JCHR (2021) 11(1), 85-89

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

The Study of Cytotoxicity of an Imidazolium Based Ionic Liquid on MCF-7 Cell Line

Elham Sajadiyeh[1], Masoumeh Tabatabaee[*1], SayedMorteza Seifati[2], Zohreh Derikvend[3]

[1]*Department of Chemistry, Yazd Branch, Islamic Azad University, Yazd, Iran*

[2]*Department of Biology, Ashkezar Branch, Islamic Azad University, Ashkezar, Iran*

[3]*Department of Chemistry, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran*

(Received: 22 December 2019 Accepted: 23 November 2020)

KEYWORDS

Imidazolium Ionic Liquids;
Cytotoxicity;
MCF-7 cell line;
IC50 values

ABSTRACT: Ionic liquids are liquid in wide range and could be applied in many fields. These compounds can be used as solvents for inorganic and organic synthesis and show biological activities. 1-ethyl-3-Methylimidazolium bromide (**1**) as an ionic liquid has been synthesized from the reaction of 1-methylimidazole and ethylbromide in toluene as solvent and under refluxed condition. The prepared compound was characterized by ^{1}H-NMR, IR spectroscopy and elemental analyses. The effect of cytotoxicity of -ethyl-3-Methylimidazolium bromide (ion liquid) on the MCF-7 cell line has been investigated. The cytotoxic activity of synthesis compound was assessed by the MTT colorimetric method in vitro assay performed in human MCF-7 breast carcinoma cell line. Dose effect of compound was investigated and results showed that the cytotoxic effect was dose-dependent. The decrease of concentration of ion liquid was followed by markedly increase of tumor cell viability. The cytotoxicity of 1-ethyl-3-Methylimidazolium bromide, expressed as IC50 values after 48 h incubation. This value was 2.91 mM for mcf-7 cell line.

INTRODUCTION

The preparation of ion liquids (ILs) is an especially active research area in recent years. These materials could be applied in many applications, due to their specific properties such as wide liquid range, high electric conductivity, the lack of vapor pressure, excellent thermal and chemical stability and large electrochemical window [1-5]. These compounds are being applied as solvents for inorganic and organic synthesis [6], for liquid–liquid extraction [7], as electrolyte in solar cells [8], catalysts [9] and in crystal engineering of coordination compounds [10-14]. Ion liquids can be used as cytotoxicity agents for some cell lines [15-18]. The properties and biological activities of ILs can be controlled bya combination of the functionalities of the organic or inorganic anions. Imidazolium based ionic liquids as the organic cationic ionic liquids are widely studied [19-23]. Anti-microbial activities of some imidazolium based ionic liquids have been studied by Rajathi and Rajendran and they found that anti-microbial activities being greatly affected by alkyl chain lengths [24]. According to our knowledge, there is no report on cytotoxicity of an imidazolium based ionic liquid on MCF-7 cell line. In the present study, the cytotoxicity of

*Corresponding author: tabatabaee@iauyazd.ac.ir (M. Tabatabaee)
DOI: 10.22034/jchr.2020.1886005.1079

1-ethyl-3-methylimidazolium bromide (**1**) (Figure 1) on MCF-7 cell line has been investigated.

MATERIALS AND METHODS

Materials and instruments

All required chemical compounds were purchased from Merck Company. 1H-NMR was recorded using a Bruker, Germany 300-MHz, IR The Shimadzu IR-470 spectrometer was uosed for recording of IR spectra and Elemental analyses were performed using a Costech ECS 4010 CHNS analyzer.

Preparation of 1-ethyl-3-Methylimidazolium bromide (1)

A similar synthetic procedure as described in literature was used for preparation of four imidazolium based ionic liquids. A mixture of 1-methylimidazole (10 mmol) and ethylbromide (11 mmol) were placed in a two necked round bottom flask including 20 mL toluene. The reaction mixture was refluxed for 10 h. The reaction mixture was cooled down and unreacted alkyl halide was decanted from the reaction mixture [22].

