hz, P–H). HRMS (ESI$^+$) *m/z*, calcd for $C_{46}H_{40}N_3O_5P$ $[M + H]^+$ 746.2778; found 746.2686.

Diphenyl (((4-(((S)-2-hydroxy-1-phenylethyl)carbamoyl) phenyl)amino)(4-morpholino-phenyl)methyl)phosphonate, 6f

(0.10 g, 57%); yellow solid recrystallized from ethanol; mp 127–129 °C; $R_f = 0.32$ (hexane: ethyl acetate: methanol, 5:4:1) ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.29 (dd, $J = 8.1$, 6.0 Hz, 1H, NH–C=O), 7.77–6.89 (m, 24H, ArH, NH), 5.66 (ddd, $J = 24.4$, 10.3, 2.0 Hz, 1H, CH-P), 5.07 (td, $J =$ 7.8, 5.5 Hz, 1H, CH–Ar), 4.91 (dt, $J = 8.0$, 5.9 Hz, 1H, OH), 3.77 (t, $J = 4.5$ Hz, 4H, CH_2-O(morph)), 3.64–3.75 (m, 2H, CH_2), and 3.09–3.21 (m, 4H, CH_2-N(morph)). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.3 (C=O), 151.2 (C-27), 150.6 (C-32), 150.4 (C-38), 150.1 (C-12), 142.3 (C-5), 130.2 (C-34, C-36, C-40, C-42), 129.8 (C-14, C-16), 129.0 (C-7, C-9), 128.4 (C-6, C-10), 127.4 (C-25, C-29), 127.1 (C-8), 125.6 (C-35, C-41), 123.4 (C-20, C-15), 121.1 (C-33, C-37), 120.9 (C-39, C-43), 115.2 (C-11, C-13), 113.7 (C-28), 113.0 (C-6), 66.5 (C-46, C-47) 65.1 (C-3), 56.1 (C-2), 53.9 (d, $J = 157.7$ Hz, C-P), and 48.7 (C-44, C-45).^{31}P NMR (243 MHz, DMSO-d_6) δ 16.59 (d, $J = 24.4$ Hz, P–H). HRMS (ESI$^+$) *m/z*, calcd for $C_{38}H_{38}N_3O_6P$ $[M + H]^+$ 664.2571; found 664.2498.

Diphenyl (((4-(((S)-2-hydroxy-1-phenylethyl)carbamoyl) phenyl)amino)(1-methyl-1H-pyrazol-4-yl)methyl) phosphonate, 6g

(0.08 g, 66%); white solid recrystallized from ethanol; mp 126–128 °C; $R_f = 0.18$ (hexane: ethyl acetate: methanol, 6:3:1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.27 (dd, $J = 8.1$, 3.4 Hz, 1H, NH–C=O), 7.81 (d, $J = 1.8$ Hz, 1H, CH-pyrazole), 7.71 (dd, $J = 8.8$, 2.0 Hz, 2H, ArH, H-10), 7.61 (s, 1H, CH-pyrazole), 7.44–6.99 (m, 15H, ArH, H-8), 6.97 (d, $J = 8.7$ Hz, 2H, ArH, H-7), 6.91 (d, $J = 10.3$ Hz, 1H, NH), 5.70 (dd, $J = 21.2$, 10.2 Hz, 1H, CH-P), 5.03 (td, $J = 7.9$, 5.5 Hz, 1H, CH–Ar), 4.88 (s, 1H, OH), 3.81 (s, 1H, CH_3), and 3.74–3.58 (m, 2H, CH_2–OH). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.3 (C=O), 150.6 (C-26), 150.5 (C-32), 150.2 (C-12), 142.3 (C-5), 138.9 (C-41), 130.7 (C-14, C-16), 130.3 (C-28, C-30), 129.8 (C-34, C-36), 129.1 (C-7, C-9), 128.4 (C-6, C-10), 127.4 (C-38), 127.1 (C-8), 125.6 (C-29, C-35), 123.4 (C-15), 121.0 (C-27, C-31), 120.9 (C-33, C-37), 115.4 (C-20), 112.7 (C-11, C-13), 65.1 (C-3), 56.1 (C-2), 46.6 (d, $J = 163.2$ Hz, C-P), and 39.1 (C-42). ^{31}P NMR (243 MHz, DMSO-d_6) δ 16.40 (d, $J = 20.9$ Hz, P–H). HRMS (ESI$^+$) *m/z*, calcd for $C_{38}H_{38}N_3O_6P$ $[M + H]^+$ 583.2105; found 583.2056.

