E-ISSN: 2664-6773
P-ISSN: 2664-6765
Impact Factor: RJIF 5.6
IJCBS 2024; 6(2): 73-75
www.chemicaljournal.org
Received: 13-06-2024
Accepted: 19-07-2024

Thammanna Gowda SS
Adichunchanagiri Institute for Molecular Medicine, Adichunchanagiri Institute of Medical Science, Adichunchanagiri University, BG Nagara, Javarana Hally, Karnataka, India

Lokehs Ravilla
Anugraha Chemicals, Bangalore. [3]Dept of Microbiology, Adichunchanagiri Institute of Medical Science, Adichunchanagiri University, Javarana Hally, Karnataka, India

Priyadharshini AN
Adichunchanagiri Institute for Molecular Medicine, Adichunchanagiri Institute of Medical Science, Adichunchanagiri University, BG Nagara, Javarana Hally, Karnataka, India

Parimala Hanumesh
Adichunchanagiri Institute for Molecular Medicine, Adichunchanagiri Institute of Medical Science, Adichunchanagiri University, BG Nagara, Javarana Hally, Karnataka, India

Shobith Rangappa
Adichunchanagiri Institute for Molecular Medicine, Adichunchanagiri Institute of Medical Science, Adichunchanagiri University, BG Nagara, Javarana Hally, Karnataka, India

Dhanalakshmi TA
Department of Microbiology, Adichunchanagiri Institute of Medical Science, Adichunchanagiri University, Javarana Hally, Karnataka, India

Corresponding Author:
Thammanna Gowda SS
Adichunchanagiri Institute for Molecular Medicine, Adichunchanagiri Institute of Medical Science, Adichunchanagiri University, BG Nagara, Javarana Hally, Karnataka, India

Evaluation of anti-hemolytic and cytotoxic effects of novel piperazine derivatives

Thammanna Gowda SS, Lokehs Ravilla, Priyadharshini AN, Parimala Hanumesh, Shobith Rangappa and Dhanalakshmi TA

DOI: https://doi.org/10.33545/26646765.2024.v6.i2a.106

Abstract
Synthetic compounds have been used as antimicrobial, anticancer, anti-inflammatory & anti antioxidant from long back. Majority of synthetic drugs like molecules causes the side effects along with their biological activity. In this context, the present study aimed to evaluate the anticytotoxic and anti-haemolytic activity of novel piperazine compound which were proved to be effective antimicrobial agents in the previous studies. Three novel piperazine molecules RL-308, RL-318 & RL-327 were used to evaluate their anticytotoxic effect on non-cancerous Human Foreskin Fibroblasts (HFF) cell lines. Interestingly they could able to exhibit that they are nontoxic up to 1.5 µg/ml. But they exhibit the low toxicity at 2 µg/ml concentration and the same level was continued up to 3 µg/ml. Among three, one piperazine derivative RL-308 was very less toxic. Furthermore, the hemolytic potential was determined against human erythrocytes, which showed that the all the three piperazine derivatives proved to be safe at 1.5 ug concentration when compared to the known hemolytic agent H_2O_2. Hence, we can conclude that the novel piperazine derivatives are non-toxic to normal human cells and don't cause lysis in human RBC cells in microgram concentrations.

Keywords: Piperazine, cytotoxic, hemolytic, RBC membrane

1. Introduction
Piperazine is a well-known heterocyclic compound has gained attention due to its diverse biological activities and diverse pharmacological activities. This compound serves as a core structure from which large number of derivatives have been synthesized, these molecules have shown significant potential as an antibacterial agent against various pathogens. The propensity & potentiality of novel piperazine derivatives against various pathogens including multidrug-resistant strains has been demonstrated in our previous studies [1, 3].
Recent research has shown that piperazine derivatives possess a broad spectrum of biological activities, including antibacterial, antifungal, and anticancer properties [2, 4]. The structural modifications of piperazine can enhance its efficacy and selectivity, leading to the development of compounds with improved therapeutic profiles. However, alongside their antibacterial potential, it is crucial to evaluate the anti-haemolytic [5] and cytotoxic effects of these derivatives to ensure their safety for clinical use.
This study aims to systematically assess the anti-haemolytic and cytotoxic properties of newly synthesized piperazine derivatives, providing valuable insights into their therapeutic potential. By establishing a comprehensive understanding of the balance between antibacterial activity and cytotoxicity, this research seeks to contribute to the identification of safe and effective piperazine-based antibacterial agents.

