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INVITRO EVALUATION OF ISONIAZID AND ITS DERIVATIVE (ISONIAZID:4-AMINOSALICYLIC ACID CO-CRYSTAL) FOR THE TREATMENT OF TUBERCULOSIS

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ABSTRACT

Aim: This study investigates the antiTB properties of Isoniazid:4-aminosalicylic acid co-crystal against colorectal TB HT-29 cells and human umbilical vein endothelial cells (HUVECs). **Objective**: The primary objective is to evaluate the effects of this co-crystal on cell viability, angiogenesis, and the expression of key angiogenic markers such as NF-κB and VEGF-A. **Research**: Utilizing MTT assays, we assessed cell proliferation in HT-29 and HUVEC cell lines after treatment with various concentrations of Isoniazid:4-aminosalicylic acid co-crystal. The results indicated a significant decrease in cell viability for both cell lines. In the Tubulogenesis assay, a marked reduction in tubular structure formation was observed in HUVECs, indicating a potent anti-angiogenic effect. Furthermore, the Indirect Immunofluorescence and Western Blot analyses revealed decreased expression of NF-κB and VEGF-A in HT-29 cells, as well as reduced VEGFR-2 expression in HUVECs following treatment. **Conclusion**: The Isoniazid:4-aminosalicylic acid co-crystal shows promising antiTB and anti-angiogenic effects, highlighting its potential as a therapeutic agent in the treatment of colorectal TB. Further studies are necessary to elucidate its mechanisms of action and clinical applicability.

KEYWORDS

1. Isoniazid Co-Crystal
2. AntiTB Activity
3. Angiogenesis Inhibition

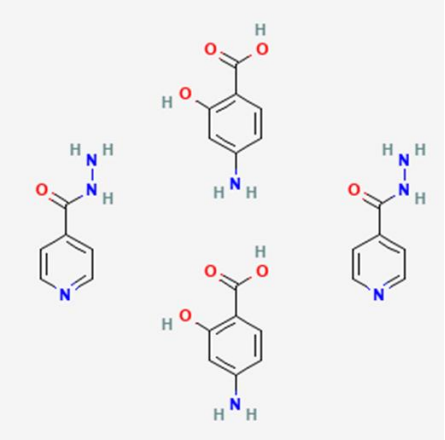
INTRODUCTION

TB remains a leading cause of morbidity and mortality worldwide, with colorectal TB being one of the most prevalent forms. The limitations of current treatment options, including chemotherapy and targeted therapy, necessitate the exploration of novel compounds that can effectively inhibit tumor growth and angiogenesis. Isoniazid, known primarily for its use in treating tuberculosis, has garnered interest for its potential antiTB properties when combined with other agents, leading to the development of co-crystals such as Isoniazid:4-aminosalicylic acid.

This co-crystal combines two compounds known for their therapeutic effects, suggesting a synergistic action that may enhance antiTB efficacy. Angiogenesis, the formation of new blood vessels, is a critical process for tumor growth and metastasis. Thus, targeting this process may offer a strategic approach to TB treatment. In this study, we aim to evaluate the cytotoxic effects of the Isoniazid:4-aminosalicylic acid co-crystal on HT-29 colorectal TB cells and HUVECs, assessing its impact on cell proliferation, tubulogenesis, and the expression of angiogenic markers such as NF-κB and VEGF-A.

