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A novel approach to determine anti-proliferative, anti-migratory and anti-microbial properties of 2-phenylethylammonium carboxylate molecular salts

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2-Phenylethylammonium (PEA) salt derivatives have been prepared between 2-phenylethylamine with various aromatic carboxylic acids (nicotinic, benzoic, salicylic, and γ-resorcylic) and confirmed by spectroscopic analyses. Additionally, the possible biological activities of these salts have been examined and the antiproliferative and antimigrative effects of salts on breast and colon cancer cell lines have been determined. Furthermore, to obtain preliminary data for the effects of the indicated compounds on cancer angiogenesis, *in vitro* migration analysis has been performed by human vascular endothelial cells. The antibacterial effects of the compounds have also been investigated. Therefore, an important contribution has been made to studies conducted to better recognize the pharmacological effects of salts.

Keywords: Antibacterial effect, Antimigratory effect, Antiproliferative effect, Cancer, Salt, Spectroscopy

In the field of pharmaceutical ingredients, solid organic salts have been studied based on properties such as solubility, chemical and thermal stability, and hygroscopicity due to non-covalent interactions[1]. Organic acids have nice donor-acceptor groups[2-4]. The structure of acid-base-mediated salts can be controlled by combining the corresponding cations and anions (consisting of organic acids, aromatic and aliphatic amine derivatives), which allows us to prepare biologically active compounds[5,6].

Phenolic acids such as salicylic (2-hydroxybenzoic), coumaric (hydroxycinnamic), and vanillic (4-hydroxy-3-methoxybenzoic) acid used as an anion source in the synthesis of salts have shown various biological properties such as antioxidant, anti-cancer, anti-bacterial, anti-mutagenic, anti-inflammatory, and anti-viral[7]. Benzoic, salicylic, and nicotinic (3-pyridine carboxylic) acids, which are active parts of some drugs, are converted into sodium salts, better soluble than free ligands, and better assimilated by human organisms[8]. Pyridine carboxylic acids and their derivatives, which form an important anthelmintic and vitamin group, also have very interesting pharmaceutical properties. Especially nicotinic acid has very interesting pharmaceutical properties that play a vital role in the metabolism of all living cells, and studies have focused on transition metal complexes[9]. Salicylates, benzoates, and nicotinates have been reported as antimicrobial compounds[10]. In addition, phosphazene-based salts with significant antiproliferative, antibacterial and antifungal effects were prepared from the reactions of fully substituted cyclotriphosphazenes with gentisic acid (2,5-dihydroxybenzoic acid)[11-13].

In this study, a series of four salts based on 2-phenylethylammonium, consisting of anions of aromatic organic acid: nicotinate **1** (C_6H_4N-COO^-), benzoate **2** (C_6H_5-COO^-), 2-hydroxybenzoate **3** (C_6H_5O-COO^-) and 2,6-dihydroxybenzoate **4** ($C_6H_5O_2$-COO^-) was synthesized. Their structure was characterized by FT-IR, ^1H and ^{13}C NMR spectroscopy and elemental analysis. The antiproliferative effects of salts were investigated *in vitro* on MDA-MB-231 and HT29 cells, which are the most commonly used breast cancer and colon cancer cell lines in medical research. Furthermore, to investigate the effects of salts on cell migration, the *in vitro* wound-healing assay by these cancer cell lines was performed. Because cell migration is involved in several pathological processes such as tumor invasion and metastasis. In addition, to obtain a preliminary data for the effects of the indicated compounds on cancer angiogenesis, *in vitro* migration analysis was performed by human vascular endothelial

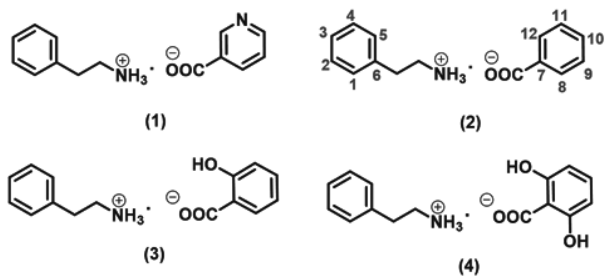
cells (HUVECs). Furthermore, in order to explore the antibacterial activity, it was studied to determine the minimal inhibitory concentrations (MIC). The salts were screened for their *in vitro* antibacterial activities against two gram-negatives and one gram-positive bacteria.

Results and Discussion

Chemistry

PEA salt derivatives were formed by transferring protons from a range of aromatic carboxylic acids to 2-phenylethylamine (Scheme 1). All the compounds are solid-state. In the case of primary amines, the formation of species containing protonated base/protonated acid pairs can cause salts to have higher melting points[14]. In this regard, the melting point of salts (except **3**) is above 100°C. All the salts were soluble in polar solvents such as water, methanol, ethanol, DMSO, *etc*. NMR (^{1}H and ^{13}C) and FT-IR spectral data confirmed the structures of the salts in concordance with elemental analysis.

FT-IR spectroscopy is one of the classic experimental methods known to be useful in providing information about the structural properties of salts. The characteristic bands of the functional groups of all the salts are presented in the "Experimental Details" and the FT-IR spectra of the salts are depicted in Figs S1-S4 (Supplementary Information). The asymmetric and symmetric stretching vibrations of the characteristic ammonium band appear in the spectral region of 3200-2500 cm^{-1} [15]. In the IR spectra of the salts, the $\nu_{as}(NH_3^+)$ stretching was observed as a medium band at about 3027 cm^{-1} while the $\nu_s(NH_3^+)$ stretching vibrations gave a broadband at about 2489 cm^{-1}. The spectrum features also two peaks at about 2165 and 2289 cm^{-1} and these correspond to stretching modes of the N–H O hydrogen bonds[16]. The band at about 1535 cm^{-1} was due to N-H bending vibrations of the salts. The salts with carboxylate anions showed two absorption bands at about 1635 cm^{-1} and 1374 cm^{-1} which are representing the asymmetric and symmetric stretches of the carboxylate group, respectively[17]. The bands at about 1588 cm^{-1}, 1480 cm^{-1}, and 756 cm^{-1} belong to the typical absorption of aromatic ring[18]. ν(C=C) stretches of 2-phenylethylammonium cation were observed at 1558 and 1480 cm^{-1}. The band at 756 cm^{-1} corresponded to the bending vibration of C-H in 2-phenylethylammonium. Besides, the stretching vibration of aromatic C-H groups was found at about 3081 and 3058 cm^{-1}.