2. Materials and Methods
2.1 Cell Culture
Human Foreskin Fibroblasts (HFF) cell lines were sourced from the American Type Culture Collection (ATCC, Manassas, VA, USA). The HFF cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Merck Millipore, Burlington, MA, USA). Then the media was supplemented with 100 units/mL penicillin, 100 mg/mL streptomycin, and 10% (v/v) fetal bovine serum (FBS). The cells were incubated at 37 °C in a 5% CO2 atmosphere.

2.1 Anti cytotoxic activity
2.1.1 MTT Assay
Cellular cytotoxicity was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-

diphenyltetrazolium bromide (MTT) assay (7). In brief, cells were plated at a density of 1 × 10^4 cells/mL in 96-well microtiter plates containing minimum essential medium with fetal bovine serum (FBS) and allowed to adhere overnight. Serial log dilutions of various test compounds were added to each well in triplicate, with final compound concentrations ranging from 1 µg/mL to 20 µg/mL. The cells were incubated with these piperazine derivatives at 37 °C in a 5% CO2 environment for 24 hours. Following incubation, MTT (Sigma, MO, USA) was added and the cells were further incubated for 4 hours. The resulting formazan crystals were dissolved using MTT solubilization solution (Sigma), and absorbance was measured at 570 nm using a 96-well imaging reader (Tecan multimode reader, USA). The cytotoxicity index was determined using the untreated cells as the negative control and the percentage of cytotoxicity in normal noncancerous cells were calculated following the method of Florento et al. Cell viability (%) was determined using the formula: [(Asample at 570 nm / Acontrol at 570 nm)] × 100.R

2.2 Anti Hemolytic activity

The anti-hemolytic potential of the extracts and fractions was assessed using a spectrophotometric method (8). Five milliliters of blood from a healthy donor were collected in EDTA vials, then centrifuged for 5 minutes at 1000×g. The supernatant was discarded, and the pellet was washed three times with PBS (0.2 M, pH 7.4) before being re-suspended in a 0.5% saline solution.

To test the piperazine derivatives RL-308, RL-318, RL-327, different concentrations of each derivative (0.5µg to 3.5µg/ml in PBS) was added to 1 mL of the erythrocyte suspension and incubated at room temperature for 20 minutes. Following this, 0.5 mL of H_2O_2 solution in buffered saline was added to induce oxidative degradation of the membrane lipids. The reaction mixture was then centrifuged at 1000×g for 10 minutes, and the absorbance of the supernatant was measured spectrophotometrically at 540 nm.

The relative hemolysis was determined by comparing it to the hemolysis observed in the H_2O_2-treated negative control, which was set at 100%. Phosphate-buffered saline was used as the positive control. Each experiment was conducted in triplicate, and the inhibitory activity of the different fractions was calculated and expressed as the percentage of hemolysis inhibition.

3. Results and Discussion

3.1 Anti cytotoxic activity

In order to determine the cytotoxicity of the piperazine derivatives, the piperazine derivatives were screened against noncancerous fibroblastic cell line, NIH/3T3 cells. Series of concentrations (0.5 to 3µg) of three piperazine derivatives were screened. The obtained results indicated that from the concentration of 0.5 ug to 1.5.ug the % viability was maintained up to 90%, from the concentration of 2 to 3 ug the % viability decreases only to 70%. Among three derivatives, 308 was shown to be significantly less toxic (Figure 1-a, b & c).