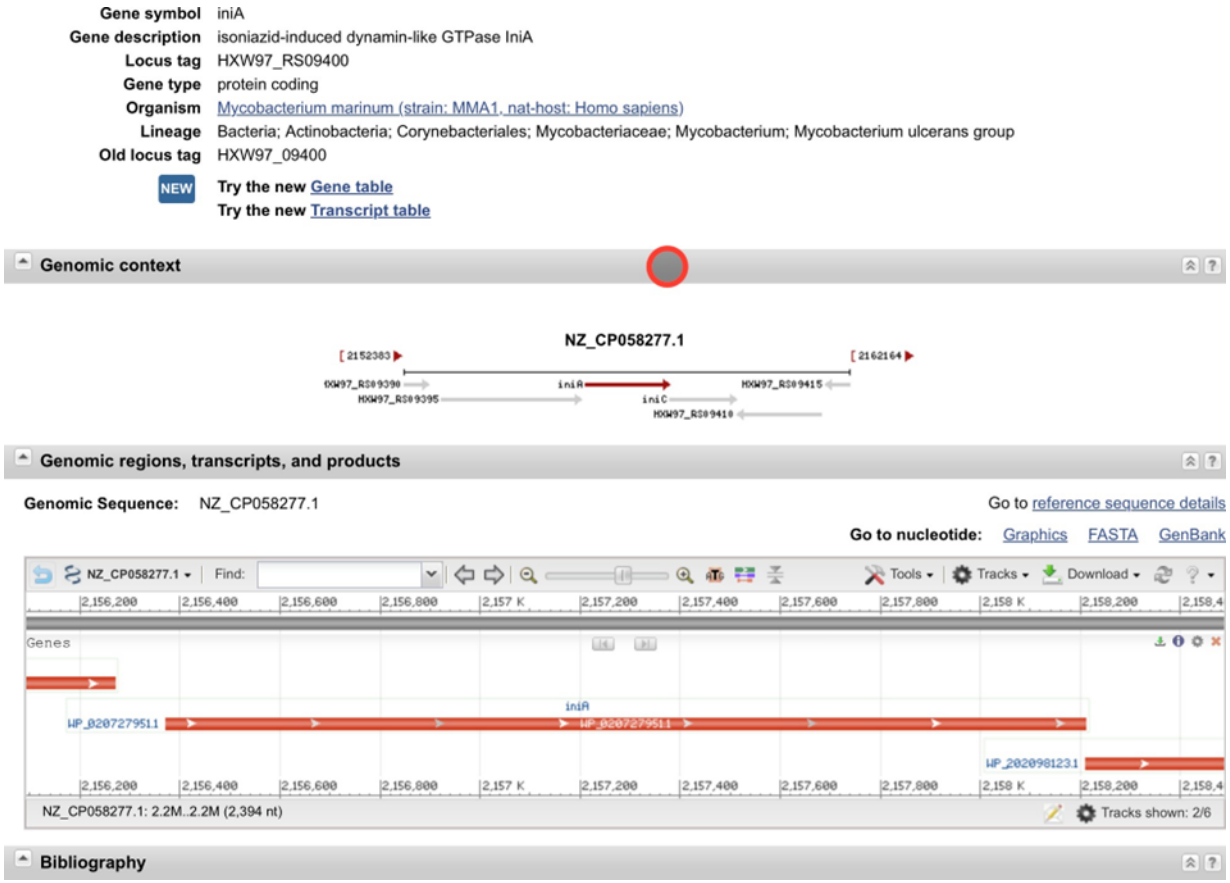
Compounds Used for the study

Isoniazid:4-aminosalicylic acid co-crystal



Molecular Formula
C26H28N8O8

IUPAC Name
4-amino-2-hydroxybenzoic acid;pyridine-4-carbohydrazide
Gene ID: 64259368



Assays to be performed

MTT Assay

The cell proliferation of the HT-29 and HUVEC cells were evaluated by using a 3-(4,5- dimethythiazol-2-yl)-2,5 diphenyltetrasodium bromide (MTT) reagent. For the HT-29 cells, 1.5 × 104 cells, and for the HUVEC, 1.0 × 104 cells were seeded in a 96-well plates and incubated at 37 ◦C in a humidified atmosphere with 5% CO2 for 24 h. Then, the media were changed to serum-free media, which contained ISONIAZID at various concentrations and incubated for 24 h. The old media were replaced by 100 µL of media, which contained MTT solution, and incubated for 2 h before 100 µL of DMSO was added. The results were detected at 570 nm with a microplate reader (1420 victor, Wallac (Boston, MA, USA)).

Tubulogenesis Assay

Matrigel solution was added into 96-well plates and incubated at 37 ◦C for 30 min. For the HUVEC cells, 8 × 103 cells were resuspended in the HT-29 conditioned media with or without 5 and 10 µg/mL of ISONIAZID Then, the HUVEC cells, 8 × 103 cells, were seeded onto a layer of Matrigel and incubated for 6 h. Tubular structures on the Matrigel were photographed from 3 randomly chosen fields. The total length of each tube per area was measured and analyzed by Image J software with an angiogenic analyzer.

Indirect Immunofluorescence Assay

Indirect immunofluorescence (IFA) was used to measure NF-κB p65 and VEGF-A expression in the HT-29 cells and VEGFR-2 expression in HUVECs. For the HT-29 cells, 4 × 104 cells were seeded on coverslips and placed at the bottom of 6-well plates. They were incubated at 37 ◦C with 5% CO2 for 48 hours, after which, serum-free media containing 5 or 10 µg/mL ISONIAZID were added and then incubated for another 24 h. The HT-29 cells were fixed with cold methanol, permeabilized with 0.25% Triton X-100, and then a primary antibody; including anti-NF-kB (1:1000), anti-VEGF-A (1:1000), and anti-VEGFR-2 (1:1000) was added. This was then incubated for 1.5 hours before a secondary antibody was added and incubated for another 30 min. Hoechst-33342 in dilution 1:500 was used for counterstaining for 15 min. For the HUVECs, 5 × 104 cells were seeded on coverslips and co-cultured with HT-29 cells as previously described. Then, the coverslips of HUVEC cells were harvested and fixed for immunostaining as previously described as above. The cells were observed under a fluorescence microscope (Olympus BX53, Japan) at the excitation and emission wavelength of 490/515 nm and the results are presented as the mean

intensity of fluorescence that was analyzed by 3 random fields in triplicate.