Diphenyl (1-((4-(((S)-2-hydroxy-1-phenylethyl)carbamoyl) phenyl)amino)-2-methylpropyl)phosphonate, 6h

(0.03 g, 21%); white solid purified by flash chromatography; mp 124–125 °C; $R_f = 0.54$ (hexane: ethyl acetate: methanol, 5:4:1). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.23–8.28 (m, 1H, NH–C=O), 7.70 (d, $J = 8.9$ Hz, 2H, ArH, H-11), 7.09–7.42 (m, 15H, ArH, H-10), 6.94 (d, $J =$ 8.5 Hz, 2H, ArH, H-9), 6.51 (d, $J = 10.5$ Hz, 1H, NH), 4.99–5.08 (m, 1H, CH–Ar), 4.89 (t, $J = 5.8$ Hz, 1H, OH), 4.44 (ddd, $J = 16.6$, 10.6, 5.3 Hz, 1H, CH-P), 3.60–3.74 (m, 2H, CH_2), 2.39 (tt, $J = 12.2$, 6.6 Hz, 1H, CH-$(CH_3)_2$), 1.14 (d, $J = 6.8$ Hz, 3H, CH_3), and 1.10 (d, $J = 6.7$ Hz, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.4 (C=O), 151.5 (C-12), 150.6 (C-26), 150.4 (C-32), 142.4 (C-5), 130.4 (C-14, C-16), 130.1 (C-28, C-30, C-34, C-36), 129.2 (C-15), 128.4 (C-7, C-9), 127.5 (C-6, C-10), 127.1 (C-8), 125.7 (C-29), 125.4 (C-35), 121.1 (C-27, C-31), 120.9 (C-33, C-37), 112.0 (C-11, C-13), 65.1 (C-3), 56.1 (C-2), 55.5 (d, $J = 152.4$ Hz, C-P), 30.3 (C-20), 20.6 (C-38), and 18.9 (C-39). ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.31 (dd, $J =$ 17.5, 7.1 Hz). HRMS (ESI$^+$) *m/z*, calcd for $C_{31}H_{33}N_2O_5P$ $[M + H]^+$ 545.2200; found 545.2088

Diphenyl (2-ethyl-1-((4-(((S)-2-hydroxy-1-phenylethyl) carbamoyl)phenyl)amino) butyl)phosphonate, 6i

(0.05 g, 31%); pale-yellow purified by flash chromatography solid; mp 88–90 °C; $R_f = 0.56$ (hexane: ethyl acetate: methanol, 5:4:1). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.26 (dd, $J = 8.1$, 3.2 Hz, 1H, NH–C=O), 7.71 (d, $J =$ 8.8 Hz, 2H, ArH, H-11), 7.45–6.93 (m, 15 H, ArH, H-10), 6.92 (d, $J = 8.6$ Hz, 2H, ArH, H-9), 6.48 (d, $J = 10.5$ Hz, 1H, NH), 5.04 (td, $J = 7.9$, 5.5 Hz, 1H, CH–Ar), 4.88 (t, $J = 5.9$ Hz, 1H, OH), 4.47 (ddd, $J = 16.4$, 10.5, 5.1 Hz, 1H, CH-P), 3.59–3.74 (m, 2H, CH_2-OH), 1.79–1.95 (m, 2H, CH_2), 1.34–1.63 (m, 3H, CH_2 and CH), and 0.81–0.99 (m, 6H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.3 (C=O), 151.1 (C-11), 150.6 (C-25), 150.4 (C-31), 142.4 (C-4), 130.4 (C-27, C-29), 130.1 (C-33, C-35), 129.3 (C-13, C-15), 128.4 (C-6, C-8), 127.5 (C-5, C-9), 127.1 (C-7), 125.7 (C-28), 125.4 (C-31), 122.7 (C-14), 121.0 (C-26, C-30), 120.9 (C-32, C-36), 111.9 (C-10, C-12), 65.1 (C-3), 56.1 (C-2), 52.5 (d, $J = 154.2$ Hz, C-P), 43.0 (C-19), 22.4 (C-37), 22.0 (C-40), 12.0 (C-38), and 11.7 (C-39). ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.81 (dd, $J = 17.6$, 8.3 Hz). HRMS (ESI$^+$) *m/z*, calcd for $C_{33}H_{37}N_2O_5P$ $[M + H]^+$ 573.2513; found 573.2477.

