Synthesis, characterization and biological evaluation of novel *N*-phenoyl phosphatidylethanolamine derivatives

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Abstract

In this study, synthesis of new *N*-phenoyl phosphatidylethanolamine derivatives was carried out by the modification of natural egg phosphatidylethanolamine (PE) with various phenolic acids. All synthesized products were characterized by NMR, FT-IR and ESI-MS spectral analysis and were evaluated for their preliminary in vitro antioxidant and cytotoxic activities. Among the active derivatives, compounds *N*-4-hydroxy-3-methoxy cinnamoyl PE, *N*-4-hydroxy-3,5-dimethoxy cinnamoyl PE and *N*-(3,4)-di hydroxy cinnamoyl PE were exhibited excellent radical scavenging activity with EC₅₀ values of 9.7, 1.5 and 2.7 µg/mL, respectively which were lower than the standard BHT and α-tocopherols. The synthesized compounds exhibited moderate to very good anticancer activity against four cancer cell lines such as HeLa, B16-F10, SKOV3 and MCF-7 using doxorubicin as a positive control. All the synthesized derivatives may have potential applications in food emulsions and the presence of phenolics has to be examined for additional antioxidant and biological activities.

Keywords Phosphatidylethanolamine · Phenolic acids · Structured phenolipids · Antioxidant activity · Cytotoxic activity

1 Introduction

N-Acylethanolamines (NAEs) are bioactive lipids which exhibit a variety of biological activities related to energy balance, inflammation, pain sensation, neuro protection, cell proliferation, apoptosis, fertility, cognition and memory depending on the acyl chain [1, 2]. Among the NAEs, *N*-acyl phospholipids represent an unusual class of phospholipids (PLs) that contain head groups modified with fatty acids [3, 4]. *N*-Acyl phosphatidylethanolamines (NAPEs) naturally occur in diverse biological systems and accumulate during cell injury or stress, such as those resulting from ischemia in animal tissues and dehydration

in plant seeds [5]. NAPEs also play signaling roles, as they are the source of *N*-acyl ethanolamides, including anandamide (*N*-arachidonoyl ethanolamide), which binds to CB1 central receptors acting as a cannabinoid agonist [6, 7]. In addition, NAPEs have been found to be interesting molecules in lipid based formulations for drug delivery applications [8]. Research reports published on the synthesis of NAPEs [9] reported interesting applications of NAPEs in biomedical and pharmaceutical areas. Head group modification of PE with fatty acids was extensively studied but similar modification with other functional compounds like phenolics is not examined so far. In one of the study, Sydow et al. [10] modified the lipoprotein

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based polyethylene glycerol-PE micelles as nano carriers for enhanced uptake efficiency into blood–brain barrier cells to study the cytotoxicity against glioma cells. This has led to an increased interest in research related to modification of PE with compounds other than fatty acids to produce novel derivatives of PE molecules for applications in biological and pharmaceutical industries. Hence, it will be interesting to investigate on the phenolic acid derivatives of PE which could produce interesting hybrid lipid molecules.

Phenolic acids (PAs) are ubiquitous natural compounds found in fruits and vegetables which are associated with color, sensory qualities, nutritional and antioxidant properties [11, 12]. Phenolic compounds and related derivatives were reported to exhibit antimicrobial, antioxidant, anticancer, antiproliferative, antitumor, antiviral and Alzheimer's disease [13–17]. Very few reports were published on phenolic compounds conjugated with PLs and these types of compounds can have interesting properties [18]. Recently, Casado et al. [19] described the transphosphatidylolation of phosphatidylcholine with phenyl tyrosol using phospholipase D to produce novel phosphatidylphenyltyrosol and the resulting molecules were found to exhibit promising antioxidant activities. In view of the biological activities of phenolic compounds and phospholipids in biomedical fields, it would be interesting to investigate on the synthesis of novel structured phenolic phospholipids by the modification of PE. In the present study, the synthesis and characterization of *N*-phenoyl phosphatidylethanolamine derivatives from egg PE is described with selected phenolic acids. The synthesized compounds were evaluated for in vitro antioxidant and cytotoxic activities.

2 Experimental

Hen eggs were obtained locally. Imidazole, *tert*-butyl diphenyl silyl chloride, 1,8-diazabicyclo [5.4.0]-undec-7-ene (DBU), tetra-*n*-butyl ammonium fluoride and oxalyl chloride were purchased from M/s Sigma Chemicals, St Louis, USA. 4-Hydroxy phenyl acetic acid, 4-hydroxy phenyl propanoic acid, cinnamic acid, 4-hydroxy-3-methoxy cinnamic acid, 4-hydroxy-3,5-dimethoxy cinnamic acid, 3,4-dihydroxy cinnamic acid, 4-hydroxy-3-methoxy benzoic acid and 4-hydroxy-3,5-dimethoxy benzoic acids were procured from Alfa Aesar, England. Sodium chloride, sodium sulphate and silica gel were purchased from SDFCL, Mumbai, India. Pre-coated silica gel plates 60 F₂₅₄ for TLC and all solvents (analytical grade) were purchased from Merck, Darmstadt, Germany. ¹H and ¹³C NMR spectra were recorded on Bruker Avance 700/500 MHz and 175/125 MHz, respectively. The NMR spectra were referenced to δ 7.26 ppm and δ 77.0 ppm in CDCl₃ solvent for

¹H and ¹³C NMR, respectively. Mass spectra was recorded using electron spray ionization on Waters e2695 Separators module (Waters, Milford, MA, USA) Mass Spectrometer. FT-IR spectrum was recorded in chloroform on a Perkin–Elmer Fourier Transform spectrum BX instrument (Model: Spectrum BX; Connecticut, USA).

2.1 Isolation of phosphatidylethanolamine

Crude egg yolk (140 g) separated from hen eggs (eight eggs) was dispersed in acetone (100 mL) and added slowly to chilled acetone (700 mL; 1:5, wt of the egg yolk/vol of acetone) at 10 °C, while stirring the contents [20]. The contents were centrifuged at 5–10 °C for 20 min. The acetone layer containing neutral lipids was decanted and the insolubles were washed with chilled acetone (3 × 700 mL) followed by centrifugation to obtain PL mixture (75.8 g). The PL mixture (5 g) was separated by silica gel column chromatography using a mixture of chloroform/methanol (90:20, v/v) as eluent to obtain the pure PE (0.9 g). Ninhydrin spray reagent was used to confirm the presence of PE on TLC.