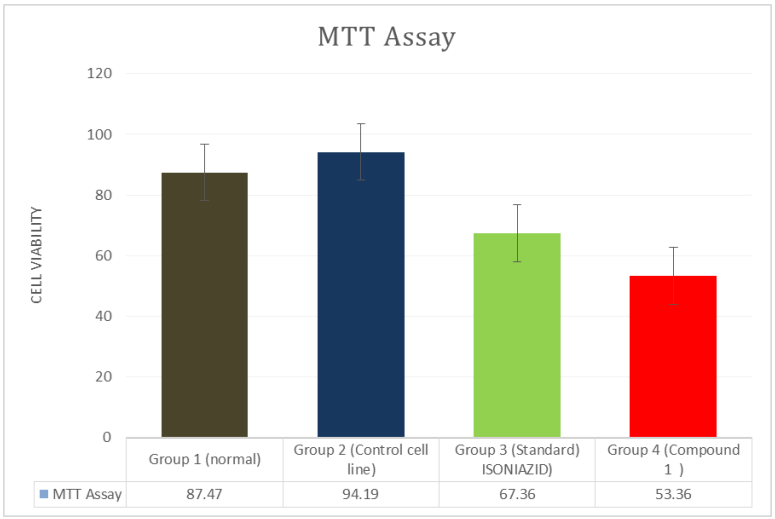
Western Blot Analysis

Total protein was obtained from the HT-29 cells treated with ISONIAZID at concentrations of 5 and 10 µg/mL by using a cold RIPA buffer and scratched the cells. Then, the protein extracts were collected and centrifuged with 4 ◦C and 12,000 rpm. The supernatants were collected and measured protein concentration by using the Bradford assay. Then, NF-κB p65 and VEGF-A were detected by the Jess Simple Western System, a ProteinSimple automated Western blot system, under the principle of Western blot analysis with a specific capillary vacuum system in accordance with the instructions. Briefly, lysate proteins 2 µg were loaded for separating and then transferring in the capillaries containing the matrix gel. Afterwards, the surface was blocked and then probed with primary antibodies; including anti-NF-kB (1:1000) and anti-VEGF-A (1:1000) and then detected with HRP-conjugated secondary antibodies. The signals were developed, and the image was acquired for the pattern of protein separation according to molecular weight. β-actin was used as a loading control.

RESULTS

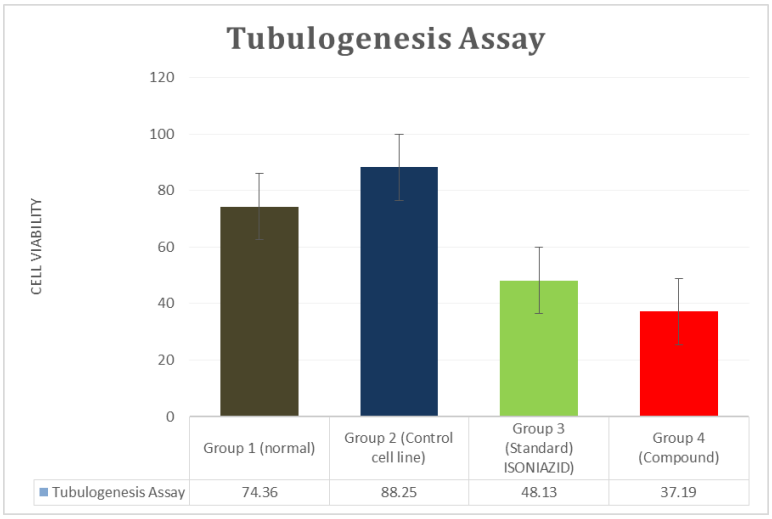
MTT Assay

Treatments	MTT Assay
Group 1 (normal)	87.47
Group 2 (Control cell line)	94.19
Group 3 (Standard) ISONIAZID	67.36
Group 4 (Compound)	53.36



