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A novel benzamide derivative SY-15 inhibits multiple myeloma cell proliferation

Nigar Abdullayeva [a,b,e,*], Kubra Gayret [b], Nazila Farhangzad [b], Ebru Dumlupinar [c], Serap Yilmaz Ozguven [d], Asuman Sunguroglu [b]

[a]Ankara University, Institute of Stem Cell, Ankara, Türkiye
[b]Ankara University, Faculty of Medicine, Department of Medical Biology, Ankara, Türkiye
[c]Ankara University, Faculty of Medicine, Department of Biostatistics, Ankara, Türkiye
[d]Trakya University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Edirne, Türkiye
[e]Ankara University, Institute of Health Sciences, Ankara, Türkiye
*Corresponding author: nigar.abd89@gmail.com (Nigar Abdullayeva)

MAIN POINTS

- The SY-15 molecule demonstrated significant cytotoxic effects in multiple myeloma cell lines, with the lowest viability observed in the RPMI 8226 cell line, while showing no notable toxicity in normal fibroblast (L929) cells.
- The molecule exhibits high selectivity and a low toxicity profile, making it a strong candidate for patients with MM who have developed resistance to existing therapies.
- Unlike classical benzamides, SY-15 may also modulate alternative apoptotic pathways, which highlights its potential for novel therapeutic combinations, particularly in drug-resistant cell lines.

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ABSTRACT

Aim: Over the past two decades, the natural course of multiple myeloma (MM) has changed significantly, primarily due to the emergence of novel therapeutic agents targeting the bone marrow microenvironment (BMM). Despite these advancements, the underlying mechanisms of drug resistance remain largely unclear. In this study, the effects of a novel benzamide derivative, SY-15, on MM cell lines were investigated, and the findings suggest that this molecule could be a promising anticancer drug candidate, warranting further research.

Materials and Methods: Multiple myeloma (MM) cell lines (MM1S, U266, H929, RPMI8226) were cultured, and the effects of various concentrations of a novel benzamide derivative on cell viability were evaluated using the MTT assay.

Results: The anticancer activity of the SY-15 molecule was evaluated in multiple myeloma (MM) cell lines following 72 hours of treatment, and for comparison, in the L929 normal fibroblast cell line. A statistically significant difference in cell viability percentages was observed among the five cell lines ($p<0.001$). The median cell viability percentage was 93.6247 for the L929 cell line, 44.4110 for the MM1S cell line, 22.4655 for H929, 31.7180 for U266, and the lowest median value was recorded in the RPMI 8226 cell line at 13.0931. Notably, SY-15 did not exhibit significant cytotoxicity in L929 fibroblast cells.

Conclusion: SY-15 has the potential to be an effective anticancer agent with high selectivity and low toxicity for the treatment of MM. It may offer a novel therapeutic option, particularly for patients who have developed resistance to other MM drugs.

Keywords: Multiple myeloma, SY-15 molecule, Anticancer drug

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INTRODUCTION

Multiple myeloma (MM) is a malignancy made up of B-lymphocytes, and it originates in the bone marrow. They usually have a poor prognosis. It leads to bone marrow suppression, destructive bone lesions, renal dysfunction, immunosuppression, and usually, death is imminent [1]. MM accounts for approximately 1.3% of all cancer diagnoses and 10% of all hematological malignancies. It is the second most common and one of the most lethal hematological cancers. Therefore, MM continues to be one of the most extensively studied cancer types [2].

According to data from the American Cancer Society, approximately 36,110 new cases of multiple myeloma are expected to be diagnosed in the United States by 2025. Of these, 20,030 will occur in men and 16,080 in women. Additionally, around 12,030 deaths are anticipated (6,540 men and 5,490 women). The lifetime risk of developing MM in the U.S. is less than 1%, with an estimated risk of 1 in 103 for men and 1

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in 131 for women. However, individual risk varies depending on personal risk factors [3].

MM is a heterogeneous disease, including progression from asymptomatic precursor stages to active symptomatic myeloma. Almost all MM patients progress from monoclonal gammopathy of undetermined significance (MGUS), an asymptomatic pre-malignant stage of the disease. MGUS is present in approximately 5% of individuals over the age of 50 and is nearly twice as common in Black individuals compared to Caucasians. MGUS carries an annual risk of approximately 1% for progression to MM or related malignancies. Since MGUS is asymptomatic, over 50% of individuals diagnosed with the condition may unknowingly carry it for more than a decade before clinical diagnosis.

