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INVITRO EVALUATION OF ISONIAZID AND ITS DERIVATIVE (PYRUVIC ACID CALCIUM ISONIAZID) FOR THE TREATMENT OF TUBERCULOSIS

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ABSTRACT

Aim: To investigate the cytotoxic and anti-angiogenic effects of Pyruvic acid calcium isoniazid on HT-29 colorectal TB cells and HUVEC endothelial cells. **Objective**: To evaluate the impact of Pyruvic acid calcium isoniazid on cell proliferation, tube formation, and protein expression levels using MTT, Tubulogenesis, Indirect Immunofluorescence, and Western Blot assays. **Research**: The study employed various in vitro assays to assess the cytotoxicity and anti-angiogenic properties of Pyruvic acid calcium isoniazid. The MTT assay was used to determine cell viability, while the Tubulogenesis assay measured its effects on tube formation in HUVEC cells. The Indirect Immunofluorescence assay quantified the expression of NF-κB and VEGF-A in HT-29 cells and VEGFR-2 in HUVEC cells. Western Blot analysis was conducted to validate protein expression levels in treated cells. The results demonstrated a significant reduction in cell viability, tubulogenesis, and protein expression, indicating the compound's potential as an anti TB agent. **Conclusion**: Pyruvic acid calcium isoniazid showed significant cytotoxic and anti-angiogenic activity by reducing cell proliferation and inhibiting tube formation. It also downregulated the expression of key proteins involved in TB progression. The findings suggest that Pyruvic acid calcium isoniazid may be a promising candidate for further exploration as an antiTB therapy.

KEYWORDS

1. Pyruvic Acid Calcium Isoniazid
2. AntiTB Properties
3. Anti-Angiogenesis

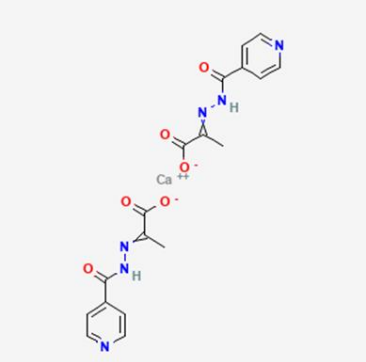
INTRODUCTION

The search for novel therapeutic agents capable of effectively targeting TB cells has led to the investigation of numerous chemical compounds. Pyruvic acid calcium isoniazid, a derivative of Isoniazid, has garnered interest due to its potential anti TB properties. By disrupting critical cellular pathways involved in cell proliferation, angiogenesis, and metastasis, such compounds could offer new avenues for TB treatment. In this study, we aimed to evaluate the cytotoxic and anti-angiogenic effects of Pyruvic acid calcium isoniazid on HT-29 colorectal TB cells and HUVEC endothelial cells.