2.2 Synthesis of protected phenolic acids

Hydroxyl group protection of phenolic acids with *tert*-butyl diphenyl silyl chloride (TBDPSCI) was carried out following a reported protocol [18]. Briefly, imidazole (1.3 eq) was added to a solution of phenolic acid (1 eq) in CH₂Cl₂ (60 mL) at 0 °C followed by drop wise addition of TBDPSCI (1.1 eq). The contents were stirred at 0 °C for 1 h. The progress of the reaction was monitored by TLC using hexane:ethyl acetate (7:3, v/v). After 1 h, the reaction was quenched with water (70 mL) and extracted with CH₂Cl₂ (3 × 60 mL). The organic layers were dried over anhydrous sodium sulphate and concentrated using rotary evaporator. The residue was purified by silica gel column chromatography using solvent system hexane:ethylacetate (84:16, v/v) to obtain the corresponding silyl ether. The products were confirmed by ¹H NMR, ¹³C NMR, ESI-MS, and FT-IR spectral data.

The spectral data for 4-(*tert*-butyl diphenyl silyloxy)-3-methoxy cinnamic acid (**2b**), 4-(*tert*-butyl diphenyl silyloxy)-3,5-dimethoxy cinnamic acid (**2c**), 3,4-di-*tert*-butyl diphenyl silyloxy cinnamic acid (**2d**), 4-(*tert*-butyl diphenyl silyloxy)-3-methoxy benzoic acid (**5b**), 4-(*tert*-butyl diphenyl silyloxy)-3,5-dimethoxy benzoic acid (**5c**), 4-(*tert*-butyl diphenyl silyloxy) phenyl acetic acid (**8e**) and 4-(*tert*-butyl diphenyl silyloxy) phenyl propanoic acid (**8f**) is provided in the supplementary material.

2.3 Synthesis of protected phenolic acid chlorides

Acid chlorides were prepared employing a reported protocol [21] from protected phenolic acids and oxalyl chloride. Briefly, the protected phenolic acids (1 eq) was dissolved in DCM (30 mL) with two drops of DMF and cooled to 0 °C and oxalyl chloride (1.2 eq) was slowly added over 15 min. The reaction was allowed to stir for 5 h. After 5 h, oxalyl chloride was evaporated under high pressure vacuum and the product acid chlorides in DCM used as next step without purification.

2.4 Synthesis of N-protected phenoyl phosphatidylethanolamine

DBU (1 eq) was added to a solution of phosphatidylethanolamine (1 eq) in CH_2Cl_2 (20 mL), followed by drop wise addition of the solution of 4-(*tert*-butyl diphenyl silyloxy) phenoyl chloride (1.2 eq) in CH_2Cl_2 (5 mL) at ambient temperature. The resultant solution was stirred for 16 h and the progress of the reaction was monitored by TLC with UV detection and further confirmed by ninhydrin negative test. After maximum conversion, the organic layer was washed with brine (3×30 mL) followed by distilled water and dried over anhydrous sodium sulphate and concentrated using rotary evaporator. The crude product was purified using silica gel column chromatography by eluting the product with chloroform: methanol (88:12, v/v) to afford pure N-protected phenoyl PE derivatives [22]. The products were confirmed by ^1H NMR, ^{13}C NMR, ESI-MS, and FT-IR spectral data.

N-Cinnamoyl phosphatidylethanolamine (11a): Yield, 77.1% (230.3 mg) as a light red semi solid. R_f 0.7 (chloroform:methanol:water, 65:25:4, v/v/v); ^1H NMR (500 MHz, CDCl_3): δ 7.58–7.48 (5H, m, Ar-H), 7.41 (1H, d, J = 15.72 Hz, Ar-CH=CH-), 6.61 (1H, d, J = 15.72 Hz, Ar-CH=CH-), 5.33 (m, $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 5.20 (1H, m, sn -2), 4.33 (2H, m, sn -1), 4.15 (2H, m, sn -3), 4.00 (2H, m, $-\text{CH}_2-\text{CH}_2-\text{NH}$), 3.58 (2H, m, $-\text{CH}_2-\text{NH}$), 2.31 (m, $-\text{CH}_2-\text{CH}_2-\text{COO}$), 1.98 (m, $-\text{CH}_2-\text{CH}=\text{CH}-$), 1.52 (m, $-\text{CH}_2-\text{CH}_2-\text{COO}$), 1.25 (m, $-\text{CH}_2-$), 0.87 (m, $-\text{CH}_2-\text{CH}_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 173.4 ($-\text{CH}_2-\text{COO}$, sn -2), 173.1 ($-\text{CH}_2-\text{COO}$, sn -1), 166.9 ($-\text{NH}-\text{CO}$), 140.5 (Ar-CH=CH-), 134.9 (Ar), 130.1 ($-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 129.6 ($-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 128.6 (Ar), 127.8 (Ar), 121.1 (Ar-CH=CH-), 70.3 (sn -2), 64.3 ($-\text{CH}_2-\text{CH}_2-\text{NH}$), 63.9 (sn -3), 62.7 (sn -1), 40.3 ($-\text{CH}_2-\text{NH}$), 33.9 ($-\text{CH}_2-\text{COO}$), 31.1 ($-\text{CH}_2-$), 29.6 ($-\text{CH}_2-$), 29.3 ($-\text{CH}_2-$), 27.1 ($-\text{CH}_2-$), 25.5 ($-\text{CH}_2-$), 22.6 ($-\text{CH}_2-$), 14.0 ($-\text{CH}_2-\text{CH}_3$). ESI-MS (m/z): 846.86 (M-1), 874.74 (M-1), 896.40 (M-1); FT-IR (neat, cm^{-1}): 3069.2 (=C-H st), 2922.1 (-C-H st), 2852.4 (-C-H st), 1735.8 (-C=O st), 1656.5 (-NH-CO- st), 1625.1 (-C=C- st), 1543.3 (Ar st), 1055.9 (P-O-C).

