# Focus on Mitochondrial Metabolism Dysfunction: Precursors of the HIV Protease Inhibitor as Promising Anticancer Candidates in Hematological Malignancies

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## Introduction

The most prevalent hematological malignancies (HMs) that have their origin in B-cells are multiple myeloma (MM) and chronic lymphocytic leukemia (CLL). MM is characterized by the presence of atypical clonal plasma cells within the bone marrow, with a proclivity for uncontrolled growth. Consequently, this results in destructive osseous bone lesions, acute kidney injury, anemia, and hypercalcemia[1]. CLL is characterized by the accumulation of aberrant monoclonal B lymphocytes (Fig. 1). While a considerable number of patients remain asymptomatic, a subset may exhibit characteristic lymphoma symptoms, acquired immunodeficiency disorders, or autoimmune complications[2]. Mitochondrial metabolism has been demonstrated to exert a pivotal influence on the development and progression of HMs. Alterations in glycolysis and oxidative phosphorylation (OXPHOS) have been associated with HM cell survival, drug resistance, and patient prognosis. In particular, elevated mitochondrial metabolism has been demonstrated to be associated with unfavorable outcomes and the development of drug resistance. The energy production of these cancer cells is dependent on mitochondrial OXPHOS, rendering this reliance a potential vulnerability that can be targeted in therapeutic interventions. Drugs targeting mitochondrial metabolism, such as those influencing OXPHOS or glucose metabolism, are currently being explored as potential therapeutic agents[3].

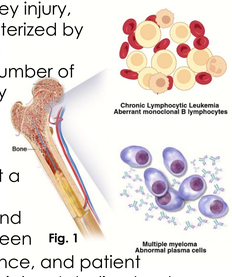


Fig. 1

## Aim

In this study, we aim to monitor the mitochondrial metabolic state of MM and CLL cell lines using the MitoTracker Red (MTR) and MitoSOX Green (MSG) fluorescent probes by flow cytometry. Furthermore, we aim to evaluate oxidative phosphorylation and glycolysis in live cells in real-time using the Agilent Seahorse XF-Pro Analyzer.

## Objectives

The objective of this study is to investigate the effect of two precursors of darunavir analogs (BupM-$NH_2$ and BnpM-$NH_2$), HIV-1 protease inhibitors, on mitochondrial metabolism alteration in MM and CLL cancer cell lines.

## Methods

Two MM cell lines (MM1S and RPMI-8226) and one CLL cell line (HG3) were seeded in a 6-well cell culture plate and treated with 30 µM of BupM-$NH_2$ and BnpM-$NH_2$. The structures of these two precursors of hydroxyethylamine derivatives 'of darunavir are (2R,3S) N-(3-amino-2-hydroxy-4-phenyl-butyl)-N-isobutyl-4-methoxy-benzenesulfonamide (BupM-$NH_2$) and (2R,3S) N-(3-amino-2-hydroxy-4-phenyl-butyl)-N-benzyl-4-methoxy-benzenesulfonamide (BnpM-$NH_2$). These compounds were synthesized, purified, and characterized by NMR (Fig. 2).

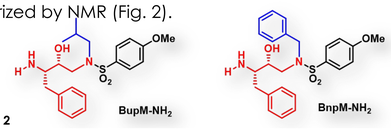


Fig. 2

Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors using Lymphocyte Separation Medium 1077 (#C44010) for density gradient centrifugation, according to manufacturer instructions. PBMCs were seeded in 6-well cell culture plate, treated as above, and used as non-pathological cells. After 24 hours of treatment, we used Invitrogen MitoTracker Red CMXRos (MTR, #M46752) to assess mitochondrial mass and Invitrogen MitoSOX Green (MSG, #M36006) for reactive oxygen species (ROS) and then evaluated by flow cytometry (Navios, Beckman Coulter). All experiments were performed including a negative control with dimethyl sulfoxide (DMSO, #67-68-5), and a positive control with rotenone (#R8875) was used only for MTR and MSG analysis. Secondly, MM1S, RPMI-8226, HG3, and PBMC cells were seeded in 96-well cell culture plate, treated for 24 h with 30 µM of BupM-$NH_2$ and BnpM-$NH_2$, and analyzed using the Seahorse XF Real-Time ATP Rate Assay Kit (#103592-100) by Agilent Seahorse XF-Pro Analyzer. It measures in real time, directly into the 96-well cell culture plate, the flux of oxygen (oxygen consumption rate, OCR) and protons (extracellular acidification rate, ECAR) of mitochondrial respiration and glycolysis, respectively (Fig. 3).

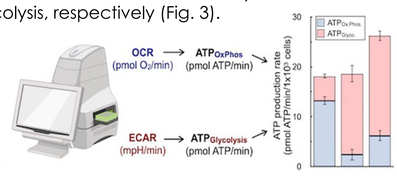


Fig. 3

## Results

We firstly performed cell viability and cell growth assays on MM1S, RPMI-8226, HG3, and non-pathological PBMC cells. Our results showed a significant decrease in proliferation and viability of about 30% and 70%, respectively, with BupM-$NH_2$ and BnpM-$NH_2$ at 30 µM, although their effect on PBMCs was milder. We further investigated superoxide anion production with MSG and mitochondrial mass using MTR by flow cytometry analysis. BupM-$NH_2$ and BnpM-$NH_2$ exhibited divergent effects on tumor cell lines. Results of the MSG assay revealed that neither compound induced ROS production after 24 h of treatment in MM cell lines. However, BnpM-$NH_2$ stimulated ROS production in HG3 cells (Fig. 4).

