Antibacterial investigation of two Benzamide derived compounds against some pathogenic bacteria

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Abstract

Antimicrobial drugs have become limited useful against nowadays pathogens; this was because of highly uptake and consumption of previously drugs which leads to gain the resistance potential by Pathogenic microbes generally and bacterial pathogens specifically. So in order to stop the mortality outcomes of these pathogens, we conducted our study to design new hopeful Benzamide derived Antibacterial drugs.

in our study we obtained two Benzamide-derived compounds and diluted them in Dimethylsulfoxide (DMSO) at the concentration of (5 and 10mg/ml) and we used Gentamicin(Genta) as positive control, and then we checked them through two standardized antibacterial (Well diffusion and MTT-Microdilution) methods in order to get the IZ and MIC values and evaluate their potential against four common standard bacterial strains which where two gram negative: P. mirabilis (ATCC 14153) and K. pneumonia (ATCC 13883) with two gram positive: E. faecalis (ATCC 29212) and S. pneumoniae (ATCC 6303).in outcomes, our finding showed that both Compound 1 and 2 had only effects on E.faecalis which was (7 and 14mm) of IZ, respectively by only diffusion method. And they didn't have effects on the rest of bacterial strains. Nevertheless our positive control (Genta) had variable inhibition and killing effect against all bacterial strains.

Keywords: *Benzamide, Antibacterial, MTT test, MIC and MBC.*

Introduction

Antimicrobial resistance among microbes is one of the major dangers these days, around the world. The most common resistance among microbial groups is antibacterial resistance, which is a group of defense mechanisms have been developed by some pathogenic bacteria (in order to survive with the existence of these drugs. However, many chemically synthetic compounds have been synthesized by researchers and had the good effect; the antibacterial resistance still is one of the global issues, and still could not be the alternatives of the previously used drugs. (Abdullah et al., 2022), (Willey et al., 2020), (Madigan et al., 2021, p.930-940) and (Zaman et al., 2017).

Klebsiella is one of the gram negative bacteria within the family Enterobacteriaceae, which is the etiological agent of Respiratory tract, urinary tract, septicemia, pneumonia and soft tissue infections. Recently, the K. pneumonia has become one of the most dangers hospital-acquired etiological agents with the high rate of resistance such as MDR and ESBL strains of K. pneumoniae (Jankauskait et al, 2016; Montgomerie, 1979; Podschun & Ullmann 1998). P. mirabilis is another member of this family which mostly causes UTI, in which the rapid MDR strains have been developed against various drugs (Girlich et al, 2020). On the other hands, S. pneumoniae as a community acquire infectious agent and E. faecalis as a nosocomial infectious agent have become among those pathogens with special urgent needs for alternative antibacterial drugs due to highly resistant rate (Miller et al., 2014) and (Cillóniz et al., 2018).

MTT assay is a colorimetric qualitative and quantitative technique that has long been used as a eukaryotic viable cell indicator, but it has recently gained popularity as a prokaryotic viable cell indicator. The stain which has originally yellow color can be utilized by some cellular specific enzymes and then being converted to Formazan crystals (purple color), which is further can be dissolved by DMSO solvent (Abdullah et al., 2022), (Grela et al, 2018) and (Benov L, 2021).

Benzamide is any compound with (C7H7NO) chemical structure, in which its derivate have various biological activities such as antibacterial, antitumor and anti-inflammatory activity, so the researchers have a lot of interest to improve previously derivate as a wish to gain new potentially biological active compound(National Center for Biotechnology Information, 2023) and (Asif, mohammad, 2016).

Our study purpose was to check and test of two Benzamide derived compounds by

colorimetric MTT improved microdilution assay on some pathogenic bacteria (P. mirabilis, S. pneumonia, E. faecalis and K. pneumoniae) in order to find their antibacterial activity toward those pathogens.