N-4-(*tert*-butyl diphenyl silyloxy)-3-methoxy cinnamoyl phosphatidylethanolamine (11b): Yield, 62.2% (193.4 mg) as a light yellow semi solid. R_f 0.75 (chloroform:methanol:water, 65:25:4, v/v/v); ^1H NMR (500 MHz, CDCl_3): δ 7.67 (4H, m, Ar-H), 7.52 (1H, d, J = 13.81 Hz, Ar-CH=CH-), 7.40 (1H, s, Ar-H), 7.36–7.30 (6H, m, Ar-H), 6.87 (1H, d, J = 7.33 Hz, Ar-H), 6.77 (1H, d, J = 7.33 Hz, Ar-H), 6.62 (1H, d, J = 13.81 Hz, Ar-CH=CH-), 5.33 (m, $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 5.17 (1H, m, sn -2), 4.32 (2H, m, sn -1), 4.11 (2H, m, sn -3), 3.93 (2H, m, $-\text{CH}_2-\text{CH}_2-\text{NH}$), 3.48 (2H, m, $-\text{CH}_2-\text{NH}$), 3.29 (3H, s, $-\text{OCH}_3$), 2.21 (m, $-\text{CH}_2-\text{CH}_2-\text{COO}$), 2.04 (m, $-\text{CH}_2-\text{CH}=\text{CH}-$), 1.51 (m, $-\text{CH}_2-\text{CH}_2-\text{COO}$), 1.24 (m, $-\text{CH}_2-$), 1.08 (9H, s, $-\text{C}(\text{CH}_3)_3$), 0.87 (m, $-\text{CH}_2-\text{CH}_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 173.6 ($-\text{CH}_2-\text{COO}$, sn -2), 173.1 ($-\text{CH}_2-\text{COO}$, sn -1), 167.1 ($-\text{NHf}-\text{CO}$), 150.5 (Ar), 146.6 (Ar), 140.9 (Ar-CH=CH-), 135.1 (Ar), 133.1 (Ar), 130.3 ($-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 129.6 (Ar), 128.1 (Ar), 127.4 (Ar), 121.1 (Ar), 120.1 (Ar), 118.5 (Ar-CH=CH-), 114.1 (Ar), 111.3 (Ar), 70.3 (sn -2), 64.4 ($-\text{CH}_2-\text{CH}_2-\text{NH}$), 63.6 (sn -3), 62.8 (sn -1), 55.1 ($-\text{OCH}_3$), 40.2 ($-\text{CH}_2-\text{NH}$), 33.9 ($-\text{CH}_2-\text{COO}$), 31.8 ($-\text{C}(\text{CH}_3)_3$), 31.4 ($-\text{CH}_2-$), 29.3 ($-\text{CH}_2-$), 29.6 ($-\text{CH}_2-$), 27.1 ($-\text{CH}_2-$), 26.5 ($-\text{C}(\text{CH}_3)_3$), 24.7 ($-\text{CH}_2-$), 22.7 ($-\text{CH}_2-$), 19.7 ($-\text{CH}_2-$), 14.0 ($-\text{CH}_2-\text{CH}_3$). ESI-MS (m/z): 1131.35 (M-1), 1159.18 (M-1), 1180.79 (M-1). FT-IR (neat, cm^{-1}): 3071.2 (=C-H st), 2926.0 (-C-H st), 2854.8 (-C-H st), 1737.4 (-C=O st), 1658.5 (-NH-CO- st), 1621.2 (-C=C- st), 1521.3 (Ar st), 1113.2 (C-O-Si), 1066.8 (P-O-C).

N-4-(*tert*-butyl diphenyl silyloxy)-3-methoxy benzoyl phosphatidylethanolamine (13b): Yield, 70.4% (214.3 mg) as a dark brown semi solid. R_f 0.75 (chloroform:methanol:water, 65:25:4, v/v/v); ^1H NMR (500 MHz, CDCl_3): δ 7.67 (4H, m, Ar-H), 7.52 (1H, s, Ar-H), 7.41 (1H, d, J = 7.33 Hz, Ar-H), 7.36–7.30 (6H, m, Ar-H), 6.77 (1H, d, J = 7.33 Hz, Ar-H), 5.33 (m, $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 5.17 (1H, m, sn -2), 4.31 (2H, m, sn -1), 4.13 (2H, m, sn -3), 3.92 (2H, m, $-\text{CH}_2-\text{CH}_2-\text{NH}$), 3.48 (2H, m, $-\text{CH}_2-\text{NH}$), 3.29 (3H, s, $-\text{OCH}_3$), 2.21 (m, $-\text{CH}_2-\text{CH}_2-\text{COO}$), 2.03 (m, $-\text{CH}_2-\text{CH}=\text{CH}-$), 1.51 (m, $-\text{CH}_2-\text{CH}_2-\text{COO}$), 1.25 (m, $-\text{CH}_2-$), 1.08 (9H, s, $-\text{C}(\text{CH}_3)_3$), 0.87 (m, $-\text{CH}_2-\text{CH}_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 173.4 ($-\text{CH}_2-\text{COO}$, sn -2), 173.1 ($-\text{CH}_2-\text{COO}$, sn -1), 167.3 ($-\text{NH}-\text{CO}$), 149.1 (Ar), 146.8 (Ar), 135.1 (Ar), 131.9 (Ar), 130.4 ($-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 129.9 (Ar), 129.6 (Ar), 128.5 (Ar), 127.4 (Ar), 126.9 (Ar), 120.6 (Ar), 114.2 (Ar), 110.9 (Ar), 70.3 (sn -2), 64.3 ($-\text{CH}_2-\text{CH}_2-\text{NH}$), 63.6 (sn -3), 62.5 (sn -1), 56.0 ($-\text{OCH}_3$), 41.2 ($-\text{CH}_2-\text{NH}$), 34.0 ($-\text{CH}_2-\text{COO}$), 31.8 ($-\text{C}(\text{CH}_3)_3$), 31.4 ($-\text{CH}_2-$), 29.6 ($-\text{CH}_2-$), 29.2 ($-\text{CH}_2-$), 27.1 ($-\text{CH}_2-$), 26.4 ($-\text{C}(\text{CH}_3)_3$), 25.5 ($-\text{CH}_2-$), 24.8 ($-\text{CH}_2-$), 22.6 ($-\text{CH}_2-$), 14.0 ($-\text{CH}_2-\text{CH}_3$). ESI-MS (m/z): 1104.84 (M-1), 1132.81 (M-1), 1154.87 (M-1). FT-IR (neat, cm^{-1}): 3011.4 (=C-H st), 2924.8 (-C-H st), 2853.9 (-C-H st), 1739.0 (-C=O st), 1646.1 (-NH-CO- st), 1623.2 (-C=C- st), 1599.1 (Ar st), 1169.7 (C-O-Si), 1069.7 (P-O-C).

The spectral data for N-4-(*tert*-butyl diphenyl silyloxy)-3,5-dimethoxy cinnamoyl phosphatidylethanolamine (**11c**), N-(3,4)-(di-*tert*-butyl diphenyl silyloxy) cinnamoyl phosphatidylethanolamine (**11d**), N-4-(*tert*-butyl diphenyl silyloxy)-3,5-dimethoxy benzoyl phosphatidylethanolamine (**13c**), N-4-(*tert*-butyl diphenyl silyloxy) phenyl acetoylethanolamine (**15e**) and N-4-(*tert*-butyl diphenyl silyloxy) phenyl propanoyl phosphatidylethanolamine (**15f**) is provided in the Supplementary material.