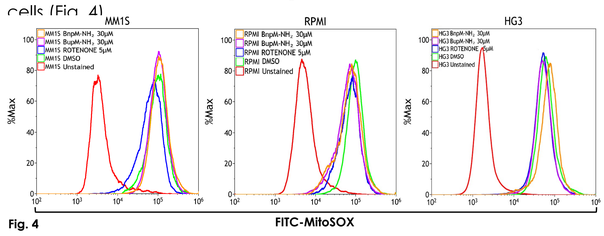


Fig. 4 FITC-MitoSOX

MTR assay results indicated a significant increase in mitochondrial mass in RPMI-8226 and HG3 cells following exposure to BnpM-$NH_2$, but not in MM1S cells. Instead, BupM-$NH_2$ reduced mitochondrial mass in MM cell lines and slightly increased it in HG3 cells (Fig. 5).

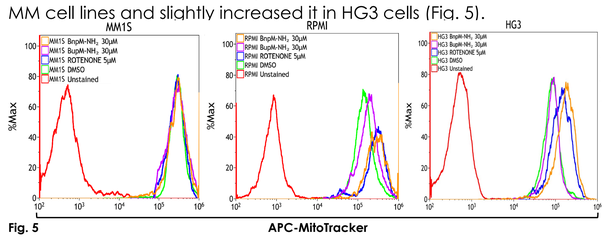


Fig. 5 APC-MitoTracker

To confirm the data, the real-time OCR and ECAR — oxidative phosphorylation and glycolysis parameters, respectively — were evaluated using the Seahorse ATP Rate Assay Kit by Agilent Seahorse XF-Pro Analyzer.
In the experimental protocol used, we observed that the mitochondrial respiration rates markedly decrease in response to BupM-$NH_2$ and BnpM-$NH_2$ in all cancer cell lines. In particular, in HG3 cells the effect of BnpM-$NH_2$ is extremely significant. It is interesting to note that there is a decrease in mitochondrial ATP production that occurs in parallel with a slight increase in the glycolytic rate. This suggests a compensatory effect in cellular survival. In contrast, the inhibition of oxidative phosphorylation indicates that aerobic glycolysis alone is not adequate for sustaining the survival of cancer cells (Fig. 6).

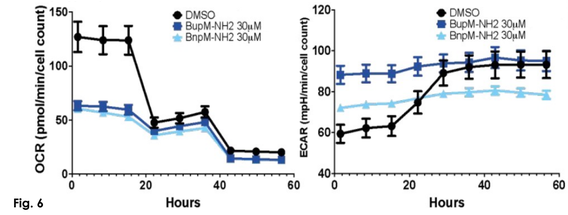


Fig. 6

On the other hand, BupM-$NH_2$ and BnpM-$NH_2$ compounds did not have a significant effect (ECAR) on PBMCs, indicating their safety on healthy cells (Fig. 7).

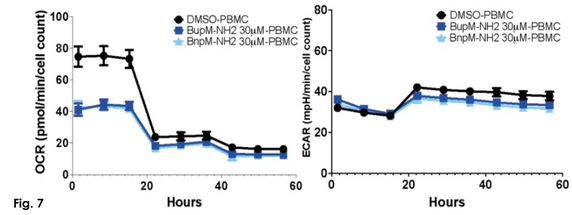


Fig. 7

## Summary

In this study, we evaluated the antitumor effects of two novel HIV protease inhibitor precursors, BupM-$NH_2$ and BnpM-$NH_2$, on multiple myeloma and chronic lymphocytic leukemia cells. Our results showed that these two compounds inhibited mitochondrial respiration and affected ROS production and mitochondrial mass. The compounds were found to be toxic mainly to MM and CLL cells, with a moderate effect on non-pathological PBMCs. Due to their greater impact on mitochondrial activity and function, these inhibitors represent new and promising therapeutic approaches for the treatment and potential eradication of MM and CLL.

## References

1. Cowan AJ, Green DJ, Kwok M, et al. Diagnosis and Management of Multiple Myeloma: A Review. JAMA. 2022;327(5):464–477.
2. Elhadary M, Elshoeibi AM, Badr A, et al. Revolutionizing chronic lymphocytic leukemia diagnosis: A deep dive into the diverse applications of machine learning. Blood Rev. 2023;62:101134.
3. Nair, R., Gupta, P., & Shanmugam, M. (2022). Mitochondrial metabolic determinants of multiple myeloma growth, survival, and therapy efficacy. Frontiers in oncology, 12, 1000106.

## Discussion

Mitochondrial metabolism dysfunction has been linked to MM progression and the transformation of CLL. Targeting mitochondrial alterations might be a promising strategy for the development of selective anticancer therapy. In this context, BupM-$NH_2$ and BnpM-$NH_2$, affecting ROS production, mitochondrial mass, and mitochondrial respiration, are promising anticancer candidates in hematological malignancies.