Scheme 1 — The scheme represents the PEA salt derivatives

The chemical shifts, multiplicities, and coupling constants are very useful for the interpretation of the ^{1}H and ^{13}C NMR signals of all the salts and presented in the "Experimental Section". The ^{1}H and ^{13}C NMR spectra of salts are given in Figs S5-S12. ^{1}H and ^{13}C NMR of the salts were performed in methanol-d_4. The ammonium CH_2 protons for all salts were observed at about δ 2.86 (PhCH$_2$CH_2) and 3.08 (PhCH_2CH$_2$), respectively. The average coupling constant, $^3J_{HH}$, is 7.8 Hz. Resonance signals of aromatic CH group protons originating from the 2-phenylethylammonium cation in the ^{1}H NMR spectrum of salts were observed in the range of δ 7.11-7.39. When the resonance signals of CH_2 group protons (δ 3.01, PhCH$_2$CH_2 and 3.30, PhCH_2CH$_2$) and aromatic C*H* group protons (δ 7.35-7.44, $H_{1\text{-}5}$) of free 2-phenylethylamine[19] compared to those of salts, it was found that the chemical shifts of salts shifted to the upfield. These changes in proton chemical shifts prove the generation of salts. Furthermore, the carboxylic acid proton (-COO*H*) at about δ 8-12 was not observed in the ^{1}H NMR spectra of the salts indicating that the carboxylic acids were converted into anticipated salts.

In the ^{13}C NMR spectra of salts, the carbonyl carbon atoms (*C*=O) were observed in the range of δ 172.38-178.08. The *C*H$_2$ carbons of 2-phenylethyl-ammonium cation for all compounds were observed at about δ 41.93 (PhCH$_2$*C*H$_2$) and 34.66 (Ph*C*H$_2$CH$_2$), respectively. When the resonance signals of CH$_2$ group protons (δ 43.60, PhCH$_2$*C*H$_2$ and 35.70, Ph*C*H$_2$CH$_2$) of free 2-phenylethylamine[19] compared to those of salts, it was found that the chemical shifts of salts shifted to the upfield.

Biological evaluation

Cell proliferation assay

In this study, synthesized salts (**1-4**) were evaluated for their antiproliferative activities against HT29, MDA-MB 231, and HUVEC cell lines using MTT

assay. The only medium was used as negative control and 5-Fu was used as a positive control. The percents of the viable cells were calculated at various concentrations (1.56 – 50 μg/mL). The quantitative values which were obtained from this assay were plotted and by GraphPad Prism 9.0.0 Software (Fig. 1, Fig. 2, and Fig. 3).

As a result of MTT cell viability experiments, it was found that all of the salts weakly inhibited colon and breast cancer cell proliferation (Fig. 1 and Fig. 2). Furthermore, it is essential that salts with higher concentrations (50 and 25 μg/mL) were more effective than lower doses in inhibiting cancer cell proliferation. Additionally, it was determined that the salts were more effective on colon cancer (HT29) than breast cancer cells (MDA-MB 231) proliferation (Fig. 1 and Fig. 2). It was determined that breast cancer cells (MDA-MB 231) were affected by the synthesized salts especially in the chronic period (48 h). Thus, these results have been shown that the related salts are more effective on colon cancer and can partially inhibit cell proliferation than breast cancer cells.