1-ethyl-3-Methylimidazolium Bromide (**1**); MP. 54-56 ºC, ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.3 (t, 3H), 3.5 (s, 3H), 5.1-6.1 (m, 5H). IR (Nujol; cm^{-1}): 3380-3500 (b), 1624 (s), 1407 (s), 1368 (s), 1154 (s), 1006 (m), 833 (s), 701 (s), 675 (s), 612 (s), 594 (s) cm^{-1}. Anal. Calcd. for $C_6H_{11}N_2Br$: C, 37.70; H, 5.76; N, 14.66 %. Found: C, 37.63; H, 5.69; N, 14.70%.

Cell culture

MCF-7 breast carcinoma cell line were maintained in DMEM High glucose (Thermo Fisher Scientific) supplemented with 10%fetal bovine serum (FBS, Sigma), penicillin (100 IU/mL), streptomycin (100 µg/mL), and in a humidified atmosphere of 95% air/5% CO_2 at 37°C. Cell number and viability was determined by trypan blue staining.

Cytotoxicity assays

Effects of the tested compound on cell viability were determined by MTT colorimetric methods [25]. The solution of MCF-7 cells diluted with DMEM High glucose medium to 10^4 cells/ml were placed in individual wells in 96-well plates. Plates incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h, and then the cells were treated with various concentrations (0.65, 1.3, 2.6, and 5.20 mM) of the test compounds in triplicate and were further incubated for 48 h. After incubation, MTT solution (5 mg/mL in PBS, 10 µL) was added into each well and were further incubated for 4 h. Supernatant were removed from the wells, and DMSO (100µL) was added and mixed thoroughly with the pipette, and incubated at 37°C for 10 min to dissolve the formazan crystals. The optical density of each well was measured at 570 nm to determine the number of viable cells. The 50% inhibitory concentration (IC50) was defined as the concentration required to cause 50% inhibition of cell growth during 48 h of treatment, was estimated using Graph Pad Prism software.

RESULTS AND DISCUSSION

1-ethyl-3-Methylimidazolium bromide (**1**) has been synthesized according to Figure 1. The ^{1}H-NMR spectrum of 1 is shown in figure 2. The hydrogens of methyl group on N1 are appeared at 3.5 ppm and the pike at 1.27 ppm is assigned to CH_3 hydrogens of ethyl methyl group. Hydrogens of CH_2 and there other hydrogens are observed at 5.96 ppm as multiplet pike.

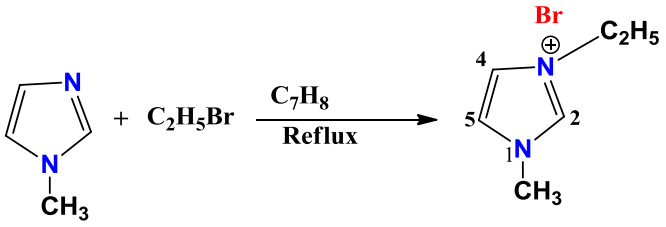

Figure 1. Preparation of 1-ethyl-3-Methylimidazolium bromide (**1**)