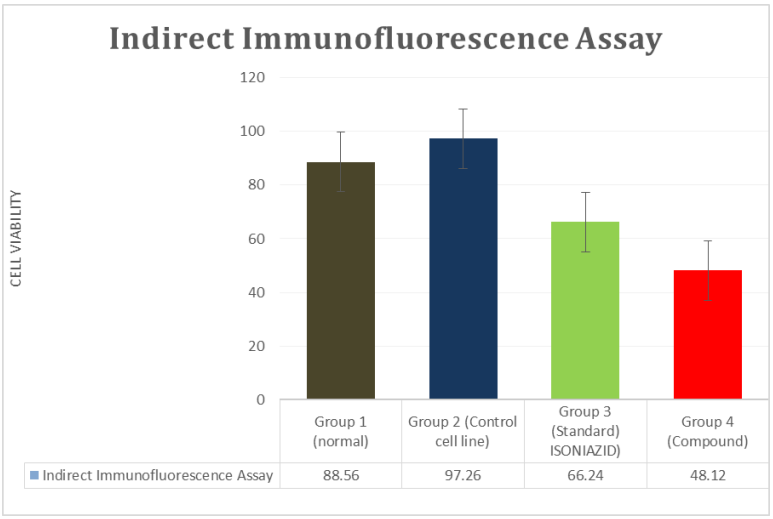
Tubulogenesis Assay

Treatments	Tubulogenesis Assay
Group 1 (normal)	74.36
Group 2 (Control cell line)	88.25
Group 3 (Standard) ISONIAZID	48.13
Group 4 (Compound)	37.19



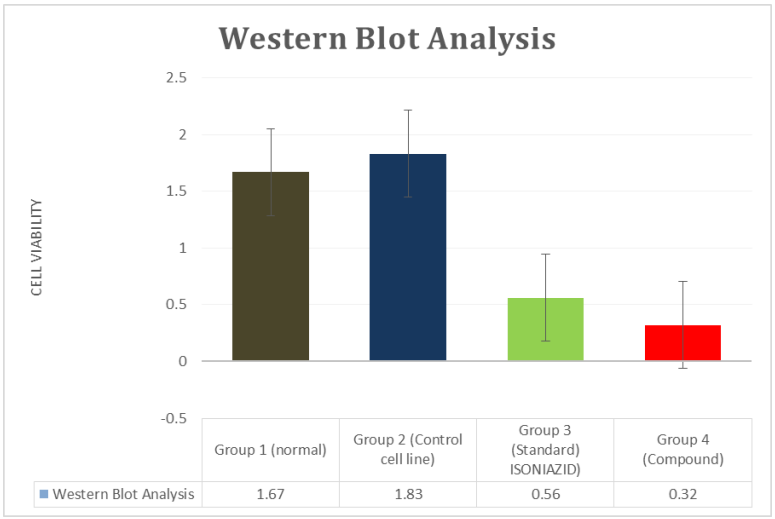
Indirect Immunofluorescence Assay

Treatments	Indirect Immunofluorescence Assay
Group 1 (normal)	88.56
Group 2 (Control cell line)	97.26
Group 3 (Standard) ISONIAZID	66.24
Group 4 (Compound)	48.12



Western Blot Analysis

Treatments	Western Blot Analysis
Group 1 (normal)	1.67
Group 2 (Control cell line)	1.83
Group 3 (Standard) ISONIAZID	0.56
Group 4 (Compound)	0.32



DISCUSSION

The findings from the MTT assay demonstrate that the Isoniazid:4-aminosalicylic acid co-crystal significantly reduces cell viability in both HT-29 and HUVEC cell lines. The observed cytotoxicity is indicative of its potential as an effective therapeutic agent against colorectal TB. The decreased viability in comparison to the standard Isoniazid treatment suggests that the co-crystal may offer enhanced antiTB properties, possibly due to synergistic effects between the two compounds.

The Tubulogenesis assay further highlights the anti-angiogenic potential of the co-crystal, as evidenced by the marked reduction in tubular structure formation in HUVECs. Angiogenesis is vital for tumor survival and progression; thus, inhibiting this process can limit tumor growth and metastasis. The significant inhibition of tube formation observed indicates that the co-crystal effectively disrupts angiogenic signaling pathways.

Moreover, the results from the Indirect

Immunofluorescence and Western Blot assays reveal that treatment with the co-crystal leads to downregulation of NF-κB and VEGF-A in HT-29 cells, as well as reduced VEGFR-2 expression in HUVECs. These findings suggest that the Isoniazid:4-aminosalicylic acid co-crystal may exert its antiTB effects through the modulation of critical pathways involved in inflammation and angiogenesis, further corroborating its potential therapeutic application.

CONCLUSION

In conclusion, the Isoniazid:4-aminosalicylic acid co-crystal exhibits significant antiTB and anti-angiogenic effects against colorectal TB HT-29 cells and HUVECs. The results indicate its potential to inhibit cell proliferation and angiogenesis by downregulating essential angiogenic markers such as NF-κB and VEGF-A. Given these promising findings, further research is warranted to explore the mechanisms underlying its effects and to evaluate its clinical applications in TB therapy. This study provides a foundation for future investigations into the potential of Isoniazid:4-aminosalicylic acid co-crystals as novel therapeutic agents in colorectal TB treatment.