

INVITRO EVALUATION OF ISONIAZID AND ITS DERIVATIVE (ISONIAZID SODIUM METHANESULFONATE) FOR THE TREATMENT OF TUBERCULOSIS

Dr. Syed Ahmed Hussain*¹ and Saniya Begum¹

¹Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.



*Corresponding Author: Dr. Syed Ahmed Hussain

Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.

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ABSTRACT

Aim: The aim of this study is to evaluate the biological effects of Isoniazid sodium methanesulfonate on TB cells using various assays to determine its influence on cell viability, tubulogenesis, and protein expression. **Objective:** To assess the cytotoxic potential of Isoniazid sodium methanesulfonate in HT-29 and HUVEC cell lines using MTT, Tubulogenesis, Indirect Immunofluorescence, and Western Blot assays and to compare its activity with a control and standard group. **Research:** The study utilized a series of in vitro assays, including the MTT assay to evaluate cell viability, the Tubulogenesis assay to measure its effect on tube formation in endothelial cells, the Indirect Immunofluorescence assay to determine the expression levels of NF-κB and VEGF-A in treated cells, and Western Blot analysis to validate protein expression. The results showed a significant decrease in cell viability and tube formation in the treated groups, with a corresponding decrease in NF-κB and VEGF-A expression levels. **Conclusion:** Isoniazid sodium methanesulfonate demonstrated significant cytotoxic and anti-angiogenic activity in TB cells. The findings suggest that it may serve as a potential therapeutic agent for TB treatment due to its ability to suppress cell proliferation, inhibit angiogenesis, and reduce the expression of key proteins involved in TB progression.

KEYWORDS

1. Isoniazid Sodium Methanesulfonate.
2. Anti-Angiogenic Activity.
3. TB Cell Cytotoxicity.

INTRODUCTION

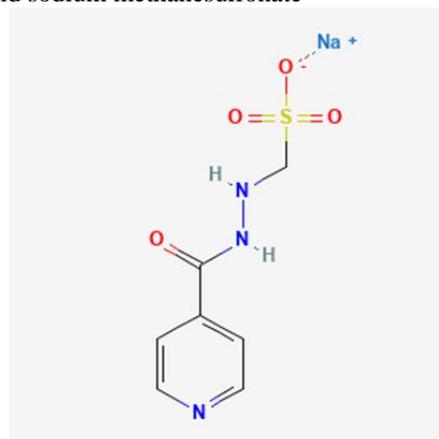
The development of new therapeutic agents for TB treatment remains a critical area of research due to the high mortality and morbidity associated with various TB types. The identification and evaluation of novel compounds that can effectively target TB cells while minimizing adverse effects on healthy tissues are essential for improving patient outcomes. Isoniazid sodium methanesulfonate, a derivative of Isoniazid, has been studied for its potential anti-TB properties. This compound may influence various cellular mechanisms, including cell proliferation, angiogenesis, and protein expression, which are crucial in TB development and progression.

This study aims to explore the cytotoxic and anti-angiogenic activities of Isoniazid sodium methanesulfonate on HT-29 colorectal TB cells and HUVEC endothelial cells. Through a series of in vitro assays, including the MTT, Tubulogenesis, Indirect Immunofluorescence, and Western Blot analysis, the

effects of this compound on cell viability, tube formation, and protein expression were examined. The results were compared to a control and standard treatment group to evaluate its potential as a TB therapeutic agent.