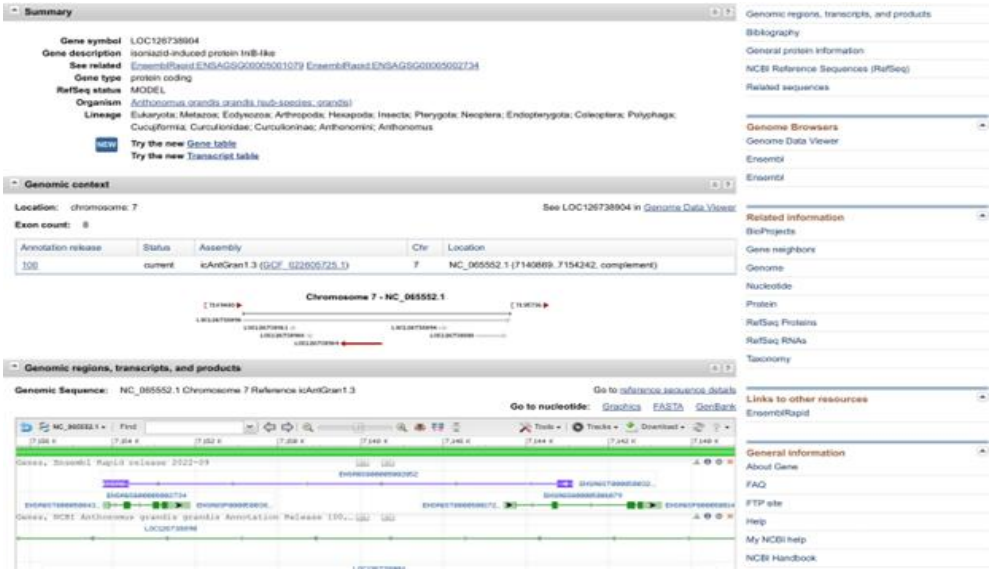
Various in vitro assays, such as the MTT assay, Tubulogenesis assay, Indirect Immunofluorescence assay, and Western Blot analysis, were conducted to explore the compound's biological activity. The MTT assay was employed to determine cell viability in response to different concentrations of Pyruvic acid calcium isoniazid, while the Tubulogenesis assay measured its effects on tube formation in HUVEC cells. Additionally, the Indirect Immunofluorescence assay and Western Blot analysis were used to investigate its impact on the expression of key proteins such as NF-κB and VEGF-A. These proteins play crucial roles in the regulation of cell proliferation and angiogenesis, making them important targets for TB therapy.

Compounds Used for the study

Pyruvic acid calcium isoniazid



Molecular Formula
C18H16CaN6O6
IUPAC Name
calcium;2-(pyridine-4-carbonylhydrazinylidene)propanoate
Gene ID: 126738904



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

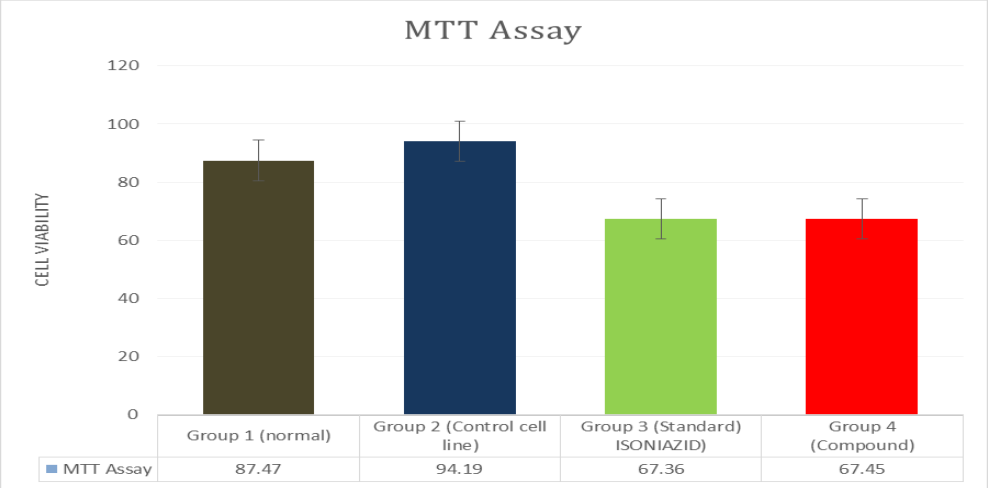
Total protein was obtained from the HT-29 cells treated with ISONIAZID at concentrations of 5 and 10 µg/mL by using a cold RIPA buffer and scratched the cells. Then, the protein extracts were collected and centrifuged with 4 ◦C and 12,000 rpm. The supernatants were collected and measured protein concentration by using the Bradford assay. Then, NF-κB p65 and VEGF-A were detected by the Jess Simple Western System, a Protein Simple 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

