Akbaş, H., Erden Tayhan, S., Bilgin, S. (2024). In Vitro Antiproliferative, Antibacterial, and Anti-Angiogenic Studies of Triethanolamine-Based Salts. *The Black Sea Journal of Sciences*, 14(3), 1192-1203.

The Black Sea Journal of Sciences, 14(3), 1192-1203, 2024. DOI: 10.31466/kfbd.1446890

KFBD

Karadeniz Fen Bilimleri Dergisi
The Black Sea Journal of Sciences
ISSN (Online): 2564-7377 https://dergipark.org.tr/tr/pub/kfbd

Araştırma Makalesi / Research Article

In Vitro Antiproliferative, Antibacterial, and Anti-Angiogenic Studies of Triethanolamine-Based Salts

Hüseyin AKBAŞ[1*], Seçil ERDEN TAYHAN[2], Sema BİLGİN[3]

Abstract

The triethanolammonium (TEA) cation associated with the anion cyclopropanecarboxylate, mercaptoacetate, trichloroacetate, and 4-dodecylbenzenesulfonate led to the formation of TEA-based salts. The antibacterial activity of the TEA salts was examined against some bacteria and the obtained results were used to calculate minimal inhibitory concentrations (MICs) values. Additionally, the antiproliferative activities of TEA salts were examined *in vitro* on breast cancer cell line. Furthermore, *in vitro* scratch assay was performed by HUVECs (human vascular endothelial cell line) to assess the anti-angiogenic effect of these salts. These compounds did not dramatically differentiate the cell viability of cancer cells. On the other hand, it was demonstrated that TEA salts blocked human endothelial cell migration slightly. Therefore, these compounds were determined to have a limited ability to inhibit angiogenesis.

Keywords: Anti-angiogenic effect, Antiproliferative effect, Antibacterial effect, Triethanolammonium salts.

Trietanolamin Esaslı Tuzların In Vitro Antiproliferatif, Antibakteriyel ve Anti-Anjiyojenik Çalışmaları

Öz

Siklopropankarboksilat, merkaptoasetat, trikloroasetat ve 4-dodesilbenzensülfonat anyonları ile trietanolamonyum (TEA) katyonu TEA esaslı tuzların oluşumuna yol açtı. TEA tuzlarının antibakteriyel aktivitesi bazı bakterilere karşı incelendi ve elde edilen sonuçlar minimum inhibitör konsantrasyon (MİK) değerlerinin hesaplanmasında kullanıldı. Ek olarak, TEA tuzlarının antiproliferatif aktiviteleri meme kanseri hücre hattı üzerinde *in vitro* olarak incelendi. Ayrıca, bu tuzların anti-anjiyogenik etkisini değerlendirmek için HUVEC'ler (insan vasküler endotelyal hücre hattı) tarafından *in vitro* çizik analizi yapılmıştır. Bu bileşikler, kanser hücrelerinin hücre canlılığını kayda değer düzeyde değiştirmedi. Öte yandan TEA tuzlarının insan endotel hücre göçünü bir miktar engellediği gösterildi. Dolayısıyla, bu bileşiklerin anjiyogenezi engelleme konusunda sınırlı yeteneğe sahip olduğu belirlendi.

Anahtar Kelimeler: Anti-anjiyojenik etki, Antiproliferatif etki, Antibakteriyel etki, Trietanolamonyum tuzları.

[1]Tokat Gaziosmanpaşa University, Science and Arts Faculty, Department of Chemistry, Tokat, Türkiye, huseyin.akbas@gop.edu.tr
[2]Tokat Gaziosmanpaşa University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Tokat, Türkiye, seçil.erden@gop.edu.tr
[3]Tokat Gaziosmanpaşa, Tokat Vocational School of Health Services, Department of Medical Laboratory Techniques, Tokat, Türkiye, sema.bilgin@gop.edu.tr

[*]Sorumlu Yazar/Corresponding Author
Geliş/Received: 04.03.2024 **Kabul/Accepted:** 18.07.2024 **Yayın/Published:** 15.09.2024