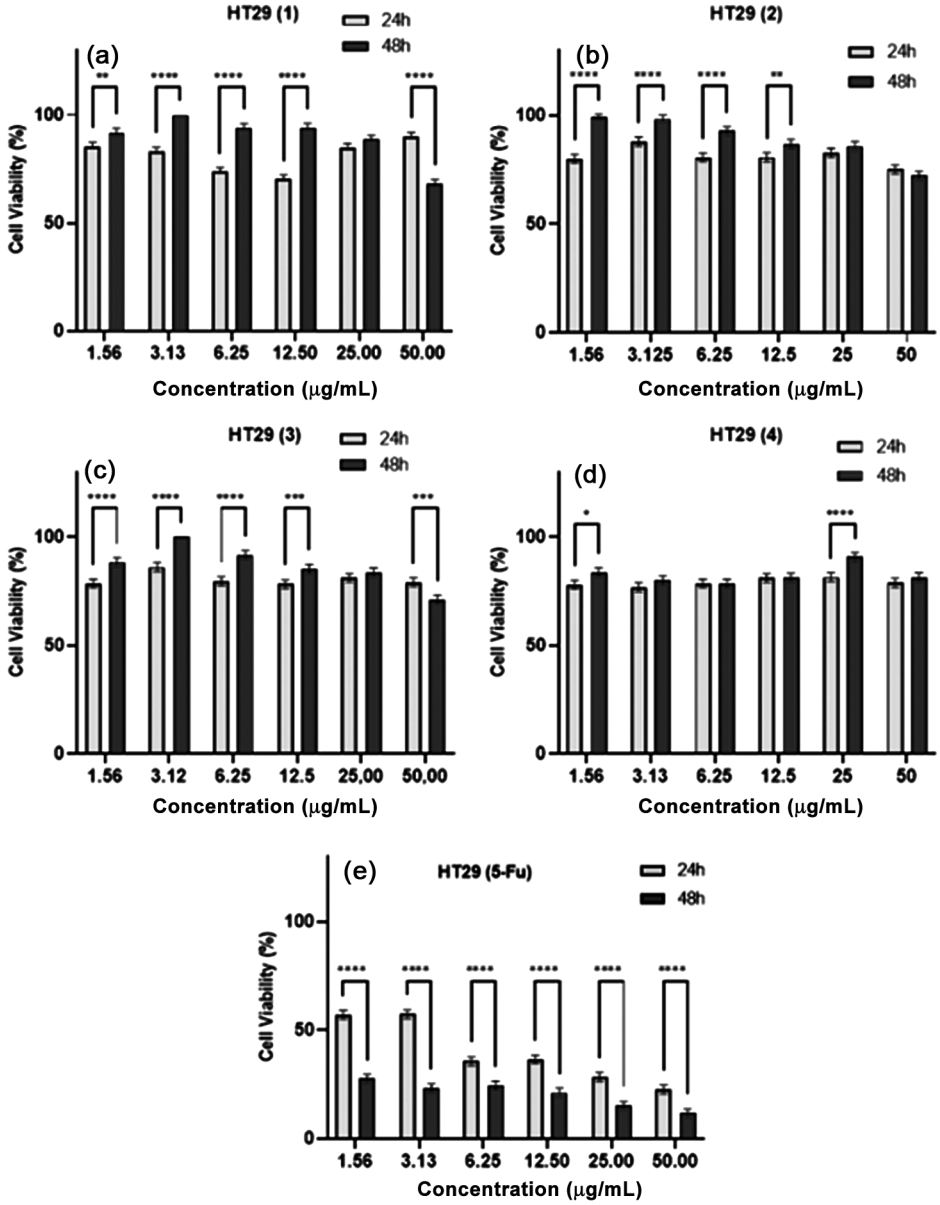


Fig. 1 — Antiproliferative effects of salts (1-4) and 5-Fu (positivie control) on HT29 cell line at different concentrations (1.56 – 50.0 μg/mL) and incubation times (24 - 48 h). All values are normalized to control untreated cells and presented as mean ± SD ($n \geq 3$). Data were analyzed using ANOVA test; **** $p \leq 0.0001$, *** $p \leq 0.005$, ** $p \leq 0.01$, * $p \leq 0.05$

In the present study, MTT analysis was also conducted to determine the possible antiproliferative effects of the synthesized salts on human vascular endothelial cells (HUVEC), which play an active role in metastasis and invasion. As a result, the anti-proliferative activity of the salts on HUVEC was determined to be higher during the chronic period (48 h) (Fig. 3). Especially, it was determined that the salts **2-4** limited cell proliferation and were at high concentrations (50 μg/mL). Additionally, cell viability was determined to be around 60% cell viability at this concentration.

Cell migration assay

In the wound healing analysis, which was performed using the most effective doses determined in the MTT assay. For example, possible anti-migratory effects of salts on colon cancer cells were investigated by salts **1**

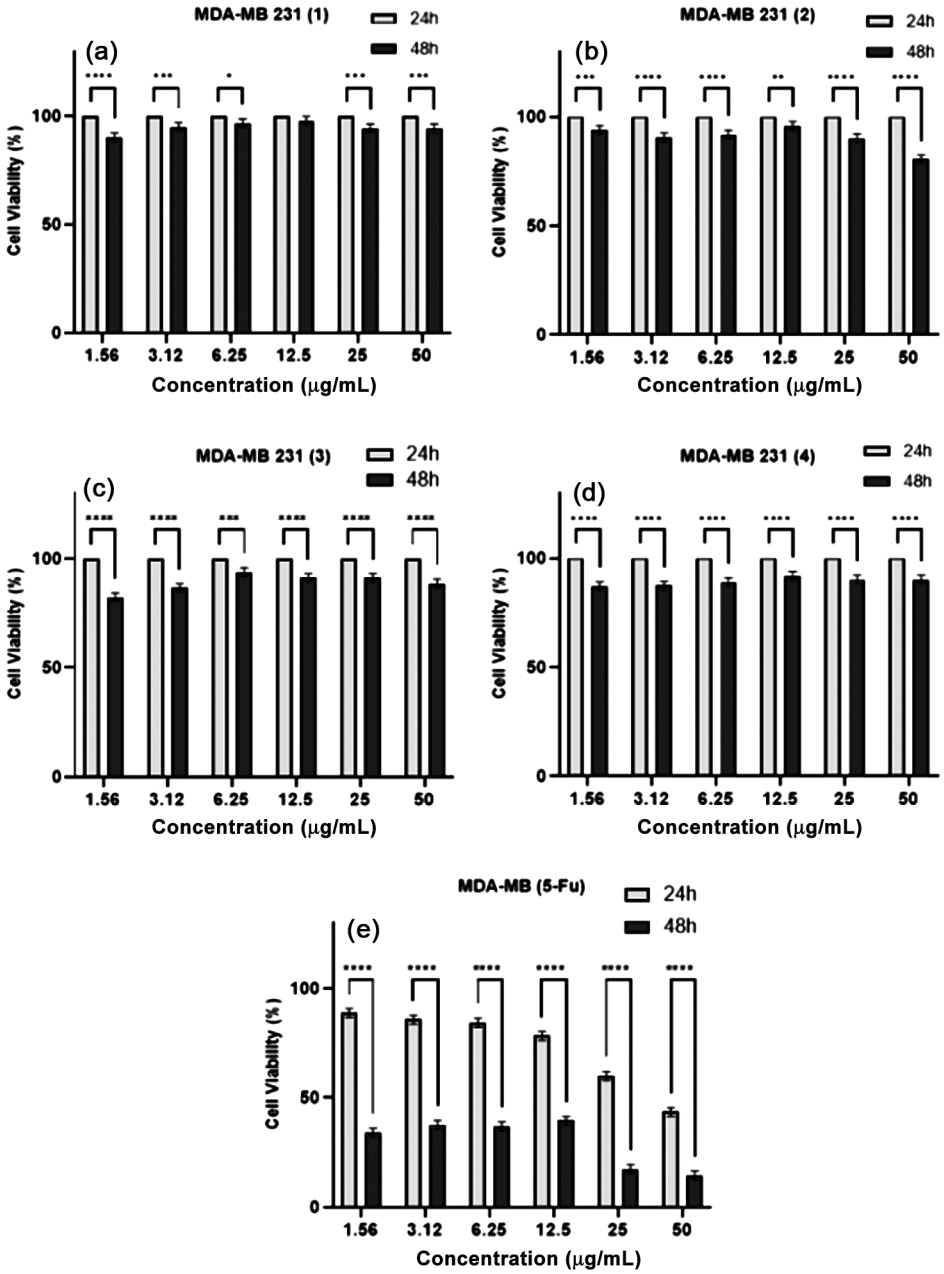


Fig. 2 — Antiproliferative effects of salts (1-4) and 5-Fu (positivie control) on MDA-MB 231 cell line at different concentrations (1.56 – 50.0 μg/mL) and incubation times (24 - 48 h). All values are normalized to control untreated cells and presented as mean ± SD ($n \geq 3$). Data were analyzed using ANOVA test; **** $p \leq 0.0001$, *** $p \leq 0.005$, ** $p \leq 0.01$, * $p \leq 0.05$