2.5 Synthesis of *N*-phenoyl phosphatidylethanolamine

N-Protected phenoyl phosphatidylethanolamine was deprotected with tetra-*n*-butyl ammonium fluoride (TBAF) as described by Li et al. [23]. Briefly, to a stirred solution of N-protected phenoyl phosphatidylethanolamine (1 eq) in THF (3 mL) was slowly added TBAF (1.0 M in THF, 1.5 eq) at 0 °C. After stirring the contents for 40 min at 0 °C, the THF solvent was removed from the reaction mixture using rotary evaporator. The crude product was extracted with CH₂Cl₂ and washed with water and brine consecutively, dried over anhydrous sodium sulphate and concentrated using rotary evaporator. The crude product was purified by silica gel column chromatography eluting the product using chloroform: methanol (80:20, v/v) to afford pure *N*-phenoyl PE derivatives. The products were confirmed by ¹H NMR, ¹³C NMR, ESI-MS, and FT-IR spectral data.

N-4-Hydroxy-3-methoxy cinnamoyl phosphatidylethanolamine (12b): Yield, 75.7% (90.3 mg) as a light yellow semi solid. *R*_f 0.7 (chloroform:methanol:water, 65:25:4, v/v/v); ¹H NMR (500 MHz, CDCl₃): δ 7.51 (1H, d, *J* = 15.86 Hz, Ar-CH=CH-), 7.37 (1H, s, Ar-H), 7.33 (1H, d, *J* = 7.65 Hz, Ar-H), 7.04 (1H, d, *J* = 7.65 Hz, Ar-H), 6.49 (1H, d, *J* = 15.86 Hz, Ar-CH=CH-), 5.32 (m, -CH₂-CH=CH-CH₂-), 5.20 (1H, m, *sn*-2), 4.30 (2H, m, *sn*-1), 4.12 (2H, m, *sn*-3), 3.98 (2H, m, -CH₂-CH₂-NH), 3.47 (2H, m, -CH₂-NH), 3.15 (3H, s, -OCH₃), 2.22 (m, -CH₂-CH₂-COO), 1.99 (m, -CH₂-CH=CH-), 1.58 (m, -CH₂-CH₂-COO), 1.24 (m, -CH₂-), 0.86 (m, -CH₂-CH₃). ¹³C NMR (125 MHz, CDCl₃): δ 173.6 (-CH₂-COO, *sn*-2), 173.1 (-CH₂-COO, *sn*-1), 167.1 (-NH-CO), 150.5 (Ar), 149.8 (Ar), 146.6 (Ar-CH=CH-), 130.3 (-CH₂-CH=CH-CH₂-), 129.6 (Ar), 120.1 (Ar), 118.5 (Ar-CH=CH-), 114.1 (Ar), 111.3 (Ar), 70.3 (*sn*-2), 64.4 (-CH₂-CH₂-NH), 63.6 (*sn*-3), 62.8 (*sn*-1), 56.3 (-OCH₃), 40.2 (-CH₂-NH), 33.9 (-CH₂-COO), 31.4 (-CH₂-), 29.6 (-CH₂-), 29.2 (-CH₂-), 27.1 (-CH₂-), 25.5 (-CH₂-), 24.7 (-CH₂-), 22.6 (-CH₂-), 19.4 (-CH₂-), 14.0 (-CH₂-CH₃). ESI-MS (*m/z*): 892.67 (M-1), 920.78 (M-1), 942.65 (M-1). FT-IR (neat, cm⁻¹): 3311.5 (-OH st), 3019.9 (=C-H st), 2921.3 (-C-H st), 2851.9 (-C-H st), 1734.7 (-C=O st), 1654.9 (-NH-CO- st), 1619.7 (-C=C- st), 1542.3 (Ar st), 1053.2 (P-O-C).

N-4-Hydroxy-3,5-dimethoxy cinnamoyl phosphatidylethanolamine (12c): Yield, 72.1% (86.5 mg) as a light brown semi solid. *R*_f 0.7 (chloroform:methanol:water, 65:25:4, v/v/v); ¹H NMR (500 MHz, CDCl₃): δ 7.51 (1H, d, *J* = 14.75 Hz, Ar-CH=CH-), 6.58 (2H, s, Ar-H), 6.22 (1H, d, *J* = 14.75 Hz, Ar-CH=CH-), 5.33 (m, -CH₂-CH=CH-CH₂-), 5.21 (1H, m, *sn*-2), 4.33 (2H, m, *sn*-1), 4.13 (2H, m, *sn*-3), 3.90 (2H, m, -CH₂-CH₂-NH), 3.48 (2H, m, -CH₂-NH), 3.09 (6H, s, -OCH₃), 2.29 (m, -CH₂-CH₂-COO), 2.01 (m, -CH₂-CH=CH-), 1.56 (m, -CH₂-CH₂-COO), 1.25 (m, -CH₂-), 0.87 (m, -CH₂-CH₃). ¹³C NMR (125 MHz, CDCl₃): δ 173.6 (-CH₂-COO, *sn*-2), 173.2 (-CH₂-COO, *sn*-1), 166.5 167.0 (-NH-CO), 151.4 (Ar), 146.6 (Ar-CH=CH-), 130.2 (-CH₂-CH=CH-CH₂-), 129.9 (Ar), 117.5 (Ar-CH=CH-), 104.3 (Ar), 70.7 (*sn*-2), 64.9 (-CH₂-CH₂-NH), 64.1 (*sn*-3), 62.3 (*sn*-1), 56.3 (-OCH₃), 40.6 (-CH₂-NH), 34.0 (-CH₂-COO), 31.1 (-CH₂-), 29.3 (-CH₂-), 29.7 (-CH₂-), 27.2 (-CH₂-), 24.8 (-CH₂-), 22.6 (-CH₂-), 14.0 (-CH₂-CH₃). ESI-MS (*m/z*): 922.07 (M-1), 950.76 (M-1), 972.51 (M-1). FT-IR (neat, cm⁻¹): 3327.5 (-OH st), 3024.1 (=C-H st), 2921.8 (-C-H st), 1734.6 (-C=O st), 1654.5 (-NH-CO- st), 1624.3 (-C=C- st), 1542.6 (Ar st), 1046.2 (P-O-C).