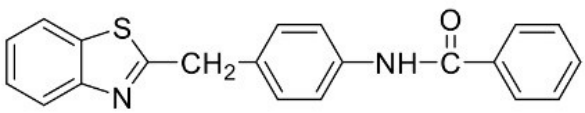
In a significant portion of patients, smoldering multiple myeloma (SMM), which is considered a clinically intermediate stage, can be identified. SMM is present in about 0.5% of the general population over the age of 40 and progresses to MM at an annual rate of 10% in the first five years, 3% in the following five years, and 1.5% per year thereafter. The rate of progression is associated with disease burden and underlying cytogenetic abnormalities; t(4;14) translocation, del(17p) deletion, and gain(1q) are considered significant risk indicators for progression from MGUS or SMM to MM [4].

Multiple myeloma (MM) is characterized by an increasing number of abnormal plasma cells. These cells produce monoclonal proteins (M-proteins), which are detectable in the blood or urine and are key in diagnosing and monitoring the disease. Beyond their diagnostic use, these abnormal proteins can cause serious complications like kidney damage and bone lesions. This highlights the need for early diagnosis and personalized treatment. In about 20% of cases, the disease spreads from the bone marrow to other organs and soft tissues, a condition known as extramedullary disease (EMD). EMD is an aggressive form of MM that complicates treatment and disease management [5].

High-resolution sequencing techniques have advanced our understanding of clonal evolution in multiple myeloma, revealing subclonal diversity and the disease's dynamic nature [6,7,8]. As knowledge of MM pathogenesis and the bone marrow microenvironment improves, various adjunctive cell therapies and new drugs have been developed. Despite recent therapeutic progress, resistance to anticancer drugs remains a major challenge in MM treatment [9]. Research efforts aim to better understand the pathways and protein expressions involved in drug resistance and to develop new therapeutic strategies. Significant progress has been achieved through the sequential or combined use of proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), monoclonal antibodies (mAbs), and autologous transplantation following high-dose therapy. However, MM is still characterized as a disease that is difficult to control, with alternating periods of remission and relapse/progression, ultimately leading to drug-resistant disease [10].

Recent studies have highlighted that benzamide derivatives induce apoptosis and reduce cell proliferation in various cancer cell lines. Benzamide derivatives are simpler chemical compounds consisting of a phenyl ring directly attached to an amide group, with various substituent groups on the phenyl ring and amide nitrogen [11]. These compounds exhibit notable antibacterial, antifungal, anticancer, and antiallergic activities and have become one of the commonly used intermediates in the synthesis of aromatic ligands [12]. They are known to be more effective against specific HDACs rather than all HDAC classes. In particular, they were shown to strongly inhibit Class I HDACs, including HDAC1, HDAC2, and HDAC3. Benzamides have been reported to induce P21WAF1 expression, cause cell cycle arrest, activate numerous pro-apoptotic genes, and exert cytotoxic effects on various cancer cell types even at very low doses. Some types of benzamides have also been found to inhibit breast cancer, similar to SAHA (suberoylanilide hydroxamic acid). Although natural products of this HDAC inhibitor family have not yet been identified, such drugs have shown promising results in clinical studies on breast cancer and lymphomas [13].

The SY-15 molecule (Figure 1) is a novel benzamide derivative first synthesized by Yılmaz et al. (2013) at Ankara University [14]. This compound was developed as a potential antitumor agent. Structurally, SY-15 features a benzamide core linked to a benzothiazole moiety through a phenyl group. The inclusion of the benzothiazole ring is particularly significant, as it may enhance the molecule's binding affinity to biological targets. This enhancement could occur through hydrophobic interactions, pi-pi stacking, and potential hydrogen bonding with amino acid residues in target proteins. In this study, we investigated the effects of the unique small molecule SY-15 on multiple myeloma (MM) cell lines, including MM1S, U266, H929, and RPMI 8226. Preliminary studies have shown that SY-15 is effective against various types of cancer, suggesting that it may possess significant anticancer activity for the treatment of multiple myeloma.Despite the effectiveness of benzamide derivatives in treating other cancers, there are few studies on their use in multiple myeloma (MM) cell lines. Consequently, there is a significant need to develop new benzamide-derived drugs for MM treatment that have novel targets, high efficacy, and minimal side effects. This study aims to highlight the potential of the small benzamide-derived molecule SY15 as a promising new antitumor agent for MM, which warrants further investigation.