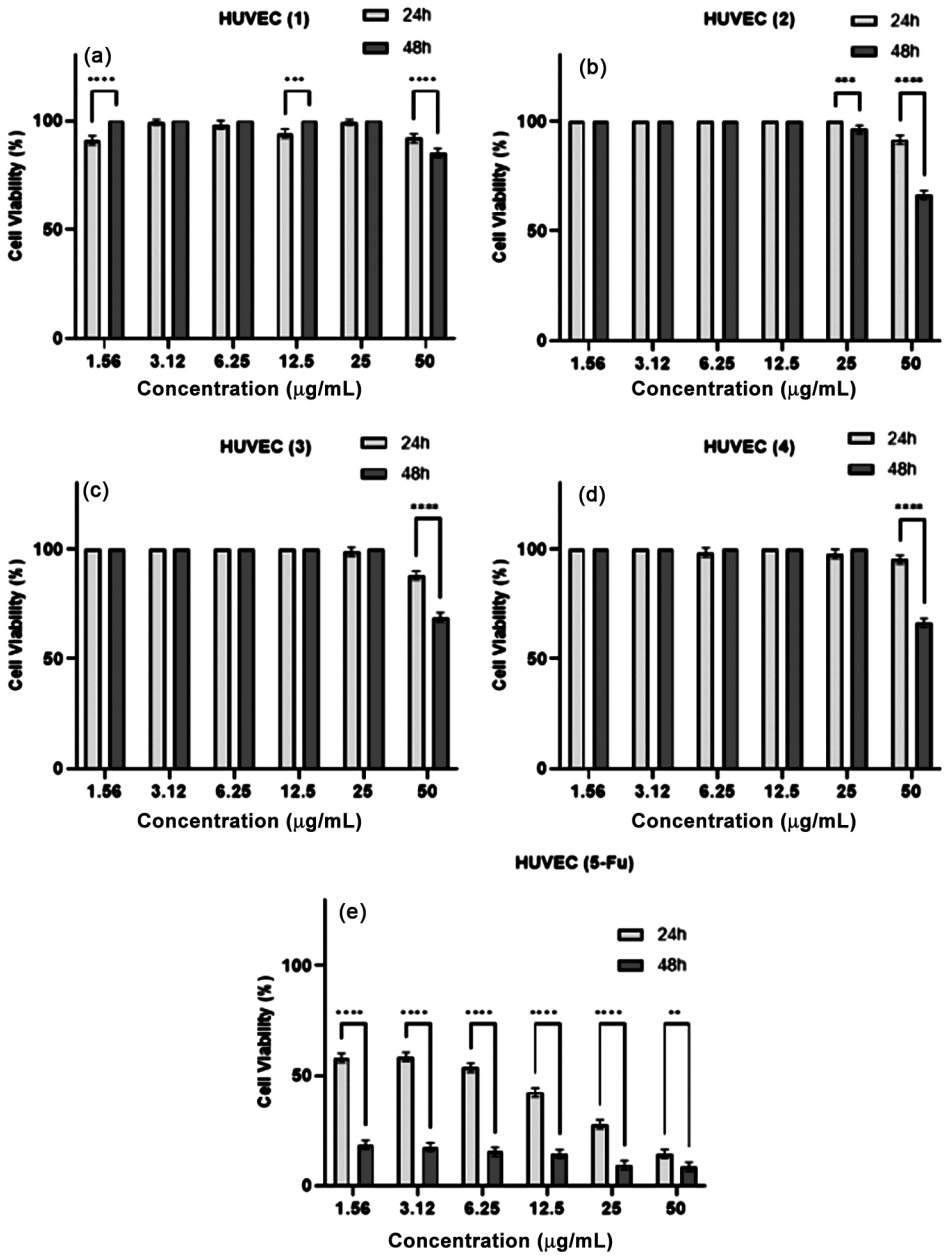


Fig. 3 — Antiproliferative effects of salts (1-4) and 5-Fu (positive control) on HUVEC cell line at different concentrations (1.56 – 50.0 μg/mL) and incubation times (24 - 48 h). All values are normalized to control untreated cells and presented as mean ± SD ($n \geq 3$). Data were analyzed using ANOVA test; **** $p \leq 0.0001$, *** $p \leq 0.005$, ** $p \leq 0.01$, * $p \leq 0.05$

and **3**. Because it was seen that the other salts (**2** and **4**) had a limited antiproliferative effect on HT29 cells in the previous experimental stage. Representative images and quantitative analysis of these analyses were shown in Fig. 4 and Fig. 5. Since the salts in the other cell line (MDA-MB 231) used in this study did not have a pronounced antiproliferative effect, migration analysis was not performed with these breast cancer cells.

The photographs shown in Fig. 4 were analyzed with the image processing program of Olympus inverted microscope and uncovered areas were calculated. The results obtained in the trials with the compound were compared with the negative control and the percentage of cancer cells migration was calculated relatively (Fig. 5).