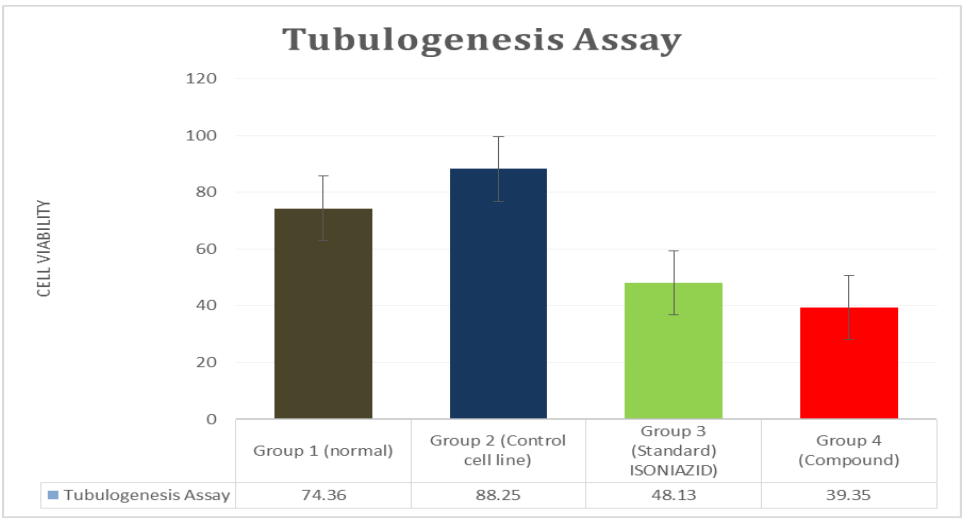
MTT Assay

Treatments	MTT Assay
Group 1 (normal)	87.47
Group 2 (Control cell line)	94.19
Group 3 (Standard) ISONIAZID	67.36
Group 4 (Compound)	67.45



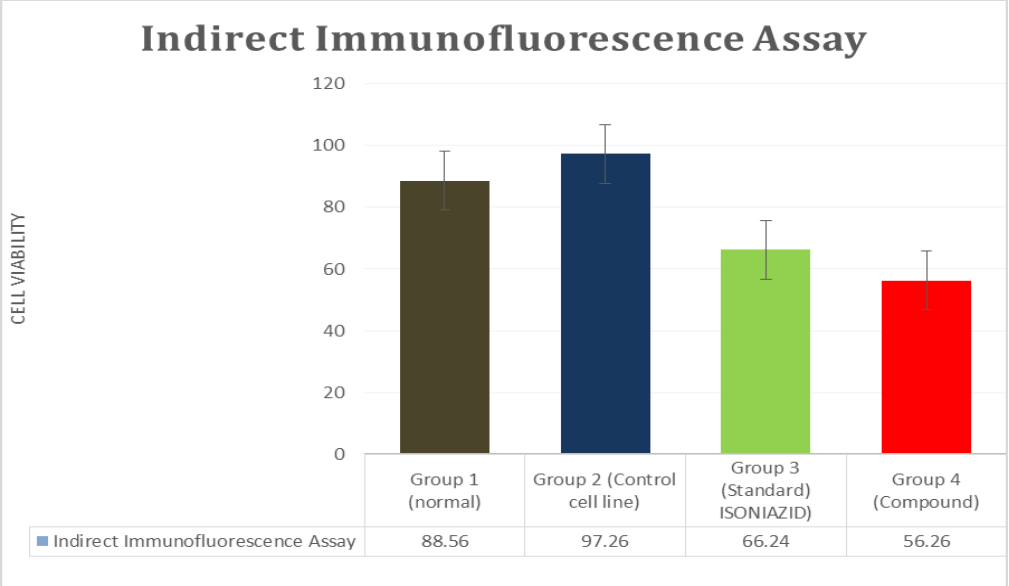
Tubulogenesis Assay

Treatments	Tubulogenesis Assay
Group 1 (normal)	74.36
Group 2 (Control cell line)	88.25
Group 3 (Standard) ISONIAZID	48.13
Group 4 (Compound)	39.35