N-(4-(benzothiazol-2-ylmethyl)phenyl)benzamide

Figure 1. Structure of SY-15 molecule.

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MATERIALS AND METHODS

Cell culture

In this study, four different MM cell lines (MM1S, U266, H929, and RPMI 8226) and the L929 normal fibroblast cell line were used. All cell lines were cultured in Roswell Park Memorial Institute medium (RPMI-1640, with L-glutamine) (Sigma, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma, USA) and antibiotics (Penicillin 100 U/mL and Streptomycin 100 µg/mL) (Gibco, USA). The cells were maintained under standard conditions in an incubator at 37°C with a gas mixture of 5% CO_2 and 95% air. Myeloma cells were maintained by adding 5 mL of fresh medium every two days until reaching 80% confluency.

MM.1S and RPMI-8226 cell lines are semi-adherent; therefore, cells must be carefully scraped before passaging. L929 cells are adherent cells and were detached by incubating with 2–3 mL of Trypsin-EDTA for 4 minutes. Viable cell counting was performed using the Trypan blue exclusion method with a hemocytometer. When the percentage of viable cells exceeded 85%, the cultures were considered ready for experiments [15].

MTT assay

The cytotoxic effects of the SY15 molecule were evaluated using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay (Roche, Germany). Cells were seeded into 96-well plates at a density of 5×10^4 cells per well and incubated overnight. Then, different concentrations of the SY15 molecule (1, 5, 10, 15, 25, 50, 75, and 100 µM) were applied to the cells and incubated for 72 hours. At the end of the incubation period, 10 µL of MTT solution (5 mg/mL) was added to each well. After a four-hour incubation, 100 µL of Sodium Dodecyl Sulfate-Hydrochloric acid (SDS-HCl) (0.01 M) solution was added to dissolve the formazan crystals. Optical density was measured at 550 nm with a reference wavelength of 690 nm using a spectrophotometric reader (Biotek, USA). Cell viability was calculated as a percentage relative to the negative control (cells treated with DMSO). Small changes in metabolic activity lead to significant changes in MTT, allowing detection of cellular stress even without direct cell death caused by toxic agents. This method is standardized for adherent or non-adherent cells grown in multi-well plates [16]. The data obtained at the end of 72 hours were analyzed.

Sample size

To detect an effect size of 0.31 (Cohen's f) for vitality percentage among cell lines with a significance level of 0.05 and a power of 0.80, a minimum total sample size of 130 (i.e., 26 for each cell line) is required. The sample size calculation was performed using G*Power (version 3.1.9.4).

Statistical analysis

Descriptive statistics for continuous variables are presented as the median (minimum, maximum). The Shapiro-Wilk test was used to assess the normality of the data. To determine significant differences between groups, the Kruskal-Wallis test was employed, followed by the Dunn's post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 30.0, Chicago, IL) and JASP (version 0.18).

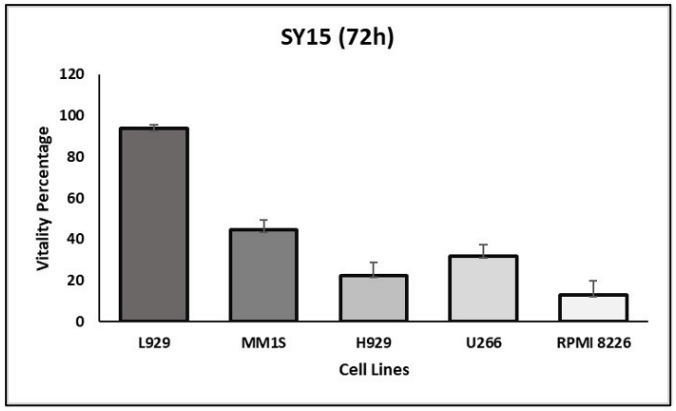

Figure 2. Viability percentages among five cell lines.