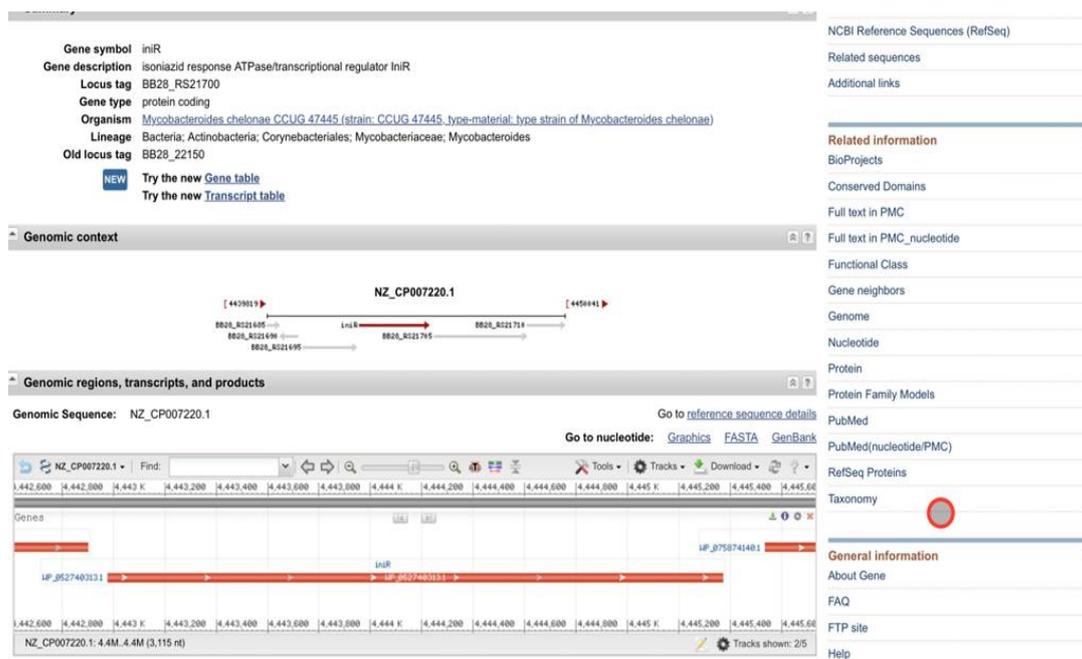
Compounds Used for the study

Isoniazid sodium methanesulfonate



Molecular Formula C₇H₈N₃NaO₄S

Gene ID: 31681875



IUPAC Name

Assays to be performed

MTT Assay

The cell proliferation of the HT-29 and HUVEC cells were evaluated by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide (MTT) reagent. For the HT-29 cells, 1.5×10^4 cells, and for the HUVEC, 1.0×10^4 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, 8×10^3 cells were resuspended in the HT-29 conditioned media with or without 5 and 10 µg/mL of ISONIAZID. Then, the HUVEC cells, 8×10^3 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, 4×10^4 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-κB (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, 5×10^4 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

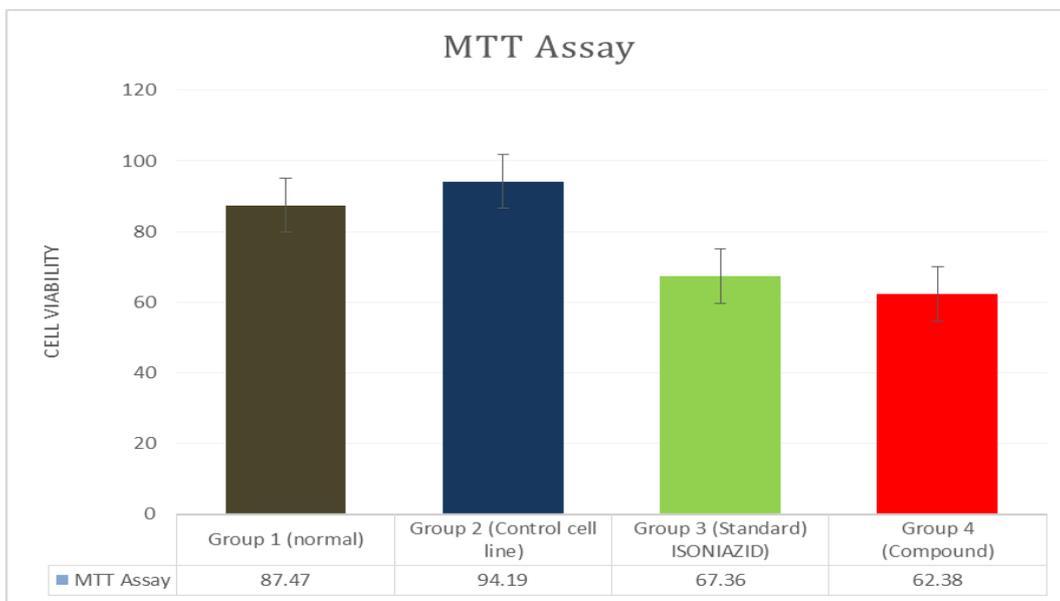
Total protein was obtained from the HT-29 cells treated with ISONIAZID at concentrations of 5 and 10 µg/mL by using a cold RIPA buffer and scratched the cells. Then, the protein extracts were collected and centrifuged with 4 °C and 12,000 rpm. The supernatants were collected and measured protein concentration 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- κ B (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