When the ability of salts **1** and **3** to inhibit the migration of colon cancer cells was examined, it was

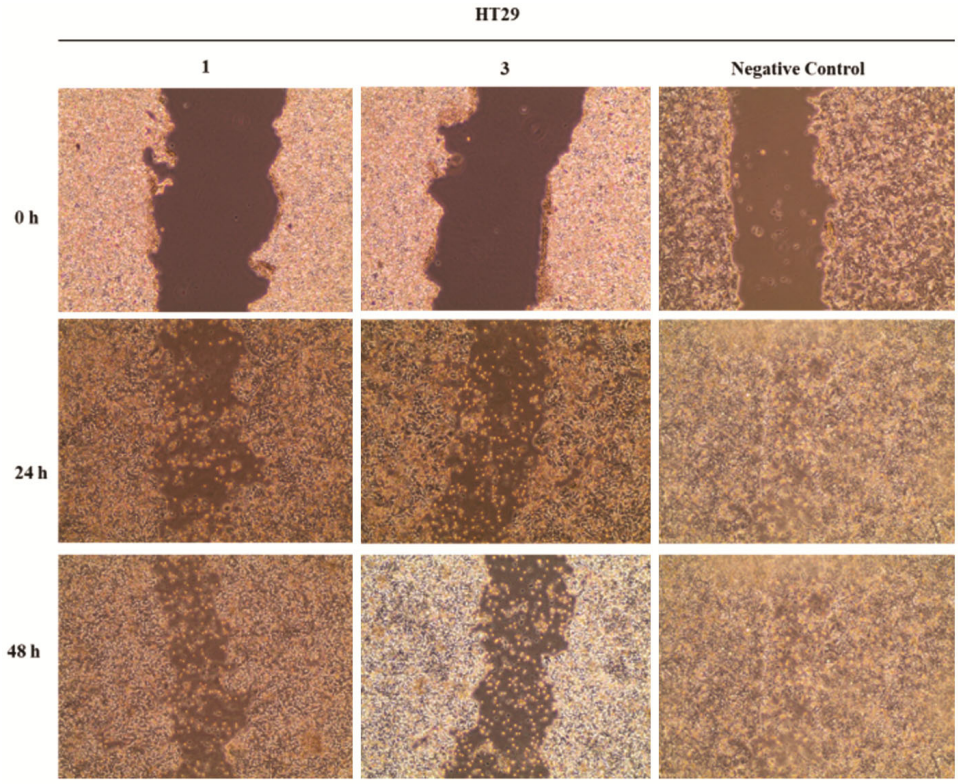


Fig. 4 — Wounded monolayers of HT29 cells incubated for 24 h and 48 h both alone (negative/untreated control) and in the presence of salts (1 and 3) at 50 µg/mL, Magnification is 4×)

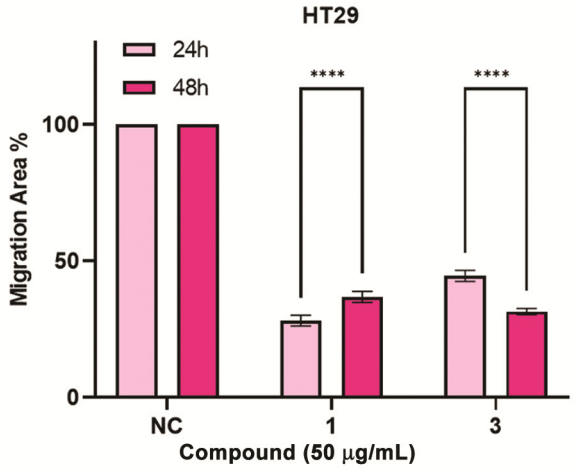


Fig. 5 — Effect of salts (1 and 3) at 50 µg/mL concentration on migration of HT29 cells in wound healing assay. Data were analyzed using ANOVA test; **** $p \leq 0.0001$

seen that this salt was prominently antimigrative at 24 and 48 h.

The possible anti-migratory effects of salts on human vascular endothelial cells were investigated by salts **2-4**. Because it was seen that salt **1** had a limited antiproliferative effect on HUVEC cells in MTT assay which is the previous experimental stage. Representative images and quantitative analysis of these analyses were shown in Fig. 6 and Fig. 7.

The photographs shown in Fig. 6 were analyzed with the image processing program of the Olympus microscope and open areas were calculated. The results obtained in studies with the compounds were compared with the negative control and the percentage of migrated cancer cells relatively (Fig. 5).

When the responses of human vascular endothelial cells to these salts which play an important role in the migration of cancer cells were investigated, it was determined that only salt **2** had an acute (24 h) anti-migratory effect. On the other hand, salts **3** and **4** had no effect at either 24 or 48 h.

Determination of antibacterial properties of PEA salts

In the present study, the antibacterial effects of the salts were examined with the aim of discovering new possible antimicrobial agents for use against some bacteria. MIC values of the salts against ATCC bacterial strains were reported in Table 1 and Fig. 8.

The MIC of the salts were determined, which were used to compare the antibacterial activities of salts against representative standard strains of gram-positive and gram-negative bacteria by the plate

