Reviewer #1:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: This manuscript "BZD9L1 benzimidazole analogue hampers colorectal tumor progression by impeding angiogenesis" was well designed and showed good results. The results found that BZD9L1showed its anti-angiogenic potential in CRC tumor and inhibited CRC tumor growth in in vitro and in vivo.

In Fig.2, A and B are mislabeled, and the authors should detected the protein expression of intercellular adhesion molecule 1 (ICAM-1, VE-cadherin and ITGA5) and SIRT1/2, besides QPCR analysis.

Thank you for picking up on the mistake. We have amended the figure legend accordingly. We agree that protein expression data is crucial to support the gene expression data. However, at this point, we apologise we could not provide protein analyses of the targets mainly due to the corresponding author's move overseas. We hope that the gene expression data is sufficient to support the xCelligence cell adhesion data at this point. Cell adhesion is one of the early steps of angiogenesis. We hope that other assays in the paper have sufficiently highlighted the anti-angiogenic potential of BZD9L1. Nevertheless, we take note of the comment to the best of our ability and have also included the significance of adhesion protein analyses and their post-translational modifications in the discussion, as below:

Lines 546-549: The effect of BZD9L1 on a panel of cell adhesion molecules and their post-translational modifications may be crucial to determine the specific mechanisms that may impact EC adhesion stability and function, thus warrants further investigations.

In animal experiments, the methods only described that "The treatments were injected intraperitoneally at a maximum volume of 250μl every three days", and how many times to inject BZD9L1.

We thank you for the comment. We have amended the dosage frequency as below:

The control group was treated with 0.5% CMC only. The treatments were injected intraperitoneally at a maximum volume of 250μl **once** every three days, alongside the measurement of weight and tumor size using the standard formula (Zhou et al. 2017):

Volume of tumor=π/6 x (Length x Width x Height)

The discussion part is mainly about the repeated description of the results, which are relatively shallow and need to be modified. For example, the mechanism, clinical application and combined treatment strategy such as oncolytic virus (doi: 10.3390/biomedicines8120593) of BZD9L1 against colon cancer need to be further discussed.

Thank you for the useful comments. The 'discussion' has been improved with the potential clinical application or combination strategy being elaborated. Please refer to lines 650-674.

There are some language and grammar errors through the manuscript. Please correct them carefully.

This has been addressed. Thank you.

Reviewer #2:
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision
Specific Comments to Authors: General comments for the author Enhanced angiogenesis is a cancer hallmark and critical for colorectal cancer (CRC) invasion and metastasis. Anti-angiogenic drugs and chemotherapy represent a standard of care for treating metastatic CRC. However, drug resistance to CRC is common in clinical practice, and the search for new drugs has been the focus of research. In this manuscript, the authors investigated the the anti-angiogenic potential of BZD9L1 on endothelial cells (EC) in vitro, ex vivo and in HCT116 CRC xenograft in vivo models. The conclusion showed that anti-angiogenic potential of BZD9L1 to reduce CRC tumor progression. Overall, this study is interesting and innovative, but it needs to be revised before it can be published.

Major concerns: 1. "Novelty" BZD9L1 has not been reported to inhibit angiogenesis. Therefore, this study is innovative. 2. "Value of findings" The existing experimental data support the value of further development of BZD9L1, so this study has a guiding significance for peers. 3. "Experimental design" Overall, the experimental design of this research is reasonable, but there are several problems, the author must improve. First, EA.hy926 is a fusion cell.

Although some studies have pointed out that it has certain vascular endothelial properties, our team found that EA.hy926 basically does not have the ability of vascular endothelial cells to form tubes in vitro and most of the surface markers of vascular endothelial cells have been lost. Therefore, I believe that the authors should use vascular endothelial cells from human CRC tissue isolated or human umbilical vein endothelial cells to supplement the in vitro toxicity and tubular experiments.

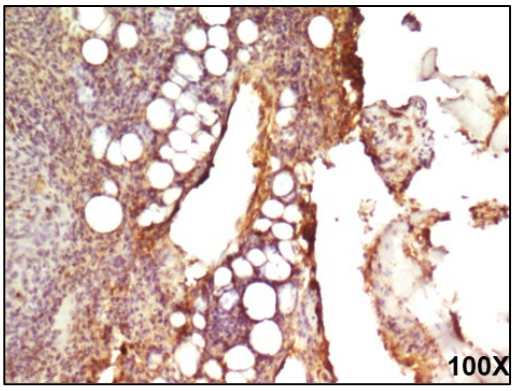
We fully agree with this comment. Hence, we have confirmed the anti-angiogenic potential of BZD9L1 on an *ex vivo* model comprising mouse choroid explants, which better recapitulates the tissue microenvironment to support the findings on EaHy926 cells. Furthermore, we have included the significance of corroborating the findings in alternative endothelial cells such as that in HUVECs or ECs from CRC tissues, in the 'Conclusion', line 699.