1. Introduction

Protatranes, or TEA salts, are representative of a class of compounds known as atranes, which consist of $[NH(CH_2CH_2OH)_3]^+$ cations and anions of protic acid (X^-) (Voronkov et al., 2009; Voronkov et al., 2014; Kondratenko et al., 2016; Gruzdev et al., 2018). Research has demonstrated that the conversion of biologically active carboxylic acids into their TEA salts increases their biological activity and broadens their action spectrum (Voronkov et al., 2002; Voronkov and Baryshok, 2010; Adamovich et al., 2018; Adamovich and Mirskova, 2018; Kondratenko and Kochina, 2021). Due to these properties, they have found wide application in medicine and agriculture as drugs with a broad spectrum of action and growth-promoting drugs. As an immunomodulator and adaptogen, Trekrezan™ (Cresacin) drug is composed of the TEA salt of 2-methylphenoxyacetic acid (Voronkov et al., 2007; Antuganov et al., 2019). Apart from that, there are cresacin's analogs chlorocreasin (triethanolammonium 2-methyl-4-chlorophenoxyacetate) (Mirskova et al., 2010), and new water-soluble aspirin analogs (ethanolammonium acetylsalicylate) without side effects (Adamovich et al., 2012). Miranda et al. (2019) developed a method for improving the biopharmaceutical properties of Furosemide, a loop diuretic widely used to treat hypertension and edema associated with kidney, liver, and heart failure. The drug and triethanolamine were combined to obtain a pharmaceutical salt, which was characterized. In addition, the TEA of 1-propylimidazole-4,5-dicarboxylic acid has been shown to have anticonvulsant properties. The acid itself has convulsant properties (Brusina et al., 2018).

The biological activities of TEA salts against some yeasts and fungi were also investigated (Mirskova et al., 2003; Mirskova et al., 2008; Akbaş et al., 2020). Alcohol yeast *Saccharomyces cerevisiae* is effectively stimulated by TEA salts (Privalova et al., 2017). Kondratenko et al. (2017) investigated the biological activities of TEA salts of biologically effective carboxylic acids (cinnamic, benzoic, oxalic, malonic, succinic, salicylic and citric acids) against *Rhizopus oryzae* fungus and caused an boost in the biomass of the fungus by 8-24%. TEA salts of cinnamic, benzoic and malonic acids were found to have a positive effect on seed germination and growth characteristics of cress (*Lepidium sativum* L.) sprouts (Kondratenko et al., 2020), and these salts were reported to exhibit selective activity against *Staphylococcus aureus* bacteria (Kondratenko et al., 2020; Lukyanova et al., 2020).

In the present study, the TEA salts consisting of tris(2-hydroxyethyl)ammonium cation $\{[NH(CH_2CH_2OH)_3]^+\}$ and anions of different organic acids have been obtained, and these are tris(2-hydroxyethyl)ammonium cyclopropanecarboxylate (**1**), tris(2-hydroxyethyl)ammonium 2-mercaptoacetate (**2**), tris(2-hydroxyethyl)ammonium 2,2,2-trichloroacetate (**3**) and tris(2-hydroxyethyl)ammonium 4-dodecylbenzenesulfonate (**4**). The microdilution method was adopted to

examine the antibacterial activities of TEA salts for some gram-positive and gram-negative bacteria. Additionally, the antiproliferative effects of TEA salts on MDA-MB-231, one of the most commonly used breast cancer cell lines in medical exploration, were examined *in vitro*. In addition, to determine whether TEA salts have an effect on angiogenesis, a preliminary *in vitro* scratch assay was performed. Human umbilical cord vein endothelial cells (HUVECs) were used in this analysis.

2. Materials and Methods

2.1. Chemicals

Triethanolamine, cyclopropanecarboxylic acid, mercaptoacetic acid, trichloroacetic acid and 4-dodecylbenzenesulfonic acid, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), 5-fluorouracil were purchased from commercial sources and used without further purification. All of other cell culture reagents were commercially obtained from Biological Industries.

2.2. Syntheses of TEA Salts

The TEA salts (**1-4**) have previously been reported (Odinokov et al., 1984, Toshimutu 1994, Akyoshi et al., 1994, Enviromental Protection Agency 2014). These salts were synthesized from stoichiometric amounts of the triethanolamine as cation and the different acids (cyclopropanecarboxylic acid, mercaptoacetic acid, trichloroacetic acid and 4-dodecylbenzenesulfonic acid) as anions. The synthesized salts were stored in a vacuum oven at 80°C for 48 h to remove excess moisture formed during the reaction, and the dried salts were sealed with laboratory parafilm to prevent moisture contamination.

2.3. Determination of TEA Salts' Antiproliferative Effects

The antiproliferative effects of TEA salts on the MDA-MB-231 breast cancer cell line were studied by MTT analysis. MDA-MB-231 breast cancer cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) for this analysis. $5x10^4$ cells/ml of cultured cells were seeded in 96-well plates and incubated overnight at 37°C in a humidified atmosphere containing 5% CO_2. They were then treated with 11 different concentrations of TEA salts (0.05-50 μg/ ml) for 24h and 48h. A stock solution of the TEA salts was prepared in DMSO (final concentration is <0.1% in culture medium) and filter sterilized. 5-Fu was chosen as the positive control reagent due to its widespread use in conventional chemotherapy. The medium used in the

plate was taken, and a medium containing 10% (v/v) MTT was added to the cells. The cells were incubated for 3h in a 5% CO_2 incubator at 37 °C in the dark. At the end of the 3h incubation, the medium containing MTT was withdrawn from the cells, and the formed formazan crystal moieties were dissolved by adding DMSO. Cell culture plates were read in a microplate reader (Tecan, Switzerland) at a wavelength of 570 nm, and absorbance values were recorded. Using the commonly known formula, the percentage of viable cells was calculated (İnan et al., 2018).