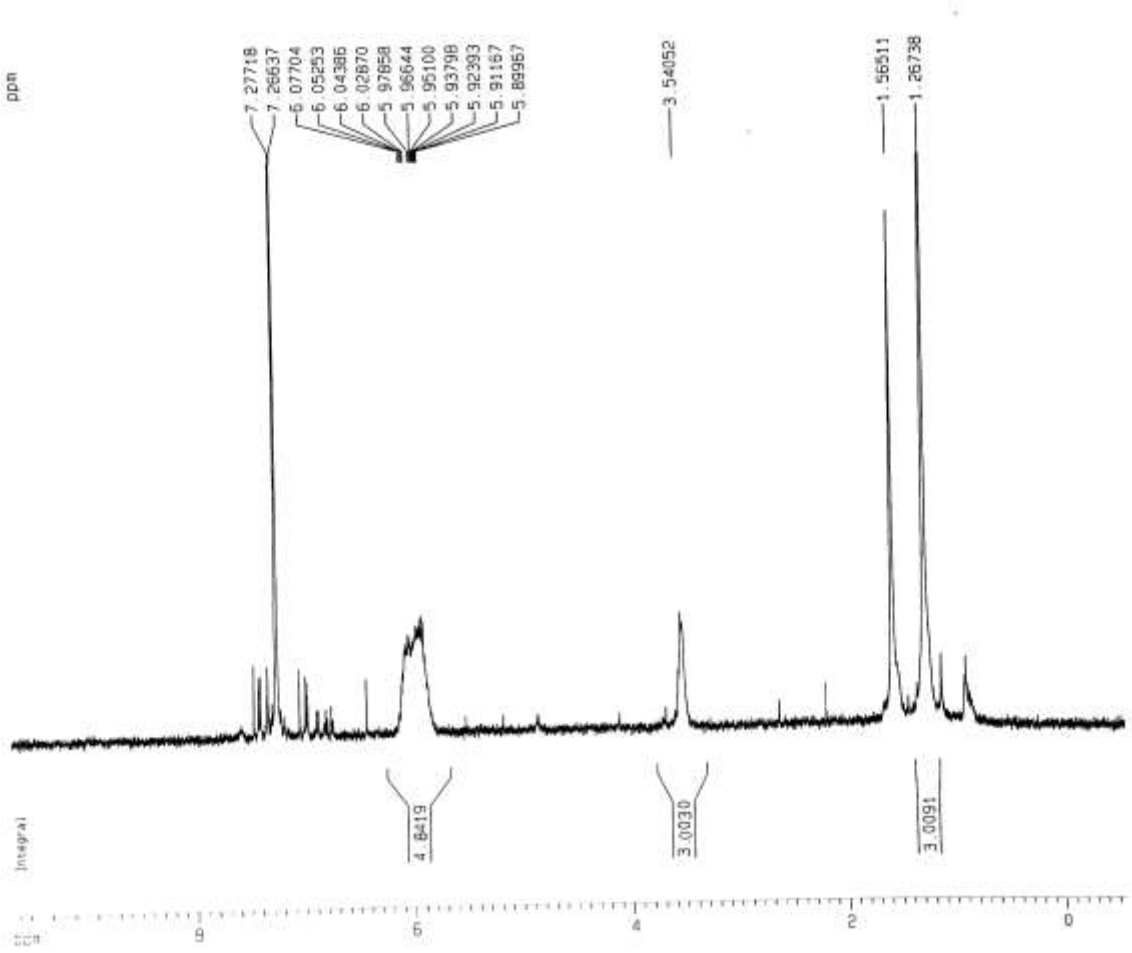

Figure 2. ^{1}H-NMR of 1-ethyl-3-Methylimidazolium bromide (**1**) in $CDCl_3$.

The IR spectrum of compound 1 shows bands at the region 1407-1624 cm^{-1} which could be related to the existence of C=C stretching frequencies and C-N stretching bond is appeared at 1154 cm^{-1}.

The cytotoxic activity of 1-ethyl-3-Methylimidazolium bromide was assessed by the MTT in vitro assay performed in human MCF-7 breast carcinoma cell line. Figure 3 shows Effects of different concentrations of 1-ethyl-3-Methylimidazolium bromide (**1**) on viability of MCF-7 cell line after 48h treatment. The cytotoxic effect was dose-dependent: the decrease of concentration of compound was followed by markedly increase of tumor cell viability. The cytotoxicity of the compound, expressed as IC50 values after 48 h incubation. Results shows IC50 value = 2.91 mM for MCF-7 cell line.

