OPEN PEER REVIEW

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Gene, Cell and Tissue
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Peer Review Report for "In-Vitro Evaluation of Biological Activity of New Series of 1, 3, 4-Oxadiazole Derivatives Against *Bap* Gene Expression in *Acinetobacter baumannii* Biofilm"

Author(s): Sedigheh Akbarnezhad Ghareh Lar, Nakisa Zarrabi Ahrabi, Yasin SarveAhrabi

Review Timeline:

Submit Date: 14 Dec 2020
Revised Date: 19 Jan 2021
Accepted Date: 30 Jan 2021

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0) 19 Dec 2020

Dear AE,
The authors investigated the inhibitory effects of oxadiazole derivatives on bacterial biofilm formation. I'm fascinated by the idea. Still. The article has low readability and has to be grammatically improved. Besides, the authors need to clarify some issues before we can proceed with publication.
1. What do you mean by "on the bacterial external and confer on bacteria the capability to form a ...". The sentence is not clear and poorly written.
2. Please re-write the objective of the study.
3. The authors have to mention the p-values with two or three decimal points in the Results and in the Abstract as well.
4. Provide MesH keywords.
5.There are sentences in the Introduction without related references.
6. Name of all genes should be italicized throughout the manuscript
7. In the Methods, the authors mentioned a time interval of 30 minutes for the annealing of primers while amplifying the BAP gene within 35 cycles. It doesn't seem correct. Please

clarify.
8. The authors need to briefly explain the synthesis process at the beginning of the Methods.
9. At the end of the Discussion, the authors stated that "Since these structures have been synthesized and studied for the first time, any studies on these derivatives were found in our database". What database are the authors referring to?
10. How did Phenyl, Naphthalene, and MethoxyPhenyl groups caused the desirable effects observed for 4a, 4d, and 4i derivatives? Please elaborate on the biological effects of these compounds and similar structures bearing these moieties.
11. Please discuss the limitations of the study.
Thanks.

Behnam Rafiee: Reviewer | **Revision** (0) 27 Dec 2020

Dear AE,
Comments for the authors:
1) The title is appropriate
2) Gene name and bacterium name must be written in italics.
3) biofilm-associated protein (Bap), Must be written in full in the introduction.
4) In material and methods, There are many misspellings and misspellings. brain heart broth is correct. greenhouse!! It is better to use more scientific terms such as incubation. Due to the poor writing of the article, it seems that the Persian version has been translated into English by a semi-skilled expert.
5) Samples from which hospitals or hospitals in Tehran were isolated. The name of the hospital must be mentioned.
6) In the results section, a graph or curve related to real-time PCR is not provided. Assuming the expression or non-expression of genes.
7) In order to complete the work, it was better to do the test related to biofilm production or non-production of biofilm with positive and negative controls and not just in real-time.
8) Figure number one related to electrophoresis is scientifically flawed, firstly, column number 7 has a band that has been distorted with a black pen !!! This is not a scientific work. Secondly, you have written column 7 of positive control !!!! In the absence of a band, there should be a negative control, a positive control that naturally exists in the band and is related to the standard strain of the relevant microorganism.
Third, you did not specify the size of the marker. The number 127 seems strange!
This photo is not acceptable to me.
9) The discussion is fairly straightforward. But a good comparison and a challenge has not been made, it should be reconsidered
In general, the article in question needs a general review in terms of writing and science, as if innovation has been observed and the techniques are appropriate.
Thanks.

Saeedeh Salimi: Associate Editor | **Revision** (0) 28 Dec 2020

Dear EIC,
According to the comments of both reviewers, the manuscript needs major revision. Indeed, the authors should revise the manuscript regarding the English edition.

Best regards

Zahra Heidari: EIC | **Revision** (0) 29 Dec 2020

Dear Author,
Based on the reviewers' and AE comments this manuscript needs major revision and English language editing. a point by point reply to the comments also must be done.
Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Reviewer 1:

1. What do you mean by "on the bacterial external and confer on bacteria the capability to form a ...". The sentence is not clear and poorly written.

A: Acinetobacter bumanni is one of the most common opportunistic pathogens in Health centres that are resistant to many antibiotics due to biofilm production.

2. Please re-write the objective of the study.

A: The aim of this study was to investigate the effect of new derivatives of 1, 3, 4-oxadiazole based on the 1, 3, 4-oxadiazole-2-yl-piridin-2-yl-methanol group on the expression of *A. baumannii Bap* gene, which is effective in promoting the biofilm formation ability of drug-resistant specimens.

3. The authors have to mention the p-values with two or three decimal points in the Results and in the Abstract as well.

A: Done in main file.

4. Provide MesH keywords.

A: Oxadiazole, *Acinetobacter baumannii*, *BAP*, Real-time PCR

5.There are sentences in the Introduction without related references.

A: Done in main file.

6. Name of all genes should be italicized throughout the manuscript

A: Done in main file.

7. In the Methods, the authors mentioned a time interval of 30 minutes for annealing of primers while amplifying the BAP gene within 35 cycles. It doesn't seem correct. Please clarify.