Biological activity

Cell culture

The human triple negative-breast cancer (MDA-MB-231), human estrogen receptor-positive breast (MCF-7), and human normal breast (MCF-10A) cell lines were purchased from the American Type Culture Collection (ATCC).

MDA-MB-231 (ATCC® HTB-26™), was cultured in Roswell Park Memorial Institute 1640 Medium GIBCO with L-glutamine, containing 10% fetal bovine serum (FBS) and 1% antibiotic solution (5000 units/mL penicillin, 5000 µg/mL streptomycin).

MCF-7 (ATCC® HTB-22™), was cultured in Dulbecco's Modified Eagle Medium GIBCO: nutrient mixture F-12 (DMEM/F12), 15 mM HEPES and L-glutamine, containing 10% FBS and 1% antibiotic solution.

MCF-10A (ATCC® CRL-10317™), was cultured in DMEM/F12 supplemented with the Mammary Epithelial Cell Culture Kit Lonza: epidermal growth factor, insulin, hydrocortisone, gentamicin sulfate-amphotericin-B (GA-1000), and bovine pituitary extract.

Cell proliferation inhibition assay

All compounds (**4a–4i** and **6a–6i**) were screened for their cell proliferation inhibition effect on MDA-MB-231, MCF-7, and MCF-10A. Briefly, once cell culture reached 80% of confluence, a plate of 96 wells was prepared by the dissociation of the cell monolayer using 0.25% trypsin (5 min incubation at 37 °C); afterward 5 mL of media was added to inactivate enzyme activity. The cell suspension was placed in a conical tube and centrifuged (1250 rpm, 5 min), the pellet was resuspended in media for cell counting using a Neubauer chamber. Once cell concentration was determined, the 96-well plates were prepared by seeding 10,000 cells/well in 100 µL (for MCF-7 and MCF-10A) and 8000 cells/well in 100 µL (for MDA-MB-231). All cell lines were incubated at 37 °C, 5% of CO_2 and 95% humidity for 24 h before adding the drug molecules. All compounds were dissolved in dimethyl sulfoxide (DMSO) to get a stock solution of 10 mg/mL concentration, further dilutions of the stock were done with culture media. Cell viability was assessed by MTT method (Riss et al. 2016; Berridge and Tan 1993), after 24 h of compounds incubation, MTT reagent (3 mg/mL in PBS) was added into each well and incubated for 3 h. Solution in all wells was discarded, and DMSO (100 µL) was added to solubilize the formazan crystals. Absorbance was measured spectrophotometrically at 570 nm using a microplate reader Gen 5. Once the screening was complete, the compounds with cytotoxicity below 100 µM were selected to determine their IC_{50}, all experiments were performed in triplicate in three different days.