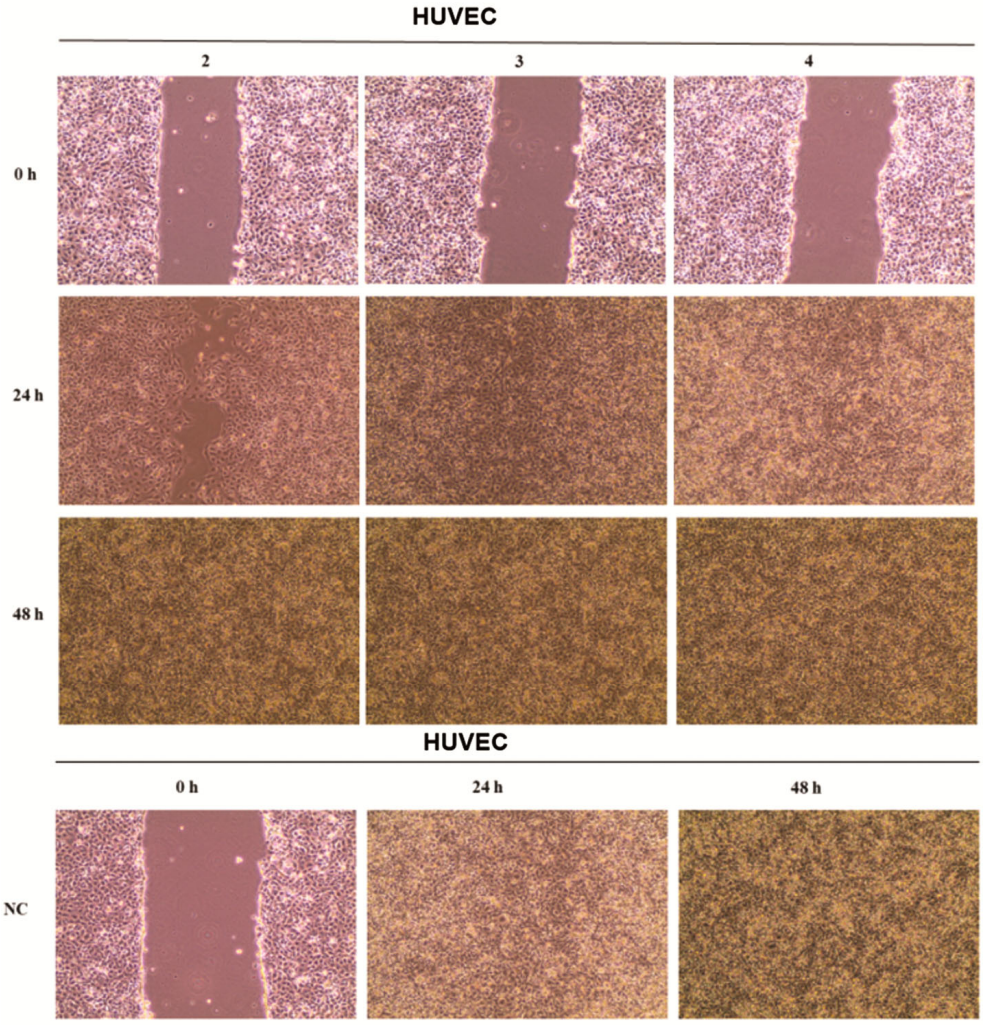


Fig. 6 — Wounded monolayers of HUVEC cells incubated for 24 h and 48 h both in the presence of salts (2,3 and 4) at 50 µg/mL (a) and negative/untreated control (b), Magnification is 4×)

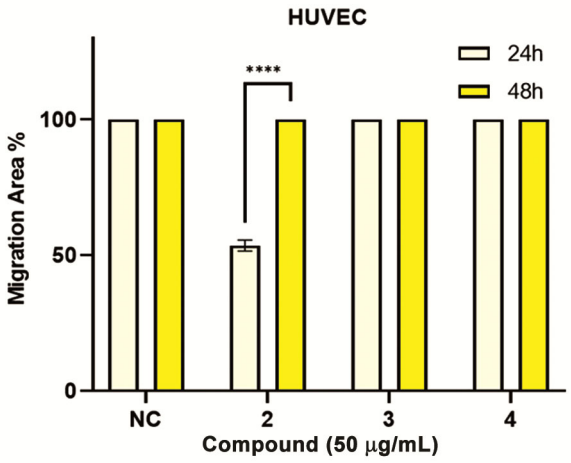


Fig. 7 — Effect of salts on migration of HUVECs at 50 µg/mL in wound healing assay. Data were analyzed using ANOVA test; **** $p \leq 0.0001$

Table 1 — The determined MIC values (mg/mL) of PEA salts ATCC bacterial strains

Compd	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
1	12.5	12.5	3.13
2	6.25	3.13	1.56
3	12.5	3.13	1.56
4	12.5	6.25	1.56

double dilution method[20]. As a result, MIC values studied in this work increase in the order: 2<1=3=4 for *Staphylococcus aureus*; 2=3<4<1 for *Pseudomonas aeruginosa*; 2=3=4<1 for *Klabsiella pneumoniae* (Table 1). The results indicate that salts could kill bacteria and inhibit their growth. Moreover, compound **1** exhibited higher MIC values relative to the others.

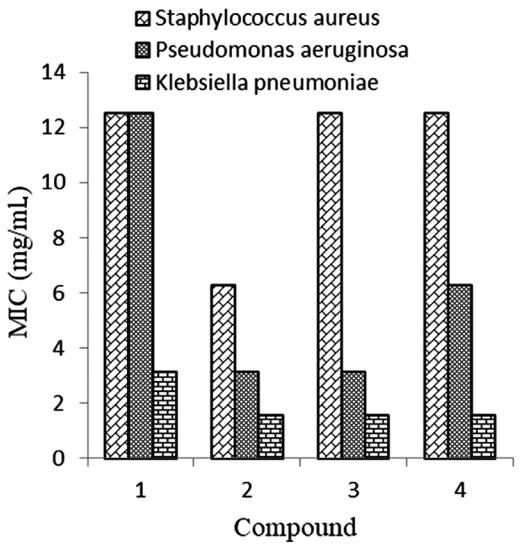


Fig. 8 — The minimal inhibitory concentration (mg/mL) of the tested PEA salts

Experimental Details

Materials and methods

2-Phenylethylamine, nicotinic acid, benzoic acid, salicylic acid and γ-resorcylic acid were purchased from commercial sources and used without further purification. FT-IR spectra were recorded on a Jasco FT-IR 4700 spectrometer in the range of 400-4000 cm^{-1}. Elemental analyses were recorded on an Elementar Vario Micro Cube elemental analyzer. ^{1}H and ^{13}C NMR spectra of the salts were measured using AC Bruker 400 MHz NMR spectrometer in methanol-d_4 at ambient temperature. The melting point of the salts were determined on a Barnstead Electrothermal instrument and are uncorrected.