2.4. Determination of Anti-Angiogenic Properties of TEA Salts

In vitro scratch assay was performed to assess the activity of TEA salts that affect angiogenesis by HUVECs (human vascular endothelial cell line) (İspir et al., 2019). In this work, firstly, the cell viability test was performed to determine the effective dose of compounds on HUVECs by MTT analysis which is described in detail above. Then taking into account the specified dose, *in vitro* scratch assay described below was applied. HUVECs were plated (5×10^4 cells/well) on six well culture dishes in an appropriate culture medium. When cells reached more than 80 % confluency, the cell monolayer was wounded by a sterile 200µl (yellow) pipette tip and all the wells were rinsed with phosphate buffer saline (PBS). Then cells were treated with the most effective dose (25µg/ml) of TEA salts which was determined by MTT cell viability assay in the previous experimental stage. Photographs were taken with a inverted light microscope every 24h for two days after the application of the salts and the percentage of the cell migration was calculated. These values were compared with negative control and evaluated whether the TEAs had antiangiogenic potential.

2.5. Antibacterial Susceptibility Testing

To find new antimicrobial drugs to utilize against some bacteria, the antibacterial properties of TEA salts were investigated in the current work. The MICs were studied to learn more about the antibacterial activity by MIC dilution methods (Erden Tayhan et al., 2018). The compounds **1-4** were evaluated for their *in vitro* antibacterial activities against two gram-negatives (G-), *Klebsiella pneumonia and Pseudomonas aeruginosa*, and one gram-positive (G+), *Staphylococcus aureus*. Bacterial strains of *Staphylococcus aureus* [ATCC 25923 (G+)], *Pseudomonas aeruginosa* [ATCC 27853 (G-)], *Klebsiella pneumonia* [ATCC 15380 (G-)] were utilized. Microorganisms were cultivated in the exponential phase in nutritional broth at 37ºC for 18 h and then were diluted with new broth medium to a final concentration of 10^4 CFU ml^{-1}. By calculating the MIC using the microdilution method in a 96-well microtiter plate, *in vitro* antibacterial activity was examined. In sterile 96-well plates with a bacterial broth medium, serial two-fold dilutions of the investigated

substances were prepared in concentrations ranging from 50 mg/ml to 0.392 mg/ml. For 24h, the inoculation plates were incubated at 37°C. Using a microplate reader (Tecan, Switzerland), the optical density of each well was measured at 620 nm after 24h. The bacterial inhibition percentage (IC %) was determined with the commonly known formula (Lavorgna et al., 2019). The tested substances were dissolved in DMSO and then diluted to a 10% DMSO concentration in a nutrient-liquid medium. The solvent control test was used to examine how 10% DMSO affected the growth of bacteria. It was observed that 10% DMSO did not prevent microbial growth. Additionally, the two-fold serial dilution test used in the current experiment caused the concentration of DMSO to fall (the working concentration was 5% and below) steadily. Each test included sterility and growth controls. Three times each of the trials were conducted and the mean results were used.

3. Findings and Discussion

3.1. The Antiproliferative Properties of TEA Salts

MTT cell viability studies revealed that TEA salts had no appreciable impact on cell viability (Figure 1). Only **1** and **4** inhibited cancer cell viability weakly at higher doses (50 and 25 µg/ml) at the first 24h. At the second day, compounds have been shown to lose their antiproliferative effects.

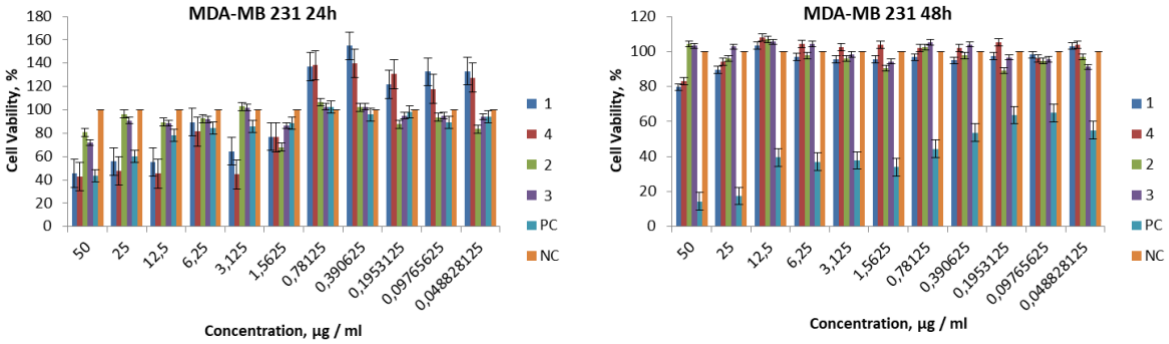

Figure 1. The effect of different concentrations of TEA salts on the viability of MDA-MB-231 at 24h and 48h (PC: positive control – 5Fu, NC: negative control).