Apoptosis assay

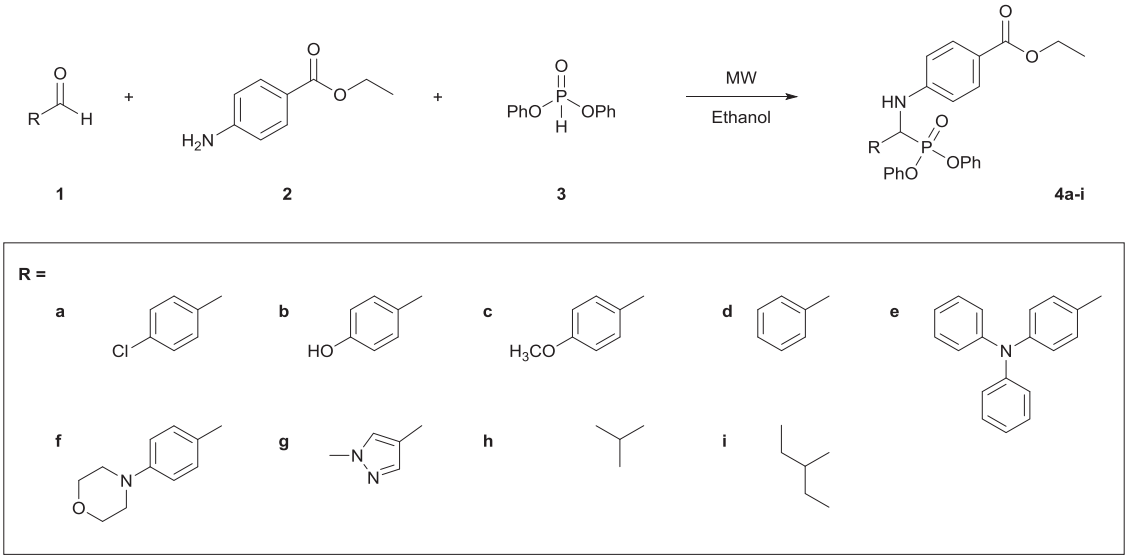
The EnzChek® Caspase-3 Assay Kit#2 from Molecular Probes was used to determine apoptosis through detection of caspase-3 activity in MCF-7. Once cell growth reached 80% of confluence, a plate of 6 wells was prepared by seeding about 1,000,000 cells/well in 2 mL of culture media. The six-well plates were incubated at 37 °C, 5% of CO_2 and 95% humidity for 24 h before adding the test drug IC_{50} of each drug molecule (36 µM), after 24 h of drugs incubation cells were then harvested, lysed and assayed as described in the kit protocol. Podophyllotoxin (36 µM) was used as positive control (apoptosis inductor), untreated cells were used as negative control.

Results and discussion

Synthesis of ester derivatives

α-Aminophosphonates **(4a–4i)** were synthesized following the one-pot Kabachnik–Fields reaction (Scheme 1).

The reaction conditions used in this work afforded yields as high as similar reactions reported using different catalysts and organic solvents. In this regard, Maddina et al. 2014 reported a protocol for the synthesis of α-aminophosphonates derivatives containing a pyrazine moiety through one-pot three components Kabachnik–Fields reaction, using MW irradiation (490 W) and toluene as a solvent; authors reported yields between 84 and 92% after 10–20 min. Subba Reddy et al. 2014 reported a similar protocol using Amberlyst-15 as catalyst (MW irradiation at 490 W) and the reported yields were 83–92%. Rezaei et al. 2011 synthesized a series of α-aminophosphonates using the same three-component method with different aromatic aldehydes, aromatic amines, and diethyl phosphite in THF as a solvent, and $FeCl_3$ as a catalyst; yields were moderate to good (73–84%). In another publication, several α-aminophosphonates were synthesized by Reddy et al. 2016, they used Triton X-100 as catalyst and reaction times of 30–60 min at 70 °C they obtained yields in the 76–86% range. Finally, Afshari et al. 2017 reported the synthesis of α-aminophosphonates using cobalt ferrite magnetic nanoparticles; the reaction conditions were 10–40 min at room temperature and yields between 70 and 95%. In summary, the reports described above provide evidence supporting the synthesis of α-aminophosphonates in good to excellent yields using either MW irradiation in organic solvents or the use of a catalyst. Our proposal is an eco-friendly approach, which also uses MW irradiation, but having the advantage of using a minimum amount of ethanol as solvent and catalyst free. The reported yields were comparable with those described in the literature (Table 1).