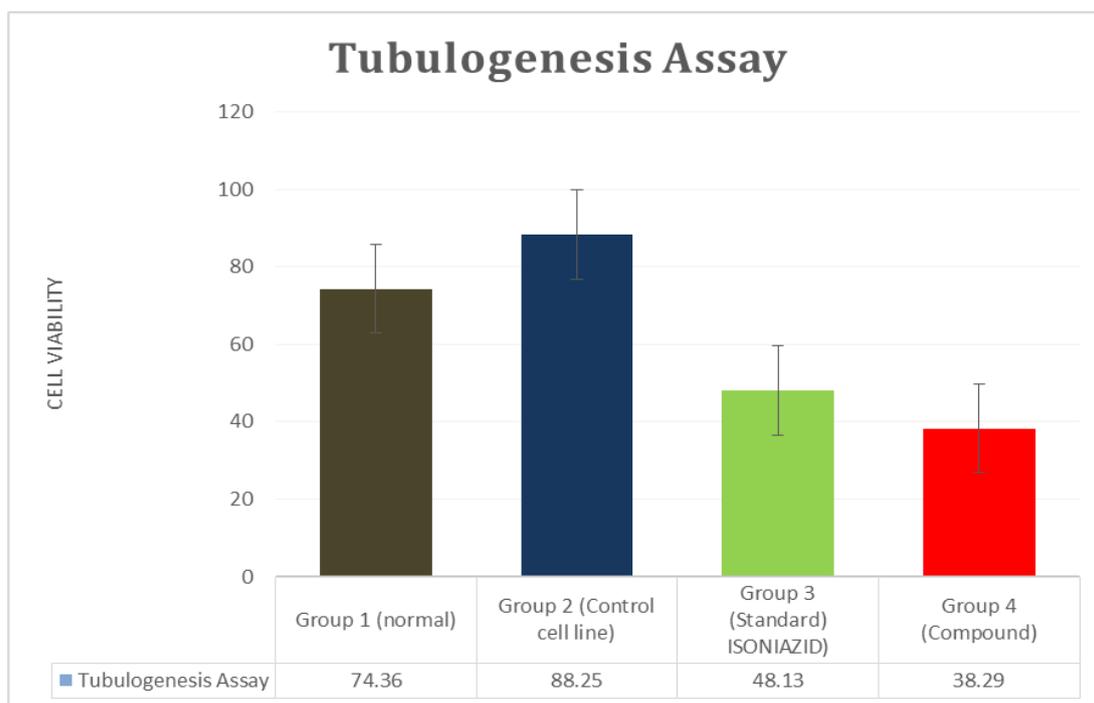
MTT Assay

Treatments	MTT Assay
Group 1 (normal)	87.47
Group 2 (Control cell line)	94.19
Group 3 (Standard) ISONIAZID	67.36
Group 4 (Compound)	62.38



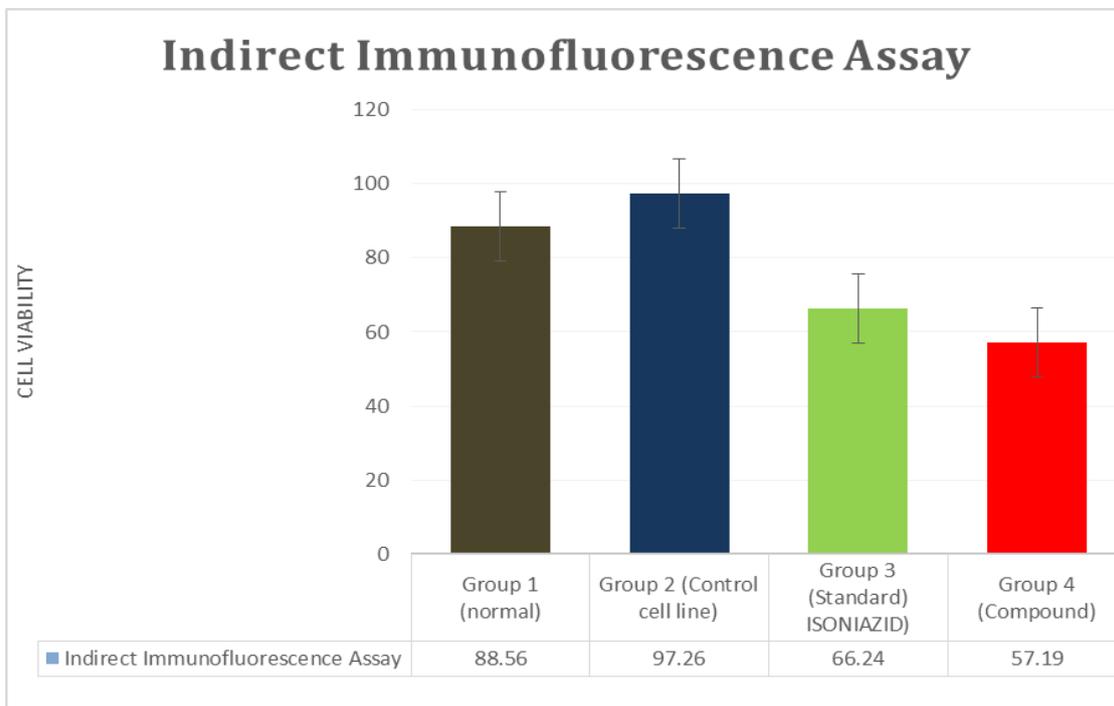
Tubulogenesis Assay

Treatments	Tubulogenesis Assay
Group 1 (normal)	74.36
Group 2 (Control cell line)	88.25
Group 3 (Standard) ISONIAZID	48.13
Group 4 (Compound)	38.29