General procedure for the synthesis of the salts

2-Phenylethylamine (1.00 g, 8.25 mmol), dissolved in methanol, was slowly added to nicotinic acid (1.02 g, 8.25 mmol), benzoic acid (1.01 g, 8.25 mmol), salicylic acid (1.14 g, 8.25 mmol) or γ-resorcylic acid (1.27 g, 8.25 mmol) in methanol solution. The mixture was stirred for 24 h in an ice bath. Methanol was removed from the mixture under reduced pressure. The compound, thus prepared was kept in a vacuum oven at 80°C for 48 h to remove excess moisture formed during the reaction. The dried compound was sealed with laboratory parafilm to prevent any moisture contamination. Also, the experimental procedure for compounds **2** and **3** have been previously described[21,22].

2-Phenylethylammonium nicotinate, 1: m.p 129°C. Anal. Calcd for $C_8H_{12}N^+(C_6H_4NO_2^-)$ (%): C, 68.83; H, 6.60; N, 11.47. Found (%): C, 69.08; H, 6.51; N, 11.16. FT-IR: 3083, 3065 (C-H arom.), 2920, 2817 (C-H aliph.), 3027, 2504 (NH_3^+), 1598, 1496 (C=C arom.), 1585 cm^{-1} (C=N arom.); ^{1}H NMR (methanol-d_4, numberings of protons are given in Scheme 1): δ 3.06 (t, 2H, $^{3}J_{HH}$= 7.8 Hz, PhCH_2CH$_2$), 2.83 (t, 2H, $^{3}J_{HH}$= 7.8 Hz, PhCH$_2$CH_2), 7.19 (m, 2H, $H_{1,5}$), 7.11 (m, 2H, $H_{2,4}$), 7.14 (m, 1H, H_3), 8.19 (dt, 1H, $^{3}J_{HH}$= 7.8 Hz, $^{4}J_{HH}$= 1.7 Hz, H_8), 7.32 (m, 1H, H_9), 8.42 (dd, 1H, $^{3}J_{HH}$= 5.0 Hz, $^{4}J_{HH}$= 1.4 Hz, H_{10}), 8.94 (d, 1H, $^{4}J_{HH}$= 1.2 Hz, H_{11}); ^{13}C NMR (methanol-d_4, numberings of carbons are given in Scheme 1): δ129.75 ($C_{1,5}$), 129.95 ($C_{2,4}$), 128.19 (C_3), 138.74 (C_6), 134.89 (C_7), 138.04 (C_8), 124.67 (C_9), 151.13 (C_{10}), 151.11 (C_{11}), 172.38 (*C*=O), 41.93 (PhCH$_2$*C*H$_2$), 34.66 (Ph*C*H$_2$CH$_2$).

2-Phenylethylammonium benzoate, 2: m.p 101°C. Anal. Calcd for $C_8H_{12}N^+(C_7H_5O_2^-)$ (%): C, 74.05; H, 7.04; N, 5.76. Found (%): C, 73.92; H, 7.44; N, 5.39. FT-IR: 3085, 3060 (C-H arom.), 2920, 2845 (C-H aliph.), 3027, 2497 (NH_3^+), 1590, 1497 cm^{-1} (C=C arom.); ^{1}H NMR (methanol-d_4, numberings of protons are given in Scheme 1): δ 3.06 (t, 2H, $^{3}J_{HH}$= 7.8 Hz, PhCH_2CH$_2$), 2.85 (t, 2H, $^{3}J_{HH}$= 7.8 Hz, PhCH$_2$CH_2), 7.22 (m, 2H, $H_{1,5}$), 7.15 (m, 2H, $H_{2,4}$), 7.17 (m, 1H, H_3), 7.30 (m, 2H, $H_{8,12}$), 7.86 (m, 2H, $H_{9,11}$), 7.26 (m, 1H, H_{10}); ^{13}C NMR (methanol-d_4, numberings of carbons are given in Scheme 1): δ 128.80 ($C_{1,5}$), 129.93 ($C_{2,4}$), 128.16 (C_3), 138.57 (C_6), 138.13 (C_7), 130.23 ($C_{8,12}$), 129.75 ($C_{9,11}$), 131.49 (C_{10}), 175.24 (*C*=O), 41.98 (PhCH$_2$*C*H$_2$), 34.79 (Ph*C*H$_2$CH$_2$).

2-Phenylethylammonium 2-hydroxybenzoate, 3: m.p 87°C. Anal. Calcd for $C_8H_{12}N^+(C_7H_5O_3^-)$ (%): C, 69.48; H, 6.61; N, 5.40. Found (%): C, 69.89; H, 6.93; N, 5.07. FT-IR: 3410 (O-H), 3087, 3061 (C-H arom.), 2924, 2857 (C-H aliph.), 3027, 2481 (NH_3^+), 1589, 1480 cm^{-1} (C=C arom.); ^{1}H NMR (methanol-d_4, numberings of protons are given in Scheme 1): δ 3.04 (t, 2H, $^{3}J_{HH}$= 7.8 Hz, PhCH_2CH$_2$), 2.82 (t, 2H, $^{3}J_{HH}$= 7.8 Hz, PhCH$_2$CH_2), 7.15 (m, 2H, $H_{1,5}$), 7.11 (m, 2H, $H_{2,4}$), 7.12 (m, 1H, H_3), 7.71 (m, 1H, H_8), 6.67 (m, 1H, H_9), 7.19 (m, 1H, H_{10}), 6.63 (m, 1H, H_{11}); ^{13}C NMR (methanol-d_4, numberings of carbons are given in Scheme 1): δ 129.73 ($C_{1,5}$), 129.93 ($C_{2,4}$), 128.17 (C_3), 137.95 (C_6), 119.85 (C_7), 131.60 (C_8), 119.04 (C_9), 133.92 (C_{10}), 117.20 (C_{11}), 162.62 (C_{12}), 176.16 (*C*=O), 41.91 (PhCH$_2$*C*H$_2$), 34.59 (Ph*C*H$_2$CH$_2$).