N-(3,4)-Di hydroxy cinnamoyl phosphatidylethanolamine (12d): Yield, 70.5% (69.4 mg) as a light yellow semi solid. *R*_f 0.7 (chloroform:methanol:water, 65:25:4, v/v/v); ¹H NMR (500 MHz, CDCl₃): δ 7.67 (1H, d, *J* = 7.24 Hz, Ar-H), 7.56 (1H, d, *J* = 13.98 Hz, Ar-CH=CH-), 7.40 (1H, s, Ar-H), 6.99 (1H, d, *J* = 7.24 Hz, Ar-H), 6.62 (1H, d, *J* = 13.98 Hz, Ar-CH=CH-), 5.33 (m, -CH₂-CH=CH-CH₂-), 5.18 (1H, m, *sn*-2), 4.29 (2H, m, *sn*-1), 4.11 (2H, m, *sn*-3), 3.93 (2H, m, -CH₂-CH₂-NH), 3.48 (2H, m, -CH₂-NH), 2.22 (m, -CH₂-CH₂-COO), 2.03 (m, -CH₂-CH=CH-), 1.51 (m, -CH₂-CH₂-COO), 1.25 (m, -CH₂-), 0.87 (m, -CH₂-CH₃). ¹³C NMR (125 MHz, CDCl₃): δ 173.5 (-CH₂-COO, *sn*-2), 173.1 (-CH₂-COO, *sn*-1), 166.7 (-NH-CO), 150.5 (Ar), 149.1 (Ar), 146.5 (Ar-CH=CH-), 130.8 (-CH₂-CH=CH-CH₂-), 129.7 (Ar), 121.9 (Ar), 117.2 (Ar-CH=CH-), 113.1 (Ar), 111.4 (Ar), 70.3 (*sn*-2), 64.5 (-CH₂-CH₂-NH), 63.8 (*sn*-3), 62.6 (*sn*-1), 40.2 (-CH₂-NH), 34.9 (-CH₂-COO), 31.4 (-CH₂-), 29.6 (-CH₂-), 29.2 (-CH₂-), 27.7 (-CH₂-), 25.6 (-CH₂-), 24.9 (-CH₂-), 22.4 (-CH₂-), 19.7 (-CH₂-), 14.1 (-CH₂-CH₃). ESI-MS (*m/z*): 878.54 (M-1), 906.76 (M-1), 928.36 (M-1). FT-IR (neat, cm⁻¹): 3334.1 (-OH st), 3016.7 (=C-H st), 2920.3 (-C-H st), 1717.9 (-C=O st), 1685.3 (-NH-CO- st), 1634.4 (-C=C- st), 1542.5 (Ar st), 1057.8 (P-O-C).

N-4-Hydroxy-3-methoxy benzoyl phosphatidylethanolamine (14b): Yield, 76.2% (90.2 mg) as a dark brown semi solid. *R*_f 0.7 (chloroform:methanol:water, 65:25:4, v/v/v); ¹H NMR (500 MHz, CDCl₃): δ 7.54 (1H, s, Ar-H), 7.46 (1H, d, *J* = 7.42 Hz, Ar-H), 6.95 (1H, d, *J* = 7.42 Hz, Ar-H), 5.35 (m, -CH₂-CH=CH-CH₂-), 5.20 (1H, m, *sn*-2), 4.35 (2H, m, *sn*-1), 4.11 (2H, m, *sn*-3), 3.94 (2H, m, -CH₂-CH₂-NH), 3.61 (2H, m, -CH₂-NH), 3.40 (3H, s, -OCH₃), 2.26 (m,

–CH₂–CH₂–COO), 2.04 (m, –CH₂–CH=CH–), 1.57 (m, –CH₂–CH₂–COO), 1.25 (m, –CH₂–), 0.87 (m, –CH₂–CH₃). ¹³C NMR (125 MHz, CDCl₃): δ 173.4, (–CH₂–COO, *sn*-2), 173.0 (–CH₂–COO, *sn*-1), 167.3 (–NH–CO), 151.2 (Ar), 149.1 (Ar), 130.4 (–CH₂–CH=CH–CH₂–), 128.9 (Ar), 120.7 (Ar), 114.3 (Ar), 111.0 (Ar), 70.2 (*sn*-2), 64.3 (–CH₂–CH₂–NH), 63.7 (*sn*-3), 62.5 (*sn*-1), 56.0 (–OCH₃), 39.7 (–CH₂–NH), 34.0 (–CH₂–COO), 31.4 (–CH₂–), 29.6 (–CH₂–), 29.3 (–CH₂–), 27.1 (–CH₂–), 25.5 (–CH₂–), 24.8 (–CH₂–), 22.6 (–CH₂–), 19.6 (–CH₂–), 14.0 (–CH₂–CH₃). ESI-MS (*m/z*): 866.59 (M-1), 894.68 (M-1), 916.76 (M-1). FT-IR (neat, cm⁻¹): 3329.5 (–OH st), 3014.2 (=C–H st), 2923.5 (–C–H st), 2853.2 (–C–H st), 1735.4 (–C=O st), 1654.2 (–NH–CO– st), 1627.4 (–C=C– st), 1542.7 (Ar st), 1056.6 (P–O–C).

N-4-Hydroxy-3,5-dimethoxy benzoyl phosphatidylethanolamine (14c): Yield, 75.4% (90.1 mg) as a light yellow semi solid. *R*_f 0.7 (chloroform:methanol:water, 65:25:4, v/v/v); ¹H NMR (500 MHz, CDCl₃): δ 6.95 (2H, s, Ar–H), 5.33 (m, –CH₂–CH=CH–CH₂–), 5.20 (1H, m, *sn*-2), 4.36 (2H, m, *sn*-1), 4.11 (2H, m, *sn*-3), 3.91 (2H, m, –CH₂–CH₂–NH), 3.59 (2H, m, –CH₂–NH), 3.45 (6H, s, –OCH₃), 2.25 (m, –CH₂–CH₂–COO), 2.00 (m, –CH₂–CH=CH–), 1.57 (m, –CH₂–CH₂–COO), 1.25 (m, –CH₂–), 0.87 (m, –CH₂–CH₃). ¹³C NMR (125 MHz, CDCl₃): δ 173.4 (–CH₂–COO, *sn*-2), 173.1 (–CH₂–COO, *sn*-1), 167.2 (–NH–CO), 146.7 (Ar), 137.5 (Ar), 129.9 (–CH₂–CH=CH–CH₂–), 129.6 (Ar), 104.9 (Ar), 70.3 (*sn*-2), 64.0 (–CH₂–CH₂–NH), 63.5 (*sn*-3), 62.6 (*sn*-1), 56.5 (–OCH₃), 41.7 (–CH₂–NH), 34.0 (–CH₂–COO), 31.4 (–CH₂–), 29.6 (–CH₂–), 29.3 (–CH₂–), 27.1 (–CH₂–), 24.8 (–CH₂–), 22.6 (–CH₂–), 19.5 (–CH₂–), 14.0 (–CH₂–CH₃). ESI-MS (*m/z*): 896.75 (M-1), 924.83 (M-1), 946.57 (M-1). FT-IR (neat, cm⁻¹): 3323.4 (–OH st), 3019.0 (=C–H st), 2922.9 (–C–H st), 2853.0 (–C–H st), 1736.0 (–C=O st), 1648.5 (–NH–CO– st), 1625.4 (–C=C– st), 1543.9 (Ar st), 1056.0 (P–O–C).