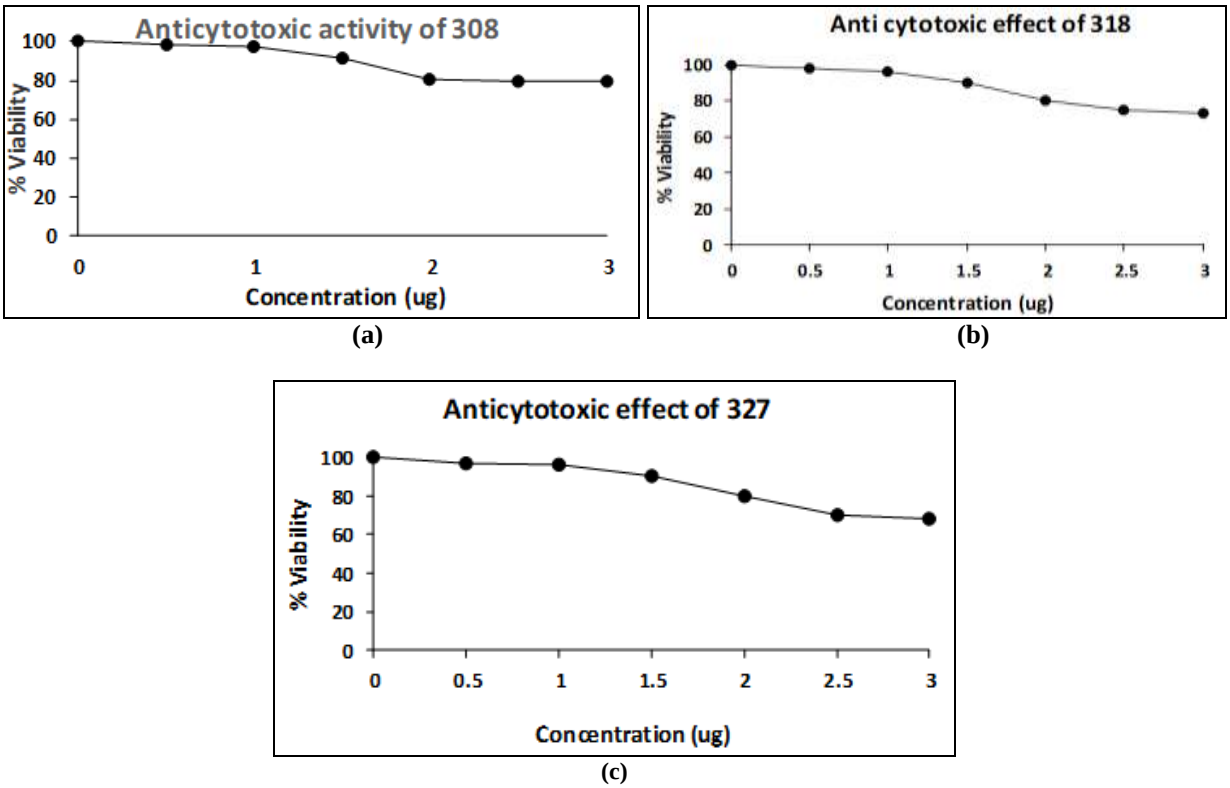

Fig 1: The potential cellular toxicity of novel piperazine derivatives a-308, b-317 & c-327 were measured using noncancerous fibroblastic cell line. Cells were treated with serial concentrations (1 to 20ug/ml) of three piperazine derivatives for 24 hours. The results shown are the average of three independent experiments

3.2 Hemolytic activity

The most abundant cells in the human body are erythrocytes, which have numerous biological and morphological features that make them ideal for drug transport applications. Polyunsaturated fatty acids (PUFAs) and hemoglobin molecules, which are redox-active oxygen transporters and strong promoters of reactive oxygen species, primarily target erythrocytes membrane. Oxidative damage to the erythrocyte membrane lipids is due to varuous reasons such as oxidative drugs, excess of transition metals, high intensity radiation,

and deficiencies in erythrocyte antioxidant coordination [9, 10]. Oxidative damage to the erythrocyte membrane lipids can lead to hemolysis. Hemolysis tends to be particularly severe when red blood cells are exposed to toxic agents like hydrogen peroxide [11].

This study aimed to evaluate whether novel piperazine derivatives RL-308, RL-318, RL 327 could prevent oxidative damage to erythrocyte membranes. And demonstrated the different levels of anti-hemolytic activity (Figure. 2). The results showed that the settling of the RBC cells in control and drug treated (1.5 µg) well labeled as 1, human RBC cells are not lysed. On the other hand, in rest of the wells, which contains the piperazine derivatives from 2 µg to 3.5 µg, the erythrocytes are lysed and are diffused in the well as a turbid liquid. Hence the results proves that the RBC cells are degraded on more than 1.5 µg/ml concentration, which is unsafe to use beyond the mentioned concentration of piperazine drug molecules.

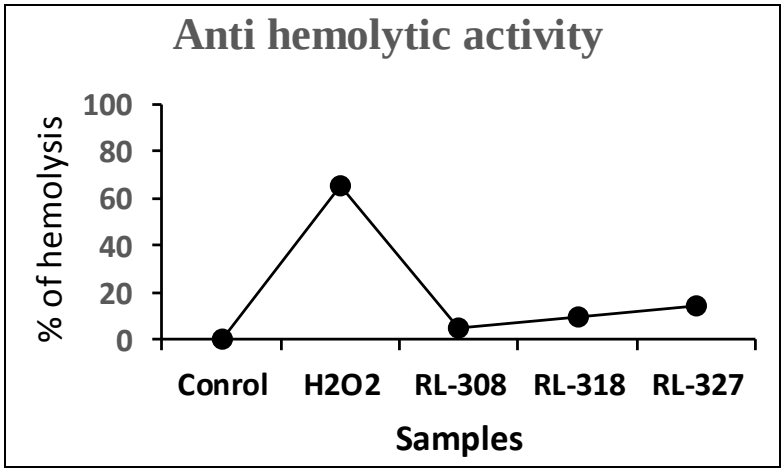

Fig 2: Hemolysis rates induced on human red blood cells (10% hematocrit) by piperazine derivatives (1.5µg/ml concentration). Values are expressed as mean ± standard deviation (n= 3)