Scheme 1 Synthesis of α-aminophosphonates **4a**–**4i**, ester derivatives

Table 1 Reaction conditions and yields of compounds **4a–4i**

Compound	Temperature (°C)	Time (min)	Yield[a] (%)
4a	80	20	82
4b	90	30	72
4c	60	20	87
4d	80	20	92
4e	80	20	58
4f	60	30	59
4g	60	30	97
4h	60	40	74
4i	80	30	90

[a]Yields after purification

The optimal temperature for the synthesis of final compounds (**4a–4i**) was determined by a series of preliminary experiments in which we increased the temperature from 80 to 120 °C. In this regard, we observed lower yields at temperatures higher than 90 °C, probably due to decomposition of the α-aminophosphonate; the new bond formed during the coupling, breaks down and the reaction equilibrium shifts to the starting materials. Based on these observations, we decided to decrease the reaction temperature to 60 °C, and for compounds **4c**, **4g**, and **4h**, we observed higher yields than those obtained at the initial conditions (80 °C). These results are consistent with those reported by Mirzaei et al. 2015, who reported the highest yields at 50–60 °C in their synthetic method. Based on these results, 60 or 80 °C could be considered as the optimal temperatures for the synthesis of most of the studied α-aminophosphonates. The only two exceptions were compounds **4e** and **4f**, probably due to steric effects produced by the *N*,*N*,*N*-triphenylamine and the 4-(morpholino)phenyl groups.

Synthesis of amide derivatives

The synthesis of a second serial of α-aminophosphonates (amide derivatives) was also performed by a Kabachnik–Fields reaction (Scheme 2) having benzamide **5** as the starting material (NH_2 component). The reaction conditions and obtained yields are shown in Table 2.

Moderated to good yields were observed for the amide derivatives (**6a–6i**), compared with the ester serial of compounds (**4a–4i**), probably due to the structure of amide **5**, which has an additional phenyl group and an OH moiety that could interfere in the interaction of the amine during the mechanistic pathway (Keglevich and Bálint 2012) that involves at first, the formation of the corresponding imine and then the interaction with the phosphite in order to obtain the desired α-aminophosphonate.

The synthesis of the benzamide **5** was done following a green chemistry method as well, based on Caldwell method (Caldwell et al. 2013). Ethyl 4-aminobenzoate and (*S*)-phenylglycinol were reacted in presence of K_3PO_4 at 70 °C for 24 h, using isopropanol as solvent the yield obtained for the benzamide **5** was 75% and it corresponds to the yields reported by Caldwell et al. 2013 (42–100%).

Cell proliferation inhibition assay

All compounds (**4a–4i** and **6a–6i**) were screened for their cell proliferation inhibition effect on MDA-MB-231, MCF-7 and MCF-10A. The preliminary evaluation was testing the α-aminophosphonates at 100 µM, once the assay was

Scheme 2 Synthesis of α-aminophosphonates **6a–6i**, amide derivatives

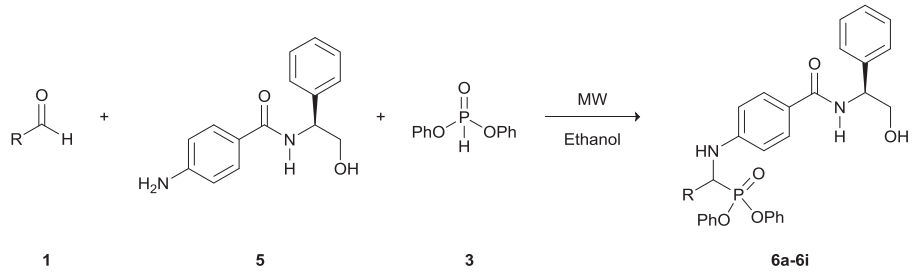


Table 2 Reaction conditions and yields of compounds **6a–6i**

Compound	Temperature (°C)	Time (min)	Yield[a] (%)
6a	60	20	80
6b	60	20	22
6c	60	20	80
6d	70	30	34
6e	70	30	49
6f	60	20	57
6g	60	30	66
6h	60	40	21
6i	60	40	31

[a]Yields after purification

complete, the compounds with cytotoxic effect below that concentration were selected to determine their IC_{50} value (μM), results are presented in Fig. 1, and IC_{50} values are listed in Table 3.