Protic molten salts have rarely been tested for anticancer studies (Elmas et al., 2020; Akbaş et al., 2023). It has also been shown that in salts with the same cation, the compounds lose their antiproliverative effects on the second day (Akbaş et al., 2020).

3.2. The Anti-angiogenic Activity of the TEA Salts

In this work, firstly the cell viability test was performed to determine the effective dose of compounds on HUVECs by MTT analysis. As a result, the percentage of cell viabilities was calculated and shown in Figure 2.

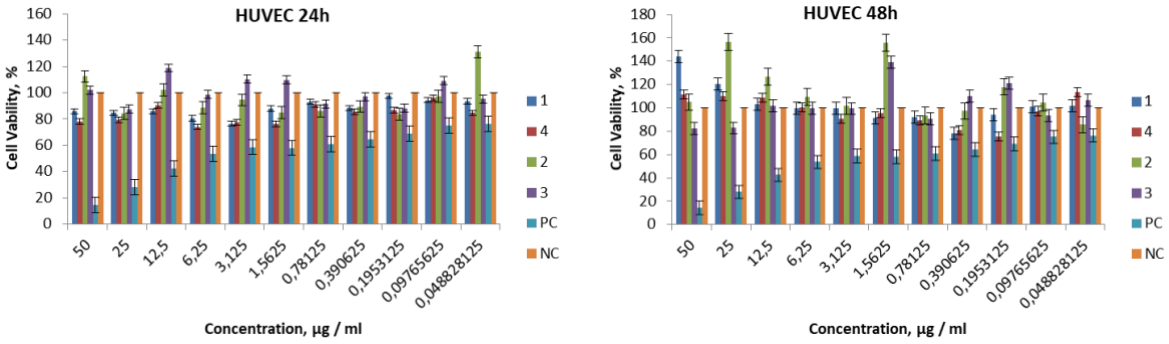

Figure 2. The effect of different concentrations of TEA salts on the viability of HUVEC at 24h and 48h (PC: positive control – 5Fu, NC: negative control).

Subsequently, taking into account the indicated dose (25 µg/ml), the cell migration assay was carried out by *in vitro* scratch analysis to determine whether TEA salts were able to inhibit HUVEC angiogenesis. Representative images and quantitative data were shown in Figure 3 and Figure 4.

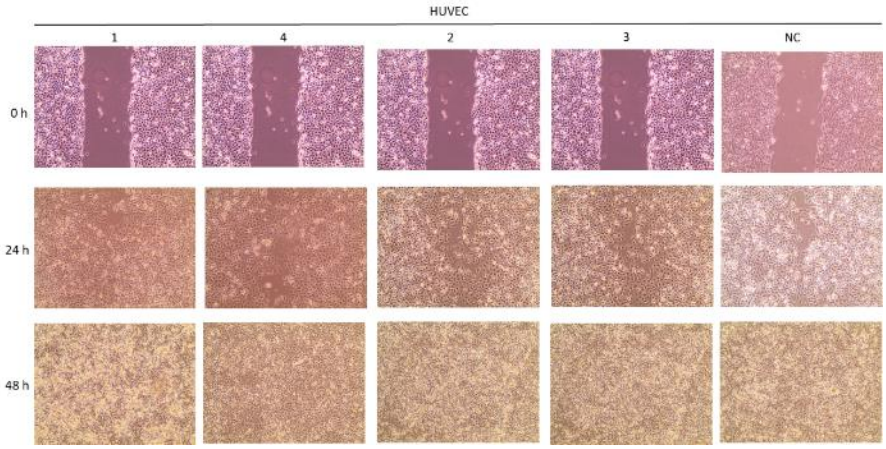

Figure 3. Effect of TEA salts on the migration of HUVEC in wound assay. Wounded monolayers of HUVEC were incubated for 24h and 48h alone (negative control) and in the presence of TEA salts (25 µg/ml). Magnification 4x.