N-4-Hydroxy phenyl acetoyl phosphatidylethanolamine (16e): Yield, 84.6% (99.9 mg) as a light red semi solid. *R*_f 0.7 (chloroform:methanol:water, 65:25:4, v/v/v); ¹H NMR (500 MHz, CDCl₃): δ 7.18 (2H, d, *J* = 7.82 Hz, Ar–H), 6.79 (2H, d, *J* = 7.82 Hz, Ar–H), 5.35 (m, –CH₂–CH=CH–CH₂–), 5.19 (1H, m, *sn*-2), 4.32 (2H, m, *sn*-1), 4.15 (2H, m, *sn*-3), 3.97 (2H, m, –CH₂–CH₂–NH), 3.64 (2H, s, Ar–CH₂–), 3.45 (2H, m, –CH₂–NH), 2.33 (m, –CH₂–CH₂–COO), 2.01 (m, –CH₂–CH=CH–), 1.60 (m, –CH₂–CH₂–COO), 1.25 (m, –CH₂–), 0.88 (m, –CH₂–CH₃). ¹³C NMR (125 MHz, CDCl₃): δ 173.5 (–CH₂–COO, *sn*-2), 172.9 (–CH₂–COO, *sn*-1), 171.6 (–NH–CO), 158.4 (Ar), 130.4 (–CH₂–CH=CH–CH₂–), 129.9 (Ar), 127.8 (Ar), 118.1 (Ar), 70.2 (*sn*-2), 64.5 (–CH₂–CH₂–NH), 63.7 (*sn*-3), 62.5 (*sn*-1), 40.0 (–CH₂–NH), 39.0 (Ar–CH₂–), 33.9 (–CH₂–COO), 31.4 (–CH₂–), 29.7 (–CH₂–), 22.6 (–CH₂–), 19.4 (–CH₂–), 14.0 (–CH₂–CH₃). ESI-MS (*m/z*): 850.57 (M-1), 878.85 (M-1), 900.57 (M-1). FT-IR (neat, cm⁻¹): 3339.6 (–OH st), 3023.2 (=C–H st), 2921.1 (–C–H st), 2851.9 (–C–H st),

1737.3 (–C=O st), 1648.7 (–NH–CO– st), 1630.4 (–C=C– st), 1543.0 (Ar st), 1058.7 (P–O–C).

N-4-Hydroxy phenyl propanoyl phosphatidylethanolamine (16f): Yield, 82.2% (97.4 mg) as a light red semi solid. *R*_f 0.7 (chloroform:methanol:water, 65:25:4, v/v/v); ¹H NMR (500 MHz, CDCl₃): δ 6.94 (2H, d, *J* = 7.70 Hz, Ar–H), 6.78 (2H, d, *J* = 7.70 Hz, Ar–H), 5.35 (m, –CH₂–CH=CH–CH₂–), 5.19 (1H, m, *sn*-2), 4.36 (2H, m, *sn*-1), 4.12 (2H, m, *sn*-3), 3.96 (2H, m, –CH₂–CH₂–NH), 3.46 (2H, m, –CH₂–NH), 2.79 (2H, t, *J* = 6.97 Hz, Ar–CH₂–CH₂–), 2.39 (2H, t, *J* = 6.72 Hz, Ar–CH₂–CH₂–), 2.26 (m, –CH₂–CH₂–COO), 2.01 (m, –CH₂–CH=CH–), 1.58 (m, –CH₂–CH₂–COO), 1.25 (m, –CH₂–), 0.87 (m, –CH₂–CH₃). ¹³C NMR (125 MHz, CDCl₃): δ 173.4 (–CH₂–COO, *sn*-2), 173.2 (–CH₂–COO, *sn*-1), 173.1 (–NH–CO), 155.3 (Ar), 131.6 (Ar), 130.3 (–CH₂–CH=CH–CH₂–), 129.1 (Ar), 115.5 (Ar), 70.2 (*sn*-2), 64.3 (–CH₂–CH₂–NH), 63.6 (*sn*-3), 62.5 (*sn*-1), 40.3 (–CH₂–NH), 38.3 (Ar–CH₂–CH₂–), 33.9 (–CH₂–COO), 31.4 (–CH₂–), 30.8 (–CH₂–), 29.6 (–CH₂–), 27.1 (–CH₂–), 25.5 (–CH₂–), 23.8 (–CH₂–), 22.6 (–CH₂–), 19.6 (–CH₂–), 14.0 (–CH₂–CH₃). ESI-MS (*m/z*): 864.71 (M-1), 892.65 (M-1), 914.69 (M-1). FT-IR (neat, cm⁻¹): 3338.3 (–OH st), 3022.7 (=C–H st), 2922.7 (–C–H st), 2853.0 (–C–H st), 1736.7 (–C=O st), 1649.5 (–NH–CO– st), 1632.4 (–C=C– st), 1543.8 (Ar st), 1056.9 (P–O–C).

2.6 Biological activities

2.6.1 Cytotoxic activity

The synthesized phenolic PE derivatives were tested for cytotoxicity assay against a panel of four different cell lines such as HeLa derived from Homo sapiens cervix adenocarcinoma cells, B16-F10 derived from Mouse skin melanoma cells (ATCC® CRL-6475™), SKOV3 derived from Human Ovarian cancer cells (ATCC® HTB-77™) and MCF7 derived from Human Breast Adenocarcinoma cells (ATCC® HTB-22™) using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay as described earlier [24]. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a 5% CO₂ incubator. After seeding of cells in 96 well culture plates, they were allowed to attach properly. Test compounds of different concentrations ranging from 1 to 50 µM were added and incubated for 24 h. The cells were then incubated with MTT (0.5 mg/mL) for 3 h and, to dissolve the insoluble formazan crystals, 100 µL dimethylsulfoxide was added to each well. Finally the absorbance of the plates was measured using a Synergy H1 multi-mode plate reader (USA). Doxorubicin was used as a positive control for comparison. The IC₅₀ values (50% inhibitory concentration) were calculated from the plotted absorbance data for the dose response curves. IC₅₀ values (in µM)

are expressed as the average of two independent experiments. The experiment was performed in triplicate and the values are expressed as the mean $\pm$ SD of at least three independent experiments.