4. Conclusion

The current study is a proof of concept that synthetic chemical derivatives can provide a substantial number of drug lead candidates with various biological activities. This is the primary study to report the potential anticytotoxic and antihemolytic effects of previously proved three piperazine derivatives. All the three derivatives showed the anticytotoxic activity against the non-cancerous Human Foreskin Fibroblastic (HFF) cell lines. Similarly, all the three derivatives exhibited the effective antihemolytic activity against the peripheral RBC cell membrane. Among three, one derivative RL-308 exhibit the anticytotoxic and antihemolytic activity very effectively. Hence these piperazine derivatives can be used as a drug molecule to treat various diseases. Further studies are required to know the mechanism of their action against anticytotoxic and antihemolytic activity.

Acknowledgments

We acknowledge Prof. MG Shivaramu, Principal, Adichunchanagiri Institute of Medical Sciences (AIMS), BG Nagar for his constant support & encouragement. We also acknowledge Prof. Prashanth K, Dean, Research, Adichunchanagiri University, BG Nagara for his scientific & moral support.

References

1. Thammanna Gowda SS, Priyadharshini AN, Lokehs Ravilla, Parimala Hanumesh, Shobith Rangappa, Dhanalakshmi TA. Antimicrobial activity of novel piperazine molecules. Int J Curr Microbiol Appl Sci. 2024;13(4):37-44.
2. Kharb R, Bansal K, Sharma AK. A valuable insight into recent advances on antimicrobial activity of piperazine derivatives. Der Pharm Chem. 2012;4(6):2470-88.
3. D'Costa VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, *et al*. Antibiotic resistance is ancient. Nature. 2011;477(7365):457-461.
4. Patel PR, Joshi H, Shah U, Patel B, Bapna M. Novel piperazine derivatives as antimicrobial agents: A comprehensive review. Asian Pac J Health Sci., 2022, 9(2).
5. Hafeez F, Zahoor AF, Rasul A, Mansha A, Noreen R, Raza Z, Ghulam Ali K, Irfan A, El-Hiti GA. Ultrasound-assisted synthesis and in silico modeling of methanesulfonyl-piperazine-based dithiocarbamates as potential anticancer, thrombolytic, and hemolytic structural motifs. Molecules. 2022 Aug;27(15):4776.
6. Walayat K, Mohsin NU, Aslam S, Ahmad M. An insight into the therapeutic potential of piperazine-based anticancer agents. Turk J Chem., 2019, 43.
7. Park JG, Kramer BS, Steinberg SM, Carmichael J, Collins JM, Minna JD, Gazdar AF, Steinberg SM. Chemosensitivity testing of human colorectal carcinoma cell lines using a tetrazolium-based colorimetric assay. Cancer Res. 1987;47(22):5875-5879.
8. El-Borai MA, Rizk HF, Ibrahim SA, Fares AK, El-Tahawy MMT, Beltagy DM. Assessment of anti-hemolytic, cytotoxicity, antioxidant activities and molecular docking study based on thienopyrazole scaffold as pharmacophore. J Mol Struct. 2021 Sep 15;1240:130602.
9. Ebrahimzadeh MA, Nabavi SM, Nabavi SF. Antioxidant activities of methanol extract of *Sambucus ebulus* L. flower. Pak J Biol Sci. 2009;12(5):447-456.
10. Hamidi M, Tajerzadeh H. Carrier erythrocytes: an overview. J Drug Deliv. 2003;10(1):9-20.
11. Naim M, Gestetner B, Bondi A, Birk Y. Antioxidative and antihemolytic activities of soybean. J Agric Food Chem. 1976;24(6):1174-1177.
12. Orrico F, Laurance S, Lopez AC, Lefevre SD, Thomson L, Möller MN, Ostuni MA. Oxidative stress in healthy and pathological red blood cells. J Agric Food Chem. 1976;24(6):1174-1177.