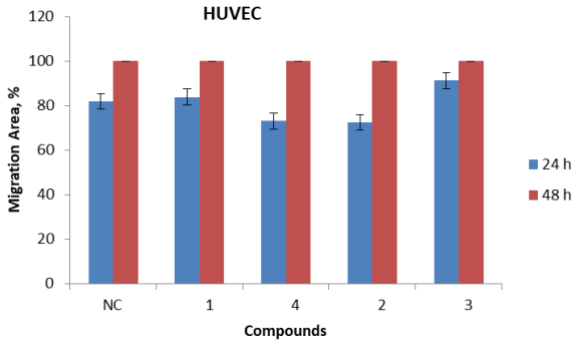

Figure 4. Effect of TEA salts on the migration of HUVEC. Wound healing assay showing inhibition of angiogenesis after 24h and 48h of exposure to TEA salts (25 µg/ml).

When Figures 3 and 4 were examined, it was shown that TEA salts (25 µg/ml) slightly inhibited HUVEC migration, which is one of the important basic steps in angiogenesis. Therefore, these compounds were determined to have a limited ability to inhibit angiogenesis.

3.3. Antibacterial Susceptibility Testing

To find new potential antimicrobial agents to utilize against some bacteria, the antibacterial activities of the TEA salts were investigated in the current study. Both G+ and G- bacteria's cell growth was suppressed by all of the synthesized compounds. The most active compounds were **2** and **3**, indicating a big reduction in bacterial growth. Additionally, the other salts, **1** and **4,** inhibited cell proliferation more slightly. The calculated percent inhibition of bacterial growth by the TEA salts was plotted and shown in Figure 5.

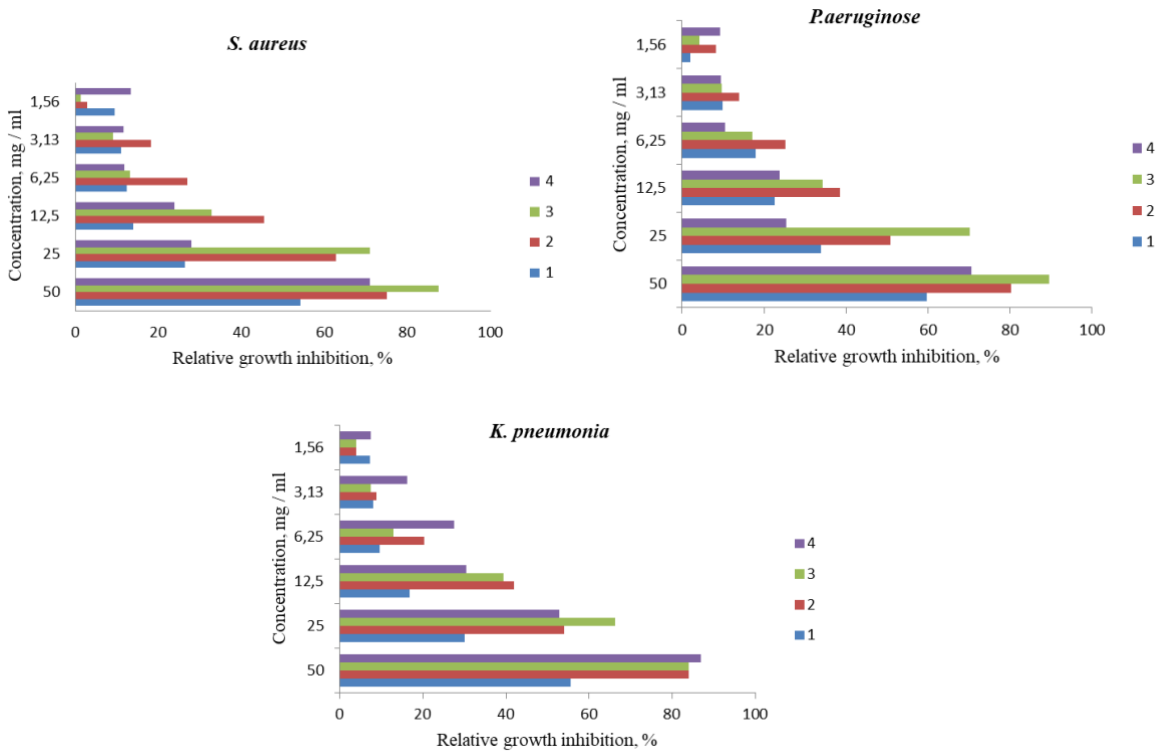

Figure 5. The calculated percent inhibition of *S. aureus* (G+), *P. aeruginose* (G-) and *K. pneumonia* (G-) growth by the TEA salts.

Minimum inhibitory concentrations of the compounds against different bacterial strains (MIC values) were reported in Table 1.

Table 1. The determined MIC values (mg/ml) of compounds for ATCC bacterial strains.