A: PCR to confirm the *Bap* gene: The presence of *Bap* gene was confirmed by molecular PCR using Sinagen fermentase kit. The reaction mixture for PCR of *Bap* gene is as follows: Master Mix (1x): 12.5 µl, Primer F (0.1 - 1µM) 1µ, Primer R (0.1 - 1µM): 1µ, Template DNA: 5µ (20pg), Sterile Deionized Water: 5.5µ, the final volume is 25µL. PCR was set up to detect the *Bap* gene according to the following method: Initial denaturation, 95°C, 120s, 1cycle, denaturation, 93°C, 45s, 35 cycles, Annealing,57°C, 60s, 35 cycles, Extension, 72°C, 30s, 35 cycles, Final Extension, 72°C, 90s, 1cycle. The primers used for the Bap and *16srRNA* genes are listed in Table 1. Finally, six strains of *A. baumannii* with Bap gene were selected for molecular studies.

8. The authors need to briefly explain the synthesis process at the beginning of the Methods.

A: Synthesis of derivatives: 1, 3, 4-oxadiazole compounds were synthesized using a single-stage, high yield method. Single-stage: 1. First, N-Iso-cyan-imino-triphenyl-phosphoran (1mmol) + 2-Pyridine-carbaldehyde (1mmoL) were dissolved in CH3COCH3 (7 mL). 2- In the next step, the carboxylic acid (1mmoL) in CH3COCH3 (10 mL) was added to the previous solution. The final solution was stirred for 12 hrs. by a magnetic stirrer at room temperature. The solvent was removed by evaporation, and the viscous residue was purified by flash column chromatography (silica gel powder: petroleum ether–ethyl acetate (4:1)) (13).

9. At the end of the Discussion, the authors stated that "Since these structures have been synthesized and studied for the first time, any studies on these derivatives were found in our database". What database are the authors referring to?

10. How did Phenyl, Naphthalene, and MethoxyPhenyl groups caused the desirable effects observed for 4a, 4d, and 4i derivatives? Please elaborate on the biological effects of these compounds and similar structures bearing these moieties.

11. Please discuss the limitations of the study.

A: In our previous study, we tested synthesis derivatives at a concentration of 1 mg/ml on a standard sample of A. baumannii, but in this study, despite a concentration 10 times lower and also on a clinical sample, amazing results were obtained. The 4i derivative in the presence of methoxyphenyl attached to the main structure had acceptable anti-biofilm properties against A. baumannii. Methoxyphenyl is one of the most effective compounds in various structures of 1, 3, 4-oxadiazoles, whose antibacterial and anti-cancer properties have already been proven. This compound (methoxyphenyl) has the ability to affect the cytoplasmic membrane, electron transport chain, metabolic activity, gene synthesis and inhibit protein synthesis. By studying these compounds on other virulence genes of A. baumannii and studying the results, we can study these compounds as well as other derivatives of these structures in the future with laboratory studies and Animal study as a treatment or adjunctive therapy in the form of tablets or additives, used in patients' food as well as hand and environment disinfectants to prevent the spread of infection caused by this bacterium in hospital settings.

Reviewer 2:

1) The title is appropriate

2) Gene name and bacterium name must be written in italics.

A: Done in main file.

3) biofilm-associated protein (Bap) , Must be written in full in the introduction.

A: The first member of this group of proteins, called BAP, has been identified in the bacterium *A. baumannii*. These proteins are located on the outer surface of bacteria. They consist of a central nucleus consisting of iterations of similar sequences. Bacteria are able to form biofilms, thus playing an important role in infectious pathogens. Structural features of these proteins include their presence on the outer surface of bacteria, high molecular weight, and the presence of a central nucleus consisting of repetitive sequences of similar sequences. These proteins give the bacterium the ability to form biofilms and thus play an important role in infectious pathogens.

4) in material and methods ,There are many misspellings and misspellings . brain heart broth is correct . greenhouse!! It is better to use more scientific terms such as incubation. Due to the poor writing of the article, it seems that the Persian version has been translated into English by a semi-skilled expert.

A: Done in main file.

5) Samples from which hospital or hospitals in Tehran were isolated. The name of the hospital must be mentioned.

A: Done in main file.

6) n the results section, a graph or curve related to real-time PCR is not provided. Assuming the expression or non-expression of genes.

A: Done in main file.

7)In order to complete the work, it was better to do the test related to biofilm production or non-production of biofilm with positive and negative controls and not just real time.

A: Done in main file.

8)Figure number one related to electrophoresis is scientifically flawed, firstly, column number 7 has a band that has been distorted with a black pen !!! This is not a scientific work. Secondly, you have written column 7 of positive control !!!! In the absence of a band, there should be a negative control, a positive control that naturally exists in the band and is related to the standard strain of the relevant microorganism. Third, you did not specify the size of the marker. The number 127 seems strange! This photo is not acceptable to me.

A: Done in main file.

9) The discussion is fairly straightforward. But a good comparison and a challenge has not been made, it should be reconsidered

A: Done in main file.

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Revision (1)

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REFEREE: Reviewer | **Revision** (1) 23 Jan 2021

Dear AE,
The study is reviewed carefully. According to comments and revisions by the author, this manuscript is accepted.
Thanks

REFEREE: Reviewer | **Revision** (1) 25 Jan 2021

Dear AE,
The manuscript is well-revised and my concerns were addressed.
Thanks.

Zahra Heidari: Associate Editor | **Revision** (1) 30 Jan 2021

Dear EIC,
Revisions were done well. Thus this manuscript can be accepted for publication in GCT.
Thanks.

Zahra Heidari: EIC | **Revision** (1) 30 Jan 2021

Dear Author,
Revisions were done well. Thus this manuscript can be accepted for publication in GCT.
Thanks.