2.6.2 Antioxidant activity

DPPH radical scavenging assay Antioxidant activity of the prepared phenolic PE derivatives was examined on the basis of the free radical scavenging effect on the sTable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical by a previously described method [25] with some modifications. Diluted working solutions of the prepared PLs and the positive controls were prepared in methanol. DPPH (1 ml, 0.002% prepared in methanol) was mixed with 1 ml of test PLs. Positive controls like BHT and α -Tocopherol was also run in parallel. The mixtures were vortexed and kept standing in the dark for 30 min. Later, the absorbance was measured at 517 nm on a Lambda 25 UV–visible spectrophotometer (Perkin–Elmer, Shelton, CT, USA). The radical scavenging activity was determined based on the decrease in the absorbance of DPPH. Lower absorbance of the reaction mixture corresponded to higher free radical scavenging activity. The DPPH radical scavenging activity was calculated using the formula of Bors and coworkers [26]: DPPH radical scavenging activity (%) = [(Absorbance of control—Absorbance of test sample)/(Absorbance of control)] $\times$ 100. The radical scavenging potential (EC₅₀ value) is the test compound concentration at which 50% of the DPPH radicals were scavenged. All experiments were performed in triplicate and values are represented as mean.

Table 1 Cytotoxic activity of *N*-phenoyl phosphatidylethanolamine derivatives

Test compounds	IC ₅₀ (μ M)			
	HeLa	B16-F10	SKOV3	MCF-7
16e	ND	23.89 $\pm$ 0.02	38.17 $\pm$ 0.76	ND
16f	ND	61.35 $\pm$ 0.86	9.38 $\pm$ 0.46	20.91 $\pm$ 2.00
11a	ND	26.50 $\pm$ 0.91	6.30 $\pm$ 0.49	16.56 $\pm$ 1.08
12b	ND	18.29 $\pm$ 0.17	9.54 $\pm$ 0.24	22.67 $\pm$ 2.44
12c	ND	34.45 $\pm$ 3.85	8.60 $\pm$ 0.72	16.86 $\pm$ 1.53
12d	ND	27.65 $\pm$ 0.15	6.19 $\pm$ 0.45	17.07 $\pm$ 1.76
14b	ND	42.25 $\pm$ 3.17	7.85 $\pm$ 0.42	16.16 $\pm$ 0.79
14c	ND	40.00 $\pm$ 2.47	10.22 $\pm$ 0.34	19.23 $\pm$ 1.46
Doxorubicin	0.8 $\pm$ 0.71	0.7 $\pm$ 0.56	0.8 $\pm$ 0.63	2.0 $\pm$ 0.81

ND: not detected; Data presented as mean $\pm$ SD, n = 3

Cell lines: HeLa, Homo sapiens cervix adenocarcinoma; B16-F10, Mouse skin melanoma (ATCC® CRL-6475™); SKOV3, Human Ovarian cancer (ATCC® HTB-77™); MCF7, Human Breast Adenocarcinoma (ATCC® HTB-22™)

3 Results and discussion

The present study was aimed at synthesis and characterization of a novel *N*-phenoyl phosphatidylethanolamine derivatives with egg PE and phenolic acids such as cinnamic acid, benzoic acid and 4-hydroxy phenyl fatty acid as substrates. All the synthesized products were characterized by NMR, FT-IR and ESI-MS spectral data and evaluated for in vitro antioxidant and cytotoxic activities. Initially, the hydroxyl group of phenolic acids was protected with TBDPSCI in presence of imidazole to obtain protected phenolic acids in 82–90% yields. The protected phenolic acid chlorides (Scheme 1) were prepared from their respective acids with oxalyl chloride and were immediately used for the next step without any purification. In the next step, amidation of PE with protected phenolic acid chlorides was carried out in presence of DBU to produce *N*-4-(*tert*-butyl diphenyl silyloxy) phenoyl PE in 60–79% yields. In the final step, TBDPS group was removed from *N*-4-(*tert*-butyl diphenyl silyloxy) phenoyl PE using TBAF as reagent to produce *N*-phenoyl PE derivatives in 70–85% yields (Scheme 2).

The prepared *N*-phenoyl PE derivatives were fully characterized and the structures of all intermediates and the final products were confirmed by NMR, FT-IR and ESI-MS spectral data. In *N*-phenoyl PE derivatives, the IR band observed at 1046–1059 cm^{−1} is characteristic for P–O–C bond. The IR bands at 1717–1738, 1648–1686 and 3311–3340 cm^{−1} are due to ester, amide and hydroxyl groups, respectively. The IR bands at 1542–1544 cm^{−1} and 1619–1635 cm^{−1} are due to aromatic and olefinic carbon–carbon double bonds, respectively.

The product *N*-cinnamoyl PE derivatives (i.e., *N*-4-hydroxy-3-methoxy cinnamoyl phosphatidylethanolamine, **12b**) were confirmed by ¹H NMR, ¹³C NMR and ESI-MS spectral data. The ¹H NMR spectra showed a multiplet at δ 2.2 ppm of the methylene groups attached to carbonyl group at *sn*-1 and *sn*-2 positions, multiplet at δ 3.4 ppm for two protons of the methylene group attached to nitrogen, singlet at δ 3.1 ppm for 3 protons of the methoxy group attached to aromatic ring, multiplet at δ 3.9 ppm for two protons of the methylene group attached to phosphate moiety, multiplet at 4.1–5.2 ppm for glycerol backbone. The multiplicity range in δ 6.4–7.5 ppm was observed which indicated the aromatic and α,β -unsaturated double bond protons. ¹³C NMR spectra showed peaks at 173.1 and 173.6 ppm for carbonyl carbon at *sn*-1 and *sn*-2 positions respectively, the peak at 167.1 ppm for amide carbon group, the peak at 56.3 ppm for methoxy group attached to aromatic ring, the peak at 40.2 ppm for methoxy group attached to nitrogen, the signals ranging in δ

Scheme 1 Synthesis of protected phenolic acid chlorides. (1) TBDPSCI, imidazole, CH₂Cl₂, 0 °C, 1 h; (2) oxalyl chloride, CH₂Cl₂, 0 °C, 5 h

111.3–150.5 ppm indicated the aromatic and double bond carbons. The ESI-MS data showed masses of 892.67 (M-1), 920.78 (M-1), 942.65 (M-1) for 1-palmitoyl-2-oleoyl-(N-4-hydroxy-3-methoxy cinnamoyl) phosphatidylethanolamine, 1-steroyl-2-oleoyl-(N-4-hydroxy-3-methoxy cinnamoyl) phosphatidylethanolamine, 1-steroyl-2-arachidonoyl-(N-4-hydroxy-3-methoxy cinnamoyl) phosphatidylethanolamine, respectively.