Compounds	*Staphylococcus aureus (G+)*	*Pseudomonas aeruginosa (G-)*	*Klebsiella pneumoniae (G-)*
1	50	50	50
2	25	50	25
3	25	25	25
4	50	50	25

By choosing the appropriate organic cation, it is possible to tune important biological differences in antibacterial properties. Whether the cation is polar or nonpolar affects the MIC values. This can be explained by the fact that cations with high polarity are more likely to remain in an aqueous solution rather than hydrophobic cell membranes and therefore bind themselves to their anions, that is, the ion trapping effect (Trapp et al., 2010; Ferraz et al., 2014). Thus, the relevant salts become trapped in an aqueous solution and hydrolyze. Moreover, salts **1**, **2**, and **3** exhibited a better MIC value compared to salts containing carboxylate anion with long alkyl chain length (Akbaş et al., 2020). With the molecular docking score, the effect of the alkyl chain and the electronegative atom in the anion directly contributed to the antimicrobial activity, which is consistent with the

experimental data. Adding a fluorine atom to an anion increases the highest antimicrobial activity among chlorine, bromine, and iodine (Kumer and Khan, 2021). In addition, almost all of the triethanolammonium salts tested in the literature exhibited noticeable activity against *Staphylococcus aureus* bacteria (Kondratenko et al., 2020). The results indicate that a possible growth inhibition occurs in the presence of salt at a low MIC concentration. It indicates the potential use of the salts used here as antibacterial drugs against resistant strains at very low concentrations.

4. Conclusions and Recommendations

Four triethanolammonium-based salts were synthesized by acid-base neutralization of triethanolamine with organic acids. These TEA salts are soluble in polar solvents (water, alcohol, etc.). The tested TEA salts were promising antibacterial compounds against *S. aureus, P. aeruginosa, K. pneumoniae.* Furthermore, **2** and **3** were the most effective derivative for inhibiting bacterial growth. The potent antibacterial activity of these four triethanolammonium-based compounds indicates their useful therapeutic application against bacterial infection. Additionally, TEA salts were investigated for their biological activity in cultured breast cancer cell lines. These compounds did not dramatically differentiate the cell viability of cancer cells. Furthermore, it was demonstrated that TEA salts blocked human endothelial cell migration slightly. Especially, **2** and **4** which contained sulfur atoms in their anions inhibited the cell migration into the denuded area more than the negative control. This result is compatible with previous publications showing that inorganic sulfur inhibits the expression and activation of angiogenesis-related genes, such as EGFR, in cancer cells (Ha et al., 2013). These findings provide significant preliminary data for using TEA salts in inhibiting angiogenesis and identifying promising drug candidates for further pharmacological evaluation. In addition, additional research is required to investigate the anti-carcinogenic effects of TEA salts containing inorganic sulfur.

Acknowledgments

This section is where one can acknowledge and thank the individuals and/or institutions who helped with and supported the author(s) with her/his/their research.

Authors' Contributions

Hüseyin Akbaş synthesized triethanolamine-based salts. Seçil Erden Tayhan and Sema Bilgin performed biological activity experiments. Hüseyin Akbaş, Seçil Erden Tayhan, and Sema Bilgin contributed to the writing of the article.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

References

Adamovich, S.N., and Mirskova, A.N., (2018). Synthesis of immunoactive tris(2-hydroxyethyl)ammonium 1-R-indol-3-ylsulfanyl(sulfonyl)acetates. *Russian Journal of Applied Chemistry*, 91, 469-472.

Adamovich, S.N., Mirskov, R.G., Mirskova, A.N., and Voronkov, M.G., (2012). Biologically active protic (2-hydroxyethyl) ammonium ionic liquids. Liquid aspirin. *Russian Chemical Bulletin*, 61, 1260-1261.

Adamovich, S.N., Oborina, E.N., and Mirskova A.N., (2018). Synthesis and pharmacological activity of tris(2-hydroxyethyl)ammonium 4-chlorophenylsulfonylacetate. *Russian Journal of Applied Chemistry*, 91, 701-705.

Akbaş, H., Yıldırım, A., Menevşe, Ş., and Gökşen, N., (2020). Antiproliferative and antimicrobial effects of tris(2-hydroxyethyl)ammonium-based protic ionic liquids with some fatty acids. *Journal of the Institute of Science and Technology*, 10, 2021-2027.

Akbaş, H., Erden Tayhan S., and Bilgin, S., (2023). A novel approach to determine anti-proliferative, anti-migratory and anti-microbial properties of 2-phenylethylammonium carboxylate molecular salts. *Indian Journal of Chemistry*, 62, 207-216.

Akyoshi, O., Takao, K., Shinichi, K., and Yasuhide, Y., (1994). Shampoos and hair rinses containing thioglycolic acid. *Espacenet*, Patent Number: JPH06116124A.

Antuganov, D., Timofeev, V., Timofeeva, K., Antuganova, Y., and Kondratenko, Y.A., (2019). Evaluation of protic ionic liquids based on triethanolammonium and tris(hydroxymethyl)methylammonium salts as buffers for ^{68}Ga-radiolabelling of PSMA-HBED-CC. *Chemistry Select*, 4, 12524-12527.