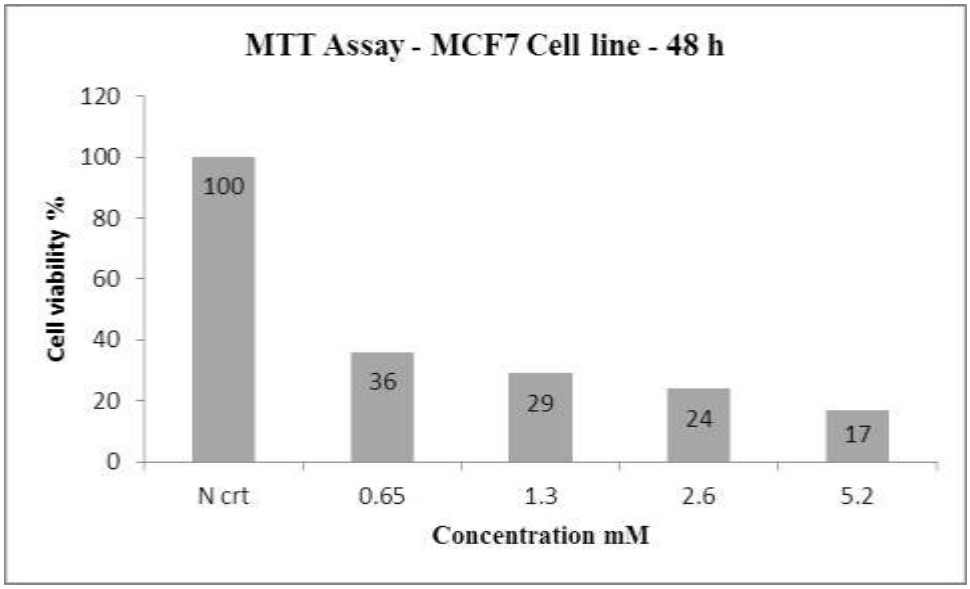

Figure 3. Effects of different concentrations of 1-ethyl-3-Methylimidazolium bromide (**1**) on viability of MCF-7 cell line after 48h treatment.

CONCLUSIONS

1-ethyl-3-Methylimidazolium bromide (**1**) as an ionic liquid has been was synthesized and cytotoxic activity of it was assessed by the MTT in vitro assay performed in human MCF-7 breast carcinoma cell line. **1** can inhibitors on MCF-7 breast cancer cell line. The tumor cell viability are increased with decreasing in concentration of the compound. The half maximal inhibitory concentration IC_{50} of 1 after 48 h is 2.91 mM.

ACKNOWLEDGEMENTS

The authors are grateful to Yazd Branch, Islamic Azad University for the support of this work.

Conflict of interests

The authors declare that they have no conflict of interest.

REFERENCES

1. Filippov A., Munavirov B., Glavatskih S., Shah F.U., Antzutkin O.N., 2020. Diffusion of Ions in Phosphonium Orthoborate Ionic Liquids Studied by 1H and ^{11}B Pulsed Field Gradient NMR. 8, article 119.

2. Naz S., Uroos M., Asim A.M., Muhammad N., Shah F.U., 2020. One-Pot Deconstruction and Conversion of Lignocellulose into Reducing Sugars by Pyridinium Based Ionic Liquid Metal Salt System. Front Chem., 8, Article 236.

3. Pérez S.A., Montalbán M.G.,Carissimi G., Licence P.,Víllora G., 2020. *In vitro* cytotoxicity assessment of monocationic and dicationic pyridinium-based ionic liquids on HeLa, MCF-7, BGM and EA. hy926 cell lines. J Hazard Mater. 385, 121513.

4. Muthayala M.K., Chhikara B.S., Parang K., Kumar A., 2012. Ionic Liquid-Supported Synthesis of Sulfonamides and Carboxamides. ACS Comb Sci. 14, 60−65.

5. Ahmed E., Ruck M, 2011. Chemistry of polynuclear transition-metal complexes in ionic liquids. Dalton Trans. 40, 9347–9357.

6. Wasserscheid P., Welton T., Ionic Liquids-New "Solutions" for Transition Metal Catalysis. Wiley-VCH, New York, 2007.

7. Visser A.E., Swatloski R.P., Reichert W.M., Mayton R., Sheff S., Wierzbicki A., Davis J.H., Rogers R.D., 2002. Task-specific ionic liquids incorporating novel cations for

the coordination and extraction of Hg^{2+} and Cd^{2+}: synthesis, characterization, and extraction studies. Environ Sci Technol. 36, 2523–2529.

8. Papageorgiou N., Athanassov Y., Armand M., Bonhôte P., Pettersson H., Azam A., Grätze M., 1996. The performance and stability of ambient temperature molten salts for solar cell applications. J Electrochem Soc. 143, 3099–3108.