Regarding cancer cell lines, compound **4b**, **6a**, **6d**, and **6h** exhibited cell proliferation inhibition activity in MDA-MB-231; among these four compounds, the ester derivative **4b** which R group corresponds to *p*-OH phenyl, was the most active one with IC_{50} of 8.15 μM in this cell line. The IC_{50} values of compound **6a**, **6h**, and **6d** were 26.61, 49.10, and 61.58 μM respectively. Although **4b** was the most active compound in MDA-MB-231, this one cannot be considered as drug candidate due to its high cell proliferation inhibition activity in the standard cell line MCF-10A, with an $IC_{50} = 3.48$ μM. A similar situation was observed for compounds **6d** and **6h**, they can inhibit the cell proliferation in the cancer cell line, but they are also cytotoxic for the normal cells; their IC_{50} is lower for MCF-10A (27.08 and 42.29 μM, respectively) than the IC_{50} for MDA-MB-231. Amide derivative **6a** which R group corresponds to *p*-Cl phenyl substituent, had $IC_{50} = 26.61$ μM in MDA-MB-231 and its IC_{50} for the normal cell line is 49.56 μM; this suggests that it is necessary almost the double of the dose to cause cell proliferation inhibition activity in the standard cell line.

Respecting MCF-7 cell line, the active compounds were **4b**, **4e**, **6c**, and **6d**; the ester derivative **4b** was cytotoxic at $IC_{50} = 13.16$ μM and highly cytotoxic in the standard cell line MCF-10A ($IC_{50} = 3.48$ μM), due to its high cell proliferation inhibition activity in the normal breast cell line this ester cannot be considered as lead compound. Interesting results were observed for compounds **4e** and **6c** which have cytotoxic effect in the cancer cell line MCF-7 ($IC_{50} = 35.72$ and 36.68 μM, respectively) and they are not cytotoxic for the normal cell line MCF-10A, their IC_{50} values are higher than 100 μM (software calculations indicated IC_{50} values >300 μM). Another interesting discovery was the amide derivative **6d**, which R corresponds to a phenyl group, it was the most active α-aminophosphonate with $IC_{50} = 0.50$ μM in MCF-7 and without producing cytotoxic effect in the normal cell line; even though its IC_{50} in MCF-10A is 27.08 μM, it is important to indicate that is necessary more than 50 times the IC_{50} of MCF-7 to present cell proliferation inhibition activity in the normal cell line and because that we considered it as good drug candidate. Evidence of the IC_{50} values suggests that the ester derivative **4e** and the amide derivatives **6c** and **6d** might be selective to cancer cell line MCF-7 and this find is probably related with their interaction with the hormone receptors present in MCF-7, but more assays must be done.

A straightforward SAR relation was not observed, but in general, the amide derivatives are more active than the ester ones, only two ester derivatives were active while four amides presented activity as described above. There is not a relationship between ester-amide and the same R group; for instance, the ester derivative with R group of *p*-OH phenyl (**4b**) was the most active in all the three cell lines tested and the amide derivative **6b** with the same R, has not cytotoxic effect in any of them. Despite the above, it seems to be a synergistic effect in some derivatives caused by (in the case of **4e**) the presence of the α-aminophosphonate, the ester part and the R group (triphenylamine) that is not observed for the corresponding amide derivative. A similar situation was observed with the amides **6c** and **6d**; it seems to be a synergistic effect caused by the presence of the α-aminophosphonate, the amide part and the R group (*p*-OCH_3 and phenyl, respectively) that is not observed for the corresponding ester derivatives.

All active compounds have IC_{50} values lower than the control Fotemustine; we selected it as a control because