2-Phenylethylammonium 2,6-dihydroxybenzoate, 4: m.p 178°C. Anal. Calcd for $C_8H_{12}N^+(C_7H_5O_4^-)$ (%): C,

65.44; H, 6.22; N, 5.09. Found (%): C, 64.96; H, 6.63; N, 5.07. FT-IR: 3258 (O-H), 3070, 3047 (C-H arom.), 2968, 2874 (C-H aliph.), 3025, 2475 (NH_3^+), 1574, 1445 cm^{-1} (C=C arom.); ^{1}H NMR (methanol-d_4, numberings of protons are given in Scheme 1): δ 3.14 (t, 2H, $^3J_{HH}$= 7.7 Hz, PhCH_2CH$_2$), 2.92 (t, 2H, $^3J_{HH}$= 7.7 Hz, PhCH$_2$CH_2), 7.29 (m, 2H, $H_{1,5}$), 7.22 (m, 2H, $H_{2,4}$), 7.23 (m, 1H, H_3), 6.21 (dd, 2H, $^3J_{HH}$= 8.2 Hz, $^4J_{HH}$= 1.8 Hz, $H_{9,11}$), 7,04 (t, 1H, $^3J_{HH}$= 8.2 Hz, H_{10}); ^{13}C NMR (methanol-d_4, numberings of carbons are given in Scheme 1): δ 129.73 ($C_{1,5}$), 129.96 ($C_{2,4}$), 128.22 (C_3), 137.86 (C_6), 105.13 (C_7), 163.14 ($C_{8,12}$), 106.95 ($C_{9,11}$), 133.77 (C_{10}), 178.08 (*C*=O), 41.91 (PhCH$_2$*C*H$_2$), 34.58 (Ph*C*H$_2$CH$_2$).

Biological evaluation

Cell proliferation assay

Cell viability was determined using the methyl-thiazolyl tetrazolium bromide (MTT) quantitative colorimetric metabolic assay[23]. HT29, MDA-MB 231, and HUVEC were seeded at a density of 5×10^3 cells/well in 96 well plates and were allowed to grow in the medium overnight. Thereafter, cells were treated with salts and 5-fluorouracil (one of the most commonly used chemotherapeutic drugs to treat cancer) dissolved in DMSO (max 0.1% of DMSO) at eleven different concentrations of 50 µg/mL-0.05 µg/mL. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 48 h. Following this incubation process, at 24th and 48th hours, cells were incubated with MTT (0.5 mg/mL) for 3 h. Cell growth was determined by measuring absorption at indicated periods of time using a microplate reader (Tecan, Switzerland). Each experiment was repeated at least three times. The proliferation assay results were expressed as the percent inhibition according to the following formula:

$$\% \text{ Inhibition} = [(\text{Absorbance of treatment}/\text{absorbance of negative control}) \times 100]$$

Cell migration assay

Metastasis is one of the malignant processes that play an important role in cancer progression. In this process, cell migration is known as one of the key mechanisms which is defined as the actual movement of individual cancer cells, cell sheets, and clusters from one location to another[24]. It involves dynamic interactions and crosstalk between various cell types, interaction with extracellular matrix molecules, and regulated production of soluble mediators and cytokines. Analysis of cancer cell migration *in vitro* is a useful assay to quantify alterations in cell migratory capacity in response to experimental manipulations. Although several methods exist to study cell migration to explain wound healing, "*in vitro* scratch assay" is known as a simple and cost-effective method. Monolayer wound healing assays were performed by plating cells in 6-well culture dishes as described previously[25]. Briefly, 5×10^4 cells were seeded per well. After the cells were allowed to attach and reach 90% confluence, a scratch was performed within the cell monolayer using a 200 µl sterile pipette tip to create an artificial wound. Later, the unattached cells were removed by washing the cells with phosphate buffer saline (PBS). In the end, the cells were treated with salts at concentrations of 50 µg/mL. This salt dose and type (**1-4**) were determined by MTT assay at the previous experimental stage. Migrated cells were observed under phase contrast inverted microscope at 0, 24, 48 h and analyzed quantitatively by image analysis software which was supplied by Olympus (Japan). Finally, percent wound healing was calculated and the results were plotted.

Determination of antibacterial properties of PEA salts

Salts were evaluated for their *in vitro* antibacterial activity against gram-positive *Staphylococcus aureus* (ATCC 25923 (G+)), gram-negative *Pseudomonas aeruginosa* (ATCC 27853 (G-)), and *Klebsiella pneumoniae* (ATCC 15380 (G-)). Antimicrobial activity was determined by the minimum inhibitory concentrations (MICs). The MIC values were defined as the lowest concentrations of the tested agent that inhibited the growth of the microorganism. Dilution methods are used to determine the MICs of salts and are the reference methods for antimicrobial susceptibility testing[26]. The study of solvent control indicated that 10% DMSO did not inhibit the growth of microorganisms. Also, in the present experiment, the concentration of DMSO was gradually decreased because of the two-fold serial dilution assay (the working concentration was 5% and lower). So, the salts synthesized for this study were solved in 10% DMSO and diluted from 50 mg/mL to 0.78 mg/mL in sterile 96-well microtiter plates containing broth medium for bacteria. After dilution of samples, the intensity of bacteria was standardized to equal a 0.5 McFarland standard (approximately 5×10^7 organisms/mL). The bacteria were then inoculated 96 well-plates and were incubated at 37°C for 24 h. After 24 h, the optical density of each well was recorded at 600 nm using a

microplate reader (Tecan, Switzerland). Each test included growth control and sterility control. The experiments were made three times, and the mean values were used.

Conclusion

A series of salts (**1-4**) of different aromatic carboxylic acids (nicotinic, benzoic, salicylic, and γ-resorcylic acid) and 2-phenylethylamine were synthesized and characterized by FT-IR, ^{1}H, and ^{13}C NMR spectroscopy and elemental analysis. Structural analysis confirms that the N unit in 2-phenylethylamine is protonated, and this has contributed to studies of the basis of aromatic acid-aromatic amine synthons in organic salts. Taken together, these findings provide important preliminary data for the use of salts in blocking cancer cell proliferation, migration, and bacterial infections. These salts offer suitable drug candidates for further pharmacological testing. Additionally, more research is needed to investigate the anti-carcinogenic, anti-migrative, and anti-microbial effects of salts.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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