9. Hallett J. P., Welton T., 2011. Room-Temperature Ionic Liquids: Solvents for Synthesis and Catalysis. Chem Rev. 111, 3508–3576.

10. Tang S., A. Babai A., Mudring A.V., 2008. Europium based ionic liquids as luminescent soft materials. Angew Chem. 120, 7743–7746.

11. Babai A., Mudring A.V., 2006. The Octanuclear Europium Cluster $[bmpyr]_6[Eu_8(\mu_4\ O)(\mu_3\ OH)_{12}(\mu_2\ OTf)_{14}\ (\mu_1\ Tf)_2](HOTf)_{1.5}$ Obtained from the Ionic Liquid [bmpyr][OTf. Z Anorg Allg Chem. 632, 1956–1958.

12. Jin K., Huang X., Pang L., Li J., Appel A., Wherland S., 2002. [Cu(I)(bpp)]BF4: the first extended coordination network prepared solvothermally in an ionic liquid solvent. Chem Commun. 2872–2873.

13. Reichert W.M., Holbrey J.D., Vigour K.B., Morgan T.D., Broker G.A., Rogers R.D., 2006. Approaches to crystallization from ionic liquids: Complex solvents–complex results or, a strategy for controlled formation of new supramolecular architectures. Chem Commun. 4767–4779.

14. Holbrey J.D., Vigour K.B., Reichert W.M., Rogers R.D., 2006. The structure of $[Co(H\text{-}tptz)Cl_3]\cdot H_2O$ (tptz=2,4,6-tri(2-pyridyl)-1,3,5-triazine) prepared by crystallization from the ionic liquid, *N*-butyl-*N*-methyl-pyrrolidinium *bis*(trifluoromethanesulfonyl)imide. J Chem Crystallogr. 36, 799–804.

15. Zhu X.W., Ge H.L., Cao Y.B., 2016. Mixture cytotoxicity assessment of ionic liquids and heavy metals in MCF-7 cells using mixtox. Chemosphere. 163, 544-51.

16. Kristina R., Železnjak J., Bubalo M.C., Redovniković I.R., Slivac I., Srček V.G., 2016. Comparative in vitro study of cholinium-based ionic liquids and deep eutectic solvents toward fish cell line. Ecotoxicology and Environmental Safety. 131, 30-36.

17. Wojciech S., Hackenberg F., Müller-Bunz H., Tacke M., 2014, Synthesis and cytotoxicity studies of p-benzyl substituted NHC–copper (I) bromide derivatives. Polyhedron. 80, 3-9.

18. Kumar R.A., Papaïconomou N., Lee J.M., Salminen J., Clark D.S., Prausnitz J.M., 2009. In vitro cytotoxicities of ionic liquids: effect of cation rings, functional groups, and anions. Environ Toxicol. 4, 388-395.

19. Sekhon B.S., 2011. Ionic liquids: Pharmaceutical and Biotechnological Applications. Asian J Pharm Biol Res, 1, 395-411.

20. Borowiecki P., Milner-Krawczyk M., Brzezińka D., Wielechowska M., Plenkiewicz J., 2013. Synthesis and Antimicrobial Activity of Imidazolium and Triazolium Chiral Ionic Liquids. Eur J Org Chem. 712–720.

21. Kaushik N.K., Attri P., Kaushik N., Choi E.H., 2012. Synthesis and Antiproliferative Activity of Ammonium and Imidazolium Ionic Liquids against T98G Brain Cancer Cells. Molecules. 17, 13727-13739.

22. Messali M., Aouad M.R., Ali A.A.S., Rezki N., Hadda T.B., Hammouti B., 2015. Synthesis, characterization, and POM analysis of novel bioactive imidazolium-based ionic liquids. Med Chem Res. 24, 1387–1395.

23. Dzyuba S.V., Kollar K. D., Sabnis S.S., 2009. Synthesis of Imidazolium Room-Temperature Ionic Liquids. J Chem Edu. 86, 856-857.

24. Rajathi K., Rajendran A., 2013, Synthesis, Characterization and Biological Evaluation of Imidazolium Based Ionic Liquids. J Chem. 2, 36-41.

25. Mosmann T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Meth. 165, 55–63.