3.1 Biological activities

3.1.1 Cytotoxic activity

The synthesized phenolic PE derivatives were tested towards their effect on growth of cancer cell lines: homo sapiens cervix adenocarcinoma HeLa, mouse skin melanoma B16-F10, human ovarian cancer SKOV3 and human breast adenocarcinoma MCF-7, by MTT assay using doxorubicin as a positive control [24]. Cell viability was measured using MTT assay after 24 h of incubation with tested compounds in concentration 1–50 µM. The MTT assay enables the detection of viable cells by measuring the activity of reducing tetrazolium salt to formazan which is proportional to the percentage of living cells. In this manner, IC₅₀ molar concentration [µM] that inhibits 50% net cell growth was determined as presented in Table 1. The compounds whose IC₅₀ values were observed to be lower

and closer to the reference drug are considered as having good cytotoxic potential.

The results in Table 1 revealed that, the phenolic PE analogues **16e-f**, **11a**, **12b-d**, and **14b-c** demonstrated moderate to very good cytotoxic activities against four cell lines. All the tested compounds did not affect the cytotoxic activity against HeLa cell lines. The compound **16e** exhibited moderate cytotoxic activity against B16-F10 and SKOV3 cell lines. The compounds **16f**, **11a**, **12b**, **12c**, **12d**, **14b** and **14c** exhibited very good cytotoxic activity against SKOV3 cell line with IC₅₀ values of 9.38, 6.30, 9.54, 8.60, 6.19, 7.85 and 10.22 µM, respectively. On the other hand, **16f**, **11a**, **12b**, **12c**, **12d**, **14b** and **14c** exhibited good cytotoxic activity against MCF-7 cell line with IC₅₀ values of 20.91, 16.56, 22.67, 16.86, 17.07, 16.16, and 19.23 µM, respectively. Among all tested derivatives, compound **12b** exhibited good cytotoxic activity against B16-F10 cell line with IC₅₀ value of 18.29 µM. The cytotoxic activity of the phenolic derivatives tested showed to be strongly dependent on their structure [27]. It is observed that the derivatives comprised of functional groups such as hydroxy, methoxy, α, β-unsaturated double bonds, amides, esters and aromatic rings. It was verified that an increase in the number of the OH ring substituent's in the compounds investigated lead to higher cytotoxic activities in the tested cell lines. The presence of a double bond is associated with an increase in cell viability as compared to the saturated compounds [28]. The results indicated that

Scheme 2 Synthesis of *N*-phenoyl phosphatidylethanolamines. (1) DBU, CH₂Cl₂, 25 °C, 16 h; (2) TBAF, THF, 0 °C, 40 min

cinnamic acid derivatives were more potent compared to benzoic acid and phenyl fatty acid derivatives. However, all synthesized derivatives were observed to exhibit lower cytotoxic activity when compared to the reference drug doxorubicin which showed IC₅₀ values in the range of 0.7–2.0 μM against the tested cell lines.

3.1.2 Antioxidant activity

The antioxidant activity of phenolic PE derivatives was evaluated by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay using BHT and α-tocopherol as standard anti-oxidants for comparison [25]. The amount of compound needed to inhibit the radicals by 50% was estimated and the values are given as their effective concentration (EC₅₀) values. The results obtained for the antioxidant evaluation are given in the Table 2.

It was observed that the synthesized phenolic PE derivatives exhibited positive results in DPPH radical scavenging assay. BHT and α-tocopherols were used as reference

Table 2 Antioxidant activity of *N*-phenoyl phosphatidylethanolamine derivatives

Test compounds	EC ₅₀ (μg/mL) (Mean ± S.D.)
16e	ND
16f	ND
11a	ND
12b	9.72 ± 0.10
12c	1.57 ± 0.03
12d	2.72 ± 0.10
14b	75.74 ± 1.5
14c	66.7 ± 0.20
BHT	28.1 ± 0.21
α-Tocopherol	11.5 ± 0.12

ND: not detected; Data presented as mean ± SD, n = 3

compounds. From Table 2, it can be observed that some of synthesized PE-N-phenolic acid derivatives were found to exhibit moderate to good antioxidant activity. Compounds **16e**, **16f** and **11a** did not show any radical scavenging activity and compounds **14b** and **14c** exhibited better activity compared to these compounds with EC₅₀ values of 75.7 and 66.7 µg/mL, respectively. Among the active derivatives, compounds **12c**, **12d** and **12b** exhibited excellent radical scavenging activity with EC₅₀ values of 1.5, 2.7 and 9.7 µg/mL, respectively which were lower than the standard BHT and α-tocopherols which showed EC₅₀ values of 28.1 and 11.5 µg/mL, respectively. According to the structure activity relationship of the prepared PE-N-phenolic acid derivatives, it can be observed that the cinnamic acid derivatives were found to be more efficient than their benzoic acid derivatives. The double bond of propanoic derivatives probably participates in stabilizing the radical by resonance as reported in the literature [29, 30]. Earlier studies on antioxidant activity of phenolipids suggest that the number, position of hydroxyl and methoxy functionality also have influence on the antioxidant activity [12, 31–33]. Previous reports also suggest that the increase of methoxy groups substantially increased the antioxidant activity of the compounds by further stabilizing the phenoxyl radical [34]. From all the tested compounds, the compounds **12c**, **12d** and **12b** exhibited the highest DPPH radical activity with EC₅₀ values of 1.5, 2.7 and 9.7 µg/mL, respectively. These types of phenolic PE derivatives can have potential applications in both food and pharmaceutical industries as they are derived from natural materials as substrates.

4 Conclusions

Novel *N*-phenoyl phosphatidylethanolamine derivatives were synthesized by modifying the head group of egg PE at amine functionality with phenolic acids for the first time. The amidation of natural egg PE with phenolic acids employing four steps to obtain *N*-phenoyl PE derivatives is described. The overall yields of the products are in the range of 34.4–60.4% from starting PE. All the products were characterized by NMR, FT-IR, and ESI mass spectral analysis. The synthesized compounds were evaluated for in vitro antioxidant and cytotoxic activities where it was found that the compounds showed moderate to good cytotoxic activity and excellent antioxidant activity. The novel *N*-phenoyl PE derivatives may have potential applications in food emulsions and at the interface of chemistry and biology. The presence of phenolics has to be examined and compared for additional antioxidant and biological activities that are reported for the *N*-acyl PE derivatives in order to know the potential of these novel compounds.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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