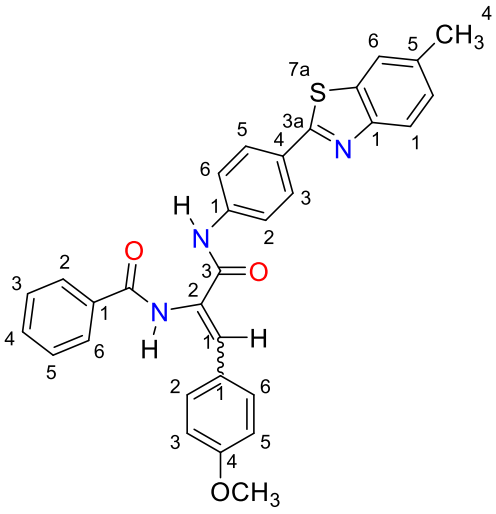
Material and Methods

Two Benzamide derived compounds have been synthesized and chemically checked up by (Ahmed and Hamad, 2023) study, and then have been provided for our study, the chemical names and their structures are presented in (figure 1); Gentamicin has been used as positive inhibition control and Dimethyl-sulfoxide (Sigma) as negative control. Our compounds have been dissolved in Dimethyl-sulfoxide at two different concentration (10mg/ml and 5mg/ml), and then sterilized by 20 micrometer size syringe filter (Minisart®, Biotech, USA). Four bacterial strain stocks which where two gram negative: P. mirabilis (ATCC 14153) and K. pneumonia (ATCC 13883) with two gram positive: E. faecalis (ATCC 29212) and S. pneumoniae (ATCC 6303), have been reactivated in Nutrient Broth and later checked through Well-diffusion assay on Mueller Hinton agar medium. Our bacterial viability indicator in Microdilution assay was a yellow tetrazolium salt, which was Methyl thiazolyl diphenyl-tetrazolium bromide (MTT) at (10 mg/mL) concentration which was used by (Abdulla et al., 2022) study. The reactivated bacterial numbers have been adjusted to McFarland (0.5) by Spectrophotometer based turbidity method. All data has been analyzed by SPSS version 23 (IBM SPSS statistics).

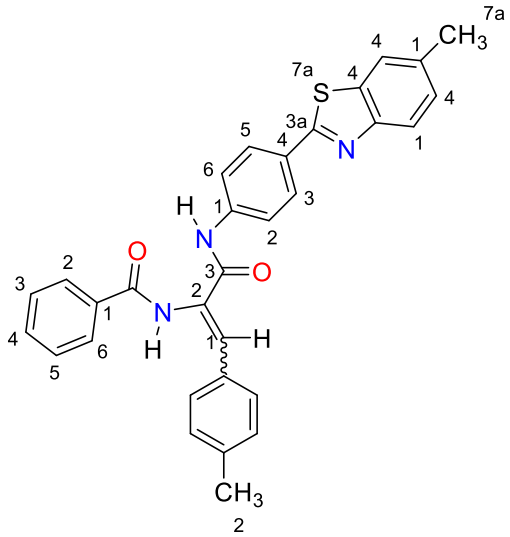
In order to start well-diffusion assay, Bacterial strains have been cultured in NB medium for reactivation, then in order to adjust bacterial number to be (McFarland 0.5), all strains have been diluted in NB. swabs from each bacterial suspension has been spread over Mueller Hinton agar plates in triplicate order, then after drying, two wells have been dug for each compound and filled with 100µl from each concentration (10mg/ml and 5mg/ml), along with positive control (Genta) and negative control (DMSO) on each plate, then incubated for 24 h at 37 C. Eventually, after incubation any clear zones around each wells has been observed and considered as inhibition zone, and compared against our positive control.

The microdilution assay in our study was a modification of (Abdulla et al., 2022) study, in which the Compounds have been diluted by two-fold to get ten different concentration from (10, 5, 2.5… to 0.02) which would diluted again 10 times in microplate wells. 10µl from each concentration have been added in wells of 96-well microplates in order. In order to apply microdilution, the bacterial numbers have been again diluted in NB to get the final number (5x 105 CFU/ml), and 100 µl of each suspension added into each wells. All plates incubated at 37 C for 18 h. then in order to observe MIC and bacterial viability, 10 µl of yellow colored MTT stain has been added to each wells and Incubated again for 4 h. eventually, in order to dissolve the purple colored formazan crystals which have been made by bacterial activity on MTT stain, 100 µl of DMSO have been added. The lowest concentration which causes zero change in MTT stain was considered as MIC of each compound, positive and negative controls.

Figure 1: Benzamide derived compounds chemical structure (C1 and C2)



N-(1-(4-methoxyphenyl)-3-((4-(6-methylbenzo[*d*]thiazol-2-yl)phenyl)amino)-3-oxoprop-1-en-2-yl)benzamide



N-(3-((4-(6-methylbenzo[*d*]thiazol-2-yl)phenyl)amino)-3-oxo-1-(*p*-tolyl)prop-1-en-2-yl)benzamide

Result and discussion

Inhibition zones (IZ) around each compounds with their controls on each plate was observes and recorded in millimeter by ruler after incubation for 24 h in Well diffusion assay, then compared with both two controls(PC and NC). On the other hands, in modified microdilution assay, after the incubation period and addition of DMSO to dissolve purple crystals, MIC were taken as the minimum amount of compound which cause total inhibition, along with MBC which indicate the least amount of chemical which kill microbes completely. ZI. MIC and MBC of all compounds with Genta is presented in (Table 1)