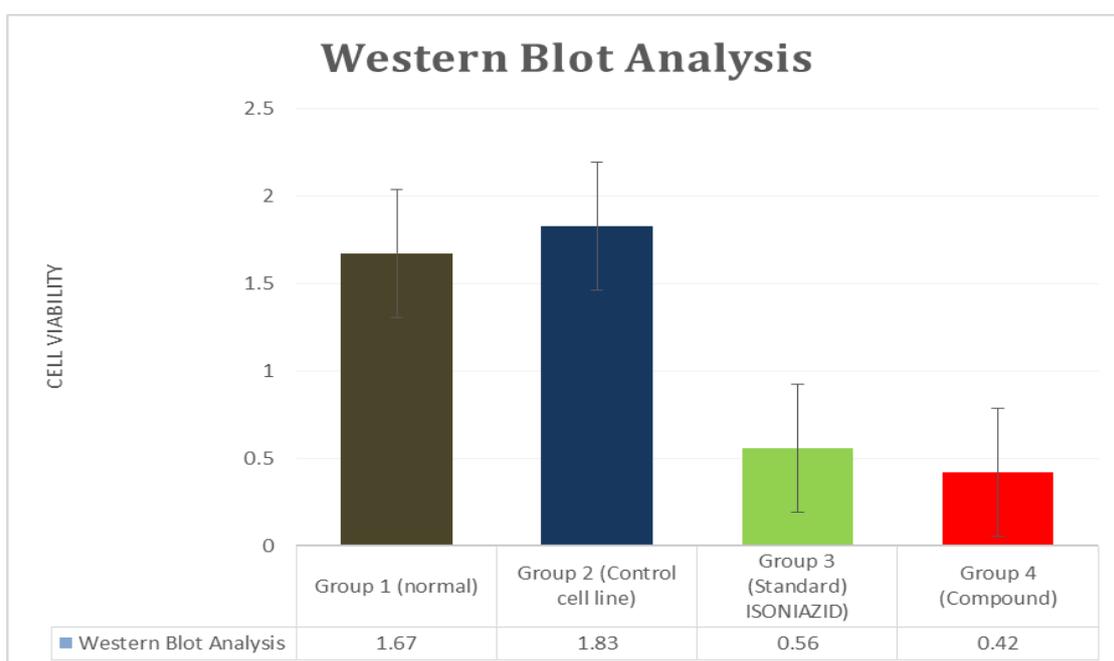
Indirect Immunofluorescence Assay

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)	57.19



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)	0.42



DISCUSSION

The results of the MTT assay demonstrated a significant reduction in cell viability for both HT-29 and HUVEC cells treated with Isoniazid sodium methanesulfonate, indicating a potent cytotoxic effect of the compound. In the tubulogenesis assay, a decrease in the total length of tubular structures was observed in the treated groups compared to the control, suggesting that the compound also possesses anti-angiogenic properties. The reduced formation of tube-like structures in HUVEC cells highlights the potential of Isoniazid sodium methanesulfonate to inhibit angiogenesis, a crucial process in tumor growth and metastasis.

The Indirect Immunofluorescence assay revealed a decrease in NF- κ B and VEGF-A expression levels in HT-29 cells and VEGFR-2 expression in HUVEC cells following treatment. This indicates that the compound interferes with the signaling pathways responsible for cell proliferation and angiogenesis. The Western Blot analysis further confirmed these findings by showing a significant reduction in NF- κ B and VEGF-A protein levels in the treated groups, supporting the hypothesis that Isoniazid sodium methanesulfonate modulates key proteins involved in TB progression.

These findings suggest that Isoniazid sodium methanesulfonate has the potential to suppress both cell proliferation and angiogenesis, making it a promising candidate for TB therapy. Its ability to target multiple pathways involved in tumor growth and development could enhance its efficacy as an antiTB agent.

CONCLUSION

The study demonstrates that Isoniazid sodium methanesulfonate exhibits significant cytotoxic and anti-angiogenic activities in HT-29 colorectal TB cells and HUVEC endothelial cells. The compound effectively reduced cell viability, inhibited tubulogenesis, and decreased the expression of key proteins such as NF- κ B and VEGF-A, which are involved in TB cell proliferation and angiogenesis. These results indicate that Isoniazid sodium methanesulfonate may serve as a potent therapeutic agent for TB treatment, either as a standalone therapy or in combination with other antiTB drugs.

Further research is recommended to explore the molecular mechanisms underlying its antiTB effects and to evaluate its efficacy in *in vivo* models. This could pave the way for the development of new therapeutic strategies incorporating Isoniazid sodium methanesulfonate as a potential treatment option for various TBs.