Brusina, M.A., Nikolaev, D.N., Fundamenskii, V.S., Gurzhii, V.V., Zolotarev, A.A., Selitrenikov, A.V., Zevatskii, Y.E., Potapkin, A.M., Ramsh, S.M., and Piotrovskii, LB., (2018). Water-soluble form of 1-alkyl(aryl)ımidazole-4,5-dicarboxylic acids. structure and anticonvulsant activity of the triethanolammonium salt of 1-propylimidazole-4,5-dicarboxylic acid. *Pharmaceutical Chemistry Journal*, 52, 299-303.

Elmas, G., Okumuş, A., Kılıç, Z., Özbeden, P., Açık, L., Çağdaş Tunalı, B., Türk, M., Çerçi, N.A., and Hökelek, T., (2020). Phosphorus-nitrogen compounds. Part 48. Syntheses of the phosphazenium salts containing 2-pyridyl pendant arm: Structural characterizations, thermal analysis, antimicrobial and cytotoxic activity studies. *Indian Journal of Chemistry -Section A*, 59, 533-550.

Environmental Protection Agency, (2014). Amine salts of alkyl (C_8-C_{24}) benzenesulfonic acid (olmithylaminopropylamine, isoprodvlamine, mono-, di-, and teiethanolamine); exemption from the requirement of a tolerance. *Federal Register*, 79, 28444-28447.

Erden Tayhan, S., Bilgin, S., and Elmastaş, M., (2018). Evaluation of the wound healing potential of teucrioside. *International Journal of Chemistry and Technology*, 2, 16-19.

Ferraz, R. , Teixeira, V., Rodrigues, D., Fernandes, R., Prudencio, C., Noronha, J.P., Petrovski, Z., and Branco, L.C., (2014). Antibacterial activity of Ionic Liquids based on ampicillin against resistant bacteria. *RSC Advances*, 4, 4301-4307.

Gruzdev, M.S., Shmukler, L.E., Kudryakova, N.O., Kolker, A.M., and Safonova, L.P., (2018). Synthesis and properties of triethanolamine-based salts with mineral and organic acids as protic ionic liquids. *Journal of Molecular Liquids*, 249, 825-830.

Ha, A.W., Hong, K.H., Kim, H.S., and Kim, W.K., (2013). Inorganic sulfur reduces cell proliferation by inhibiting of ErbB2 and ErbB3 protein and mRNA expression in MDA-MB-231 human breast cancer cells. *Nutrition Research and Practice*, 7, 89-95.

İnan, A., Sünbül, A.B., İkiz, M., Erden Tayhan, S., Bilgin, S., Elmastaş, M., Sayın, K., Ceyhan, G., Köse, M., and İspir, E., (2018). Half-sandwich Ruthenium(II) arene complexes bearing the azo-azomethine ligands: Electrochemical, computational, antiproliferative and antioxidant properties. *Journal of Organometallic Chemistry*, 870, 76-89.

İspir, E., İkiz, M., Inan, A., Sünbül, A.B., Erden Tayhan, S., Bilgin, S., Köse, M., Elmastaş, M., (2019). Synthesis, structural characterization, electrochemical, photoluminescence, antiproliferative and antioxidant properties of Co(II), Cu(II) and Zn(II) complexes bearing the azo-azomethine ligands. *Journal of Molecular Structure*, 1182, 63-71.

Kondratenko, Y., Kochina, T., Fundamensky, V., Ignatyev, I., Panikorovskii, T., and Nyanikova G., (2016). Triethanolammonium salicylate-protic alkanolammonium ionic liquid. *Journal of Molecular Liquids*, 221, 1218-1124.

Kondratenko, Y.A., and Kochina, T.A., (2021). Contribution of the scientific school of academician m.g. Voronkov to the development of the chemistry of biologically active atranes (protatranes and hydrometallatranes) (a review). *Russian Journal of General Chemistry*, 91, 2331-2351.

Kondratenko, Y.A., Gurzhii, V.V., Panova, G.G., Anikina, L.M., Udalova, O.R., Krutikov, V.I., Ugolkov, V.L., and Kochina, T.A., (2020). Biological activity of protic ionic liquids based on tris(2-hydroxyethyl)ammonium salts and the crystal structure of tris(2-hydroxyethyl)ammonium malate. *Russian Journal of Inorganic Chemistry*, 65, 1407-1415.

Kondratenko, Y.A., Nyanikova, G.G., Molchanova, K.V., and Kochina, TA., (2017). Characteristics of protic ionic liquids based on triethanolammonium salts of biologically active carboxylic acids and their impact on the growth properties of the Rhizopus oryzae fungus. *Glass Physics and Chemistry*, 43, 445-451.