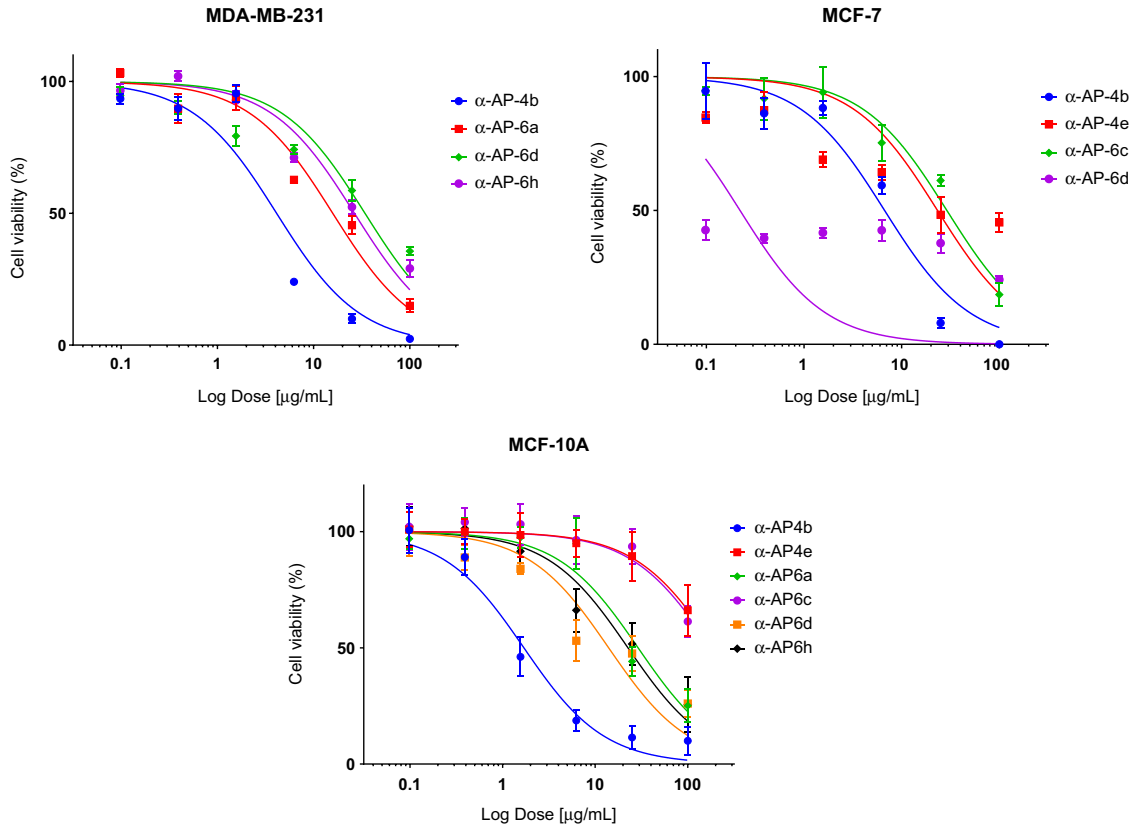


Fig. 1 Dose-response curve obtained in MDA-MB-231, MCF-7, and MCF-10A cells after exposure to active compounds. Data are expressed as mean value ± SD from three independent experiments, each performed in triplicate, determined by MTT assay, nonlinear regression was used and log(inhibitor) versus normalized response was plotted

their chemical structure similarities with the synthesized α-aminophosphonates; they all belong to the same family of compounds. Furthermore, Fotemustine is an effective drug that has been used mainly for the treatment of disseminated malignant melanoma phase I, II, and III trials with good overall response rates and survival (Fischel et al. 1993; Avril et al. 2004; Guida et al. 2018), it also has noticeable results in CNSL and glioblastoma multiforme treatments (Marinelli et al. 2018; Wu et al. 2018). Finally, Fotemustine was not evaluated in MDA-MB-231, MCF-7, and MCF-10A cell lines before and it is crucial to extend the investigation of promising candidate drugs that can be used as an alternative in breast cancer.

Apoptosis assay

Caspases are a family of cysteine proteases that act as common death effector molecules (Fulda and Debatin 2006) and their activation during the apoptotic process results in irreversible cell death (Magedov et al. 2007). The activation of caspases plays a crucial role in the biological events associated with apoptosis. In order to evaluate if α-aminophosphonates lead to the apoptosis pathway, the caspase-3 activity was examined, using a caspase-3 specific substrate,

Table 3 Cell proliferation inhibition of α-Aminophosphonates

Compound	IC_{50} (μM)		
	MDA-MB-231	MCF-7	MCF-10A
4a	>100	>100	>100
4b	8.15 ± 0.36	13.16 ± 0.53	3.48 ± 0.62
4c	>100	>100	>100
4d	>100	>100	>100
4e	>100	35.72 ± 16.65	>100 (364.75 ± 88.40)[a]
4f	>100	>100	>100
4g	>100	>100	>100
4h	>100	>100	>100
4i	>100	>100	>100
6a	26.61 ± 5.08	>100	49.56 ± 10.41
6b	>100	>100	>100
6c	>100	36.68 ± 12.31	>100 (308.40 ± 57.28)[a]
6d	61.58 ± 14.93	0.50 ± 0.22	27.08 ± 8.31
6e	>100	>100	>100
6f	>100	>100	>100
6g	>100	>100	>100
6h	49.10 ± 3.94	>100	42.29 ± 9.95
6i	>100	>100	>100
Fotemustine	851 ± 75.86	812.61 ± 64.93	1367.96 ± 86.32