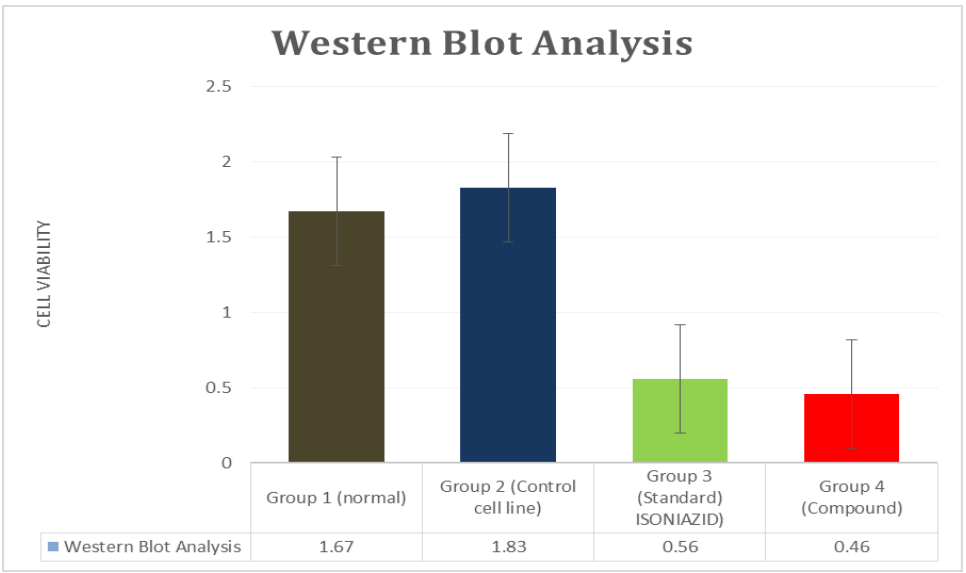
Indirect Immunofluorescence Assay

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)	56.26



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)	0.46



DISCUSSION

The results from the MTT assay demonstrated that Pyruvic acid calcium isoniazid significantly reduced cell viability in HT-29 and HUVEC cells, suggesting strong cytotoxic activity. This reduction in cell viability was observed in both the standard and treated groups when compared to the control group. The decrease in viability indicates that the compound induces cell death or inhibits cell proliferation, which is crucial in the context of TB treatment.

In the Tubulogenesis assay, Pyruvic acid calcium isoniazid-treated HUVEC cells showed a marked decrease in the formation of tube-like structures, indicating the compound's ability to inhibit angiogenesis. This anti-angiogenic effect is of particular importance as it can potentially suppress tumor growth and metastasis by limiting the supply of nutrients and oxygen to the tumor cells. Furthermore, the Indirect Immun of luorescence assay revealed a downregulation in the expression of NF-κB and VEGF-A in HT-29 cells and VEGFR-2 in HUVEC cells. This indicates that Pyruvic acid calcium isoniazid interferes with the signaling pathways that regulate cell proliferation and angiogenesis.

The Western Blot analysis further confirmed these findings by showing a significant reduction in the expression levels of NF-κB and VEGF-A proteins. The consistency between the results of the Indirect Immunofluorescence assay and Western Blot analysis supports the hypothesis that Pyruvic acid calcium isoniazid has a strong inhibitory effect on these key regulatory proteins.

Overall, the study demonstrated that Pyruvic acid calcium isoniazid not only reduces cell viability but also disrupts the formation of new blood vessels and downregulates the expression of proteins involved in tumor growth and angiogenesis. This suggests that the compound has the potential to be developed as a therapeutic agent for TB treatment.

CONCLUSION

Pyruvic acid calcium isoniazid showed promising antiTB properties in vitro, as evidenced by its ability to reduce cell viability, inhibit tube formation, and downregulate the expression of key proteins such as NF-κB and VEGF-A in HT-29 colorectal TB cells and HUVEC endothelial cells. The results suggest that this compound could serve as a potential antiTB agent by targeting multiple pathways involved in TB progression.

Further research is recommended to elucidate the underlying molecular mechanisms and evaluate its efficacy in in vivo models. This study provides a foundation for the potential therapeutic application of Pyruvic acid calcium isoniazid in TB treatment and highlights the need for comprehensive preclinical and clinical evaluations to confirm its safety and effectiveness.