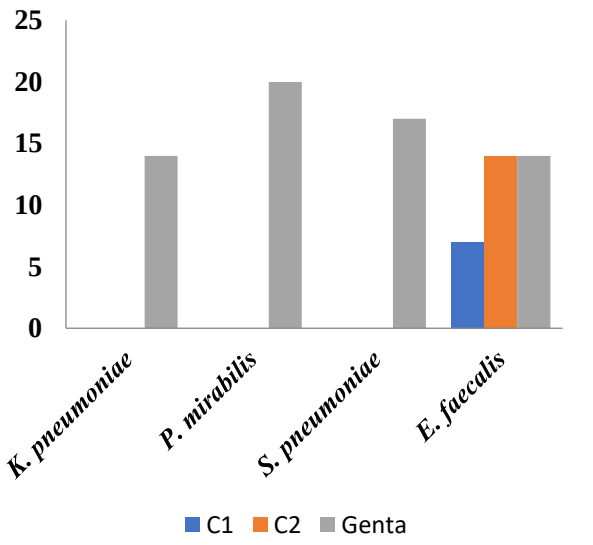
Table 1: Zone of inhibition, MIC and MBC of all compounds with Gentamicin.

Compound	Bacterial strains											
	K. pneumoniae			P. mirabilis			S. pneumoniae			E. faecalis		
	IZ (mm)	MIC (µg)	MBC (µg)	IZ (mm)	MIC (µg)	MBC (µg)	IZ (mm)	MIC (µg)	MBC (µg)	IZ (mm)	MIC (µg)	MBC (µg)
C1	/	/	/	/	/	/	/	/	/	7	/	/
C2	/	/	/	/	/	/	/	/	/	14	/	/
Genta	14	/	/	20	16	16	17	64	64	14	64	64

IZ=Inhibition zone, MIC=Minimum inhibition amount, µg= Microgram and MBC=Minimum bactericidal amount

In our study, out of both two synthesized Benzamide-derived compounds which have been checked in our study against all our tested standard bacterial strains by using both standardized antibacterial method (Well-diffusion and Microdilution), in outcomes both compounds didn't have any effect against all standard bacteria, in exception of E. faecalis in which the IZ means for C1 and C2 were (7 and 14mm). On the other hands, there weren't any MIC and MBC for E. faecalis such as the rest of other bacterial strains. While on the other side, our positive control (Genta) showed inhibition effect by both two methods, in which the IZ mean vales for P. mirabilis, S. pneumonia, E. faecalis and K. pneumoniae were 20, 14, 14 and 17mm, respectively. And the most effective MIC and MBC values were (16 µg) for P. mirabilis, while on the other hands, the worst result was for K. Pneumoniae which were zero effect. The inhibition zone means value have been illustrated in (figure 2).

Figure 2: The inhibition zone Mean values for both compounds and positive control(Genta)



In our study, after testing our compounds by both standardized method, we determined that they don't have actual antibacterial potential against both gram stained bacterial strains with the exception of E. faecali, in which C2 showed inhibition zone close to positive control (Genta) which was 17mm, even though neither of Compounds didn't show any MIC effect against all bacterial strains in our study. While in other studies Benzamide derived Compounds showed better IZs, such studies was conducted by (Sener et al., 2000) in which some compounds of benzacetamides

and benzamide (2a- 2p) were synthesized and tested against some pathogenic bacteria in which K. pneumoniae and E. faecalis by microdilution assay and in their results the MIC was in a range of (25-50 µg/ml).and another example is the study of (Butta et al ., 2019), in which some of azolylbenzamide compounds have been synthesized and prepared at two concentration 50 and 100 µg/ml and then tested against two gram positive(S. aureus and B. subtilis) and two gram negative (K. pneumoniae and P. aeruginosa) bacteria through both standard well- diffusion method such as our study and broth macrodilution assay in contrast to our study, and in outcomes there were variable effect against all bacteria , the most prominent one was against K. pneumoniae which was (9-23mm) of IZ values for all compounds in the exception of 11b compound.

MTT stain is one of the indicators which is used nowadays in order to observe the viability of living (mammalian or bacteria) cells inside of culture medium such studies has conducted by (Abdullah et al., 2022), which used Modified MTT-Microdilution assay in order to measure the bacterial inhibitions caused by tested chemical compounds. Another example was the study conducted by (Florento et al., 2012), which used MTT stain in cancer study. So in our study we used MTT stain protocol for checking the bacterial cell viability by evaluating MIC value toward our both benzamide derived compounds.

Conclusion and Outlook

Antimicrobial drugs have become limited useful against nowadays pathogens, this was because of highly uptake and consumption of previously drugs which leads to gain the resistance potential by Pathogenic microbes generally and bacterial pathogens specifically. So in order to stop the mortality outcomes of these pathogens, we conducted our study to design new hopeful benzamide derived Antibacterial drugs.in our study we obtained two benzamide derived compounds and checked through two standardized antibacterial (Well diffusion and Microdilution) methods in order to get the IZ and MIC values and evaluate their potential against four common standard bacterial strains.in outcomes, our finding showed that both C1 and C2 had only effects on E.faecalis which was (7 and 14mm) of IZ, respectively only by diffusion assay. and they didn’t have effect on the rest of bacterial strains.

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