[a]The extrapolated value was obtained using the software PRISM

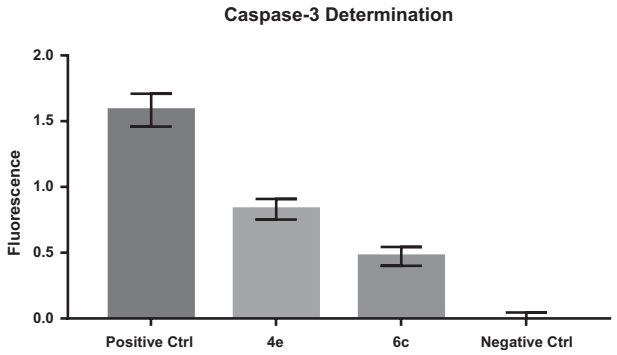


Fig. 2 Caspase-3 determination. MCF-7 cells exposed to IC_{50} of podophyllotoxin (positive control), compound **4e** and **6c**, negative control are untreated cells. Data are expressed as mean value ± SD of the mean value of three independent experiments, each performed in triplicate

Z-DEVD-R110, which is cleaved to afford a fluorescent product. As shown in Fig. 2, caspase-3 activity was markedly elevated when cells were exposed to the IC_{50} (36 μM) of compound **4e** and **6c**, the fluorescence is directly proportional to the expression of caspase-3. The intrinsic apoptosis observed for the untreated cells (negative control) is part of the normal cell regulation, and the background fluorescence has been subtracted from each value. The fluorescence lectures of podophyllotoxin, compounds **4e** and **6c** clearly show that the growth-inhibitory activity can be attributed to apoptosis induction through caspase-3 activation. These results match with some references that establish α-aminophosphonates are capable of inducing apoptosis; Huang et al. 2013 reported that α-aminophosphonates showed cell apoptosis and cell cycle analysis indicated that one compound could arrest the cell cycle in G1 stage. Ye et al. 2014 showed that α-aminophosphonates drive cells directly into apoptosis. Fang et al. 2016 investigated the apoptosis-inducing activity of α-aminophosphonates by Acridine orange/Ethidium bromide staining and indicated that these compounds induced death cell through apoptosis.

At this moment, compounds **4e** and **6c** seem to be useful lead compounds, despite their molecular weight and the presence of the phenyl rings (which contribute to increase their hydrophobicity), their IC_{50} values are low (around 36 μM), they are cytotoxic only for MCF-7, they are not cytotoxic for the normal cell line and they induced apoptosis; regarding to the organic synthesis, the MW method is easy, fast, yields are good, and these α-aminophosphonates can be synthesized through green chemistry.

Conclusion

In summary, we were able to synthesize 18 novel α-aminophosphonates by an eco-friendly "one-pot" three-component reaction, under catalyst-free conditions using microwave irradiation. The cell proliferation inhibition assay showed that in general, the amide derivatives are more active than ester ones, but no relationship between ester-amide and the R group was found. For all active compounds (**4b**, **6a**, **6d**, and **6h** for MDA-MB-231 and **4b**, **4e**, **6c**, and **6d** for MCF-7), it seems to be a synergistic effect caused by the presence of the α-aminophosphonate, the corresponding R group and either the ester or amide part of each molecule. Based on the results of cell proliferation inhibition and apoptosis assays, we consider compounds **4e** and **6c** as our lead compounds, and even though further studies are needed in order to determine the action mechanism, our work provide valuable information for further design and development of future potent α-aminophosphonates as potential anticancer agents.

Acknowledgements The authors thank the National Council for Science and Technology (CONACYT) for its financial support via Project 180854. ELLC, also thanks CONACYT for a Graduate Scholarship 229877.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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