Kumer, A., and Khan, M.W., (2021). Synthesis, characterization, antimicrobial activity and computational exploration of ortho toludinium carboxylate ionic liquids. *Journal of Molecular Structure*, 1245, 131087.

Lavorgna, M., Iacovino, R., Russo, C., Donato, C.D., Piscitelli, C., and Isidori, M., (2019). A new approach for improving the antibacterial and tumor cytotoxic activities of pipemidic acid by including it in trimethyl-β-cyclodextrin. *International Journal of Molecular Sciences*, 20, 416.

Lukyanova, S.V., Gefan, N.G., Adamovich, S.N., Oborina, E.N., Khaptanova, N.V., Kuznetsov, V.I., Ostyak, A.S., Kosilko, V.S., and Balakhonov S.V., (2020). Study of the effect of a biologically active compound tris(2-hydroxyethyl)ammonium 4-chlorophenylsulfanylacetate on the growth of listeria monocytogenes and staphylococcus aureus. *Acta Biomedica Scientifica*, 5, 47-53.

Miranda, J.A., Garnero, C., Chattah, A.K., Oliveria, Y.S., Ayala, A.P., and Longhi, M.R., (2019). Furosemide: triethanolamine salt as a strategy to improve the biopharmaceutical properties and photostability of the drug. *Crystal Growth & Design*, 19, 2060-2068.

Mirskova, A.N., Adamovich, S.N., Mirskov, R.G., and Voronkov, M.G., (2010). Tris-(2-hydroxyethyl)ammonium 2-methyl- and 2-methyl-4-chlorophenoxyacetate serve as effective inhibitors of thrombocyte aggregation and antioxidants. *Doklady Biological Sciences*, 433, 244-246.

Mirskova, A.N., Levkovskaya, G.G., Mirskov, R.G., and Voronkov, M.G., (2008). Hydroxyalkylammonium salts of organylsulfanyl(sulfonyl)acetic acids-New stimulators of biological processes. *Russian Journal of Organic Chemistry*, 44, 1478-1485.

Mirskova, A.N., Levkovskaya, G.G., Stupina, A.G., Chkhenkeli, V.A., and Voronkov, M.G., (2003). The effect of tris-(2-hydroxyethyl)ammonium aroxy-, arylthio-, and arylsulfonylacetates on the vital activity of bifidobacteria. *Doklady Biological Sciences*, 390, 239-241.

Odinokov, S.E., Glazunov, V.P., and Nabiullin, A.A., (1984). Infrared spectroscopic studies of hydrogen bonding in triethylammonium salts. Part 3.-Strong hydrogen bonding. *Journal of the Chemical Society, Faraday Transactions 2: Molecular and Chemical Physics*, 80, 899-908.

Privalova, E.A., Tiguntseva, N.P., Adamovich, S.N., Mirskov, R.G., and Mirskova, A.N., (2017). Tris(2-hydroxyethyl)ammonium arylchalcogenylacetates, growth stimulants of alcohol yeast Saccharomyces cerevisiae, *Russian Chemical Bulletin*, 66, 1320-1324.

Toshimitsu, K., (1994). Water-base ink composition for ball-point pen. *Espacenet*, Patent Number: JPH06100826A.

Trapp, S., Franco, A., and Mackay, D., (2010). Activity-based concept for transport and partitioning of ıonizing organics. *Environmental Science and Technology*, 44, 6123-6129.

Voronkov, M.G., Adamovich, S.N., and Ushakov, I.A., (2014). Aroxyprotatranes: aroxy derivatives of tris-(2-hydroxyethyl)ammonium. *Russian Journal of General Chemistry*, 84, 340-342.

Voronkov, M.G., Albanov, A.I., Aksamentova, T.N., Adamovich, S.N., Chipanina, N.N., Mirskov, R.G., Kochina, T.A., Vrazhnov, D.V., and Litvinov, M.Y., (2009). Tris(2-hydroxyethyl)ammonium salts: 2,8,9-trihydroprotatranes. *Russian Journal of General Chemistry*, 79, 2339-2346.

Voronkov, M.G., and Baryshok, V.P., (2010). Atranes as a new generation of biologically active substances. *Herald of the Russian Academy of Sciences*, 80, 514-521.

Voronkov, M.G., Kolesnikova, O.P., Rasulov, M.M., and Mirscova, A.N., (2007). Pharmacological activity and clinical effects of trecrezan and other tris-(2-hydroxyethyl)ammonium salts of arylheteroacetic acids (A review). *Pharmaceutical Chemistry Journal*, 41, 244-248.

Voronkov, M.G., Mirskova, A.N., and Levkovskaya, G.G., (2002). Carcinostatic, protective, and adaptive activities of tris-(2-hydroxyethyl)ammonium salts of arylheteroacetic acids. *Doklady Biological Sciences*, 386, 404-406.