Second, because BZD9L1 itself has cytotoxic effects, it is reasonable to inhibit the tumorigenesis of CRC cells in vivo. To highlight the potential anti-angiogenic effect of BZD9L1, the authors should further examine the number and status of CD31-positive vessels in the xenografts tissue by IHC.

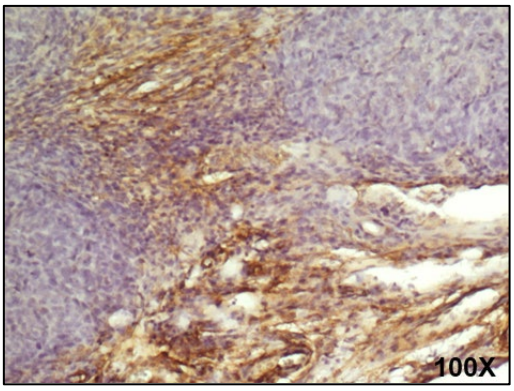
We agree with this comment. We have performed the proposed experiment to determine the protein expression of CD34 on the xenograft tissues. Unfortunately, due to the necrotic state of the tissues, we were unable to analyse the data accurately, as also verified by our pathologist (Professor Gurjeet Kaur). Hence at this point, the alternative would be to determine the anti-angiogenic potential of BZD9L1 *in vivo* via gene expression studies of EC markers. Please see below:

Inconclusive CD34 protein expression in the tumor sections

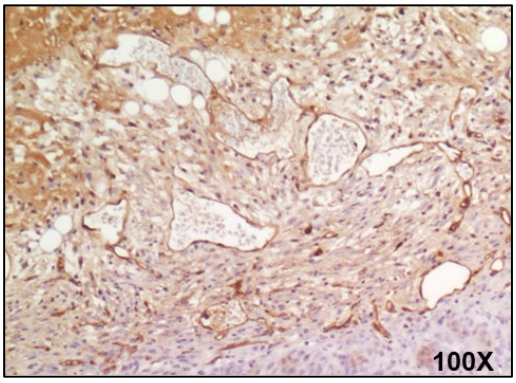
CD34 protein expression is meant to indicate the formation of vascular endothelial cells. Anti-CD34 antibody is a highly sensitive marker for endothelial cell differentiation and has also been studied as a marker for vascular tumors. However, in this study, we did not manage to evaluate the vessel size and density due to necrosis that overlaps the clear expression of the CD34 protein. Therefore, the CD34 protein expression could not be quantified, and the result remained inconclusive.



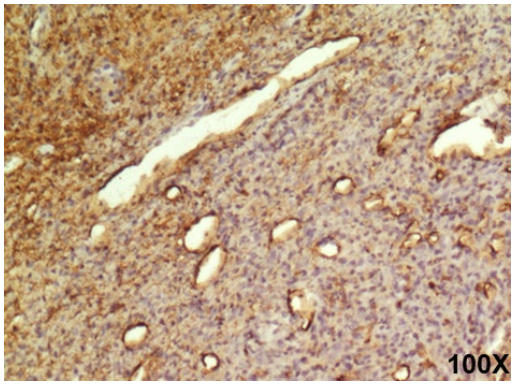
Vehicle control



BZD9L1 (50mg/kg)



BZD9L1 (250mg/kg)



Sunitinib, positive control

(40mg/kg)

Third, the authors need to consider whether to add VEGFA to rescue in some in vitro experiments.

Thank you for the comments. At this point, BZD9L1 did not significantly affect VEGF expression based on the Quantibody Human Angiogenesis Array (RayBiotech, Inc, Norcross, GA) , hence the proposed experiment may not add value to the study, which was designed with the primary objective of reporting the basic mechanism and mode of action of BZD9L1 as a potential anti-angiogenic agent in drug discovery. However, future studies can include rescue experiments specific to cytokines modulated by BZD9L1 for academic purposes.

Other amendments/note to editor:

- We have also included the data on hSIRT1 and hSIRT2 gene expression in the xenograft samples to show that BZD9L1 not only downregulated the expression of these SIRTs in mouse tissues but also in HCT116 xenograft of human origin to render the shrinkage of tumours collectively.

- We have included Dr Sasidharan Sreenivasan as an author instead of placing him under acknowledgement for his significant scientific contributions to animal work.

- Acknowledgement: We thank Mr. David Chung Tze Yang from Genomax Technologies Sdn. Bhd. for technical support on the xCELLigence Real-Time Cell Analysis (RTCA) instrument (Agilent Technologies, United States).