RESULTS

At the end of the 72nd hour, the activity of the SY-15 molecule in multiple myeloma cells was examined. A statistically significant difference was observed among the five cell lines in terms of vitality percentages ($p<0.001$) (Figure 2). The median vitality percentage of the L929 cell line was 93.6247 (83.9708, 126.4116), that of the MM1S cell line was 44.4110 (27.2785, 105.3916), H929 had a median of 22.4655 (12.4898, 103.4063), U266 had 31.7180 (27.2775, 103.9295), and RPMI 8226 showed the lowest median value of 13.0931 (11.1188, 101.8358). Statistically significant differences between groups were observed for the following comparisons, RPMI 8226 vs U266 ($p=0.003$), RPMI 8226 vs MM1S ($p<0.001$), RPMI 8226 vs L929 ($p<0.001$), RPMI 8226 vs MM1S ($p=0.002$), RPMI 8226 vs L929 ($p<0.001$), H929 vs L929 ($p<0.001$) and U266 vs L929 ($p=0.005$) (Table 1) (Figure 3).

A significant difference in vitality percentage was observed between the doses in the L929 cell line ($p=0.144$) (Figure 4a). The median vitality percentage at the 0 µM dose was 102.1857 (83.9708, 113.8433), 1 µM dose was 110.0182 (109.6539, 126.4116), 5 µM dose was 102.7322 (93.4426, 108.0145), 10 µM dose was 99.6357 (92.1675, 100.1821), 15 µM dose was 93.2604 (91.8032, 95.0819), 25 µM dose was 92.1675 (91.4389, 100.7285), 50 µM dose was 92.7140 (88.7067, 93.6247), 75 µM dose was 93.0783 (92.5318, 97.2677) and 100 µM dose was 88.8888 (84.1530, 94.1712) (Table 2).

A significant difference in vitality percentage was observed between the doses in the MM1S cell line ($p=0.005$) (Figure 4b). The median vitality percentage at the 0 µM dose was 99.8684 (98.6285, 105.3916); at 1 µM, it was 99.1921 (92.9926,

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Table 1. Viability percentages among five cell lines.

	Cell Lines					p value
	L929	MM1S	H929	U266	RPMI 8226	
Vitality Percentage Median (min-max)	93.6247 (83.9708-126.4116)	44.4110 (27.2785-105.3916)	22.4655 (12.4898-103.4063)	31.7180 (27.2775-103.9295)	13.0931 (11.1188-101.8358)	<0.001$^{\alpha}$

min: minimum max:maximum, $^{\alpha}$ Kruskal-Wallis Test.

Table 2. Percentage of viability among all doses in all cell lines.

Doses	Cell Lines				
	L929	MM1S	H929	U266	RPMI 8226
0 µM	102.1857 (83.9708-113.8433)	99.8684 (98.6285-105.3916)	103.0819 (102.3519-103.4063)	99.4889 (99.3832-99.9118)	101.5240 (96.6401-101.8358)
1 µM	110.0182 (109.6539-126.4116)	99.1921 (92.9926-100.7702)	87.3479 (81.3463-90.9975)	97.1623 (83.3127-103.9295)	88.2230 (84.6899-93.4187)
5 µM	102.7322 (93.4426-108.0145)	58.9517 (56.4719-71.6888)	41.0381 (36.9018-44.4444)	59.3127 (55.8237-61.6387)	19.1201 (18.9123-21.1984)
10 µM	99.6357 (92.1675-100.1821)	46.1018 (44.4110-63.2350)	28.7915 (24.4120-29.4403)	34.0440 (31.7180-34.8898)	13.0931 (11.1188-13.9244)
15 µM	93.2604 (91.8032-95.0819)	44.0728 (35.6190-53.9921)	22.4655 (18.8158-25.7907)	32.6696 (30.6607-32.8810)	12.4696 (12.3657-12.9892)
25 µM	92.1675 (91.4389-100.7285)	43.9601 (33.2519-44.8619)	17.7615 (17.2749-19.0592)	29.4977 (27.2775-29.6035)	12.3657 (12.1579-12.3657)
50 µM	92.7140 (88.7067-93.6247)	38.0988 (33.3646-38.6624)	17.2749 (13.2197-21.5733)	28.1233 (27.9111-28.1233)	12.4696 (12.0540-12.5736)
75 µM	93.0783 (92.5318-97.2677)	31.8993 (27.2778-39.0000)	17.7615 (14.7607-18.4914)	28.4405 (28.2290-28.9691)	12.0540 (11.8462-12.2618)
100 µM	88.8888 (84.1530-94.1712)	33.3646 (29.8703-35.84444)	19.7891 (14.4898-20.5190)	27.2775 (27.2775-27.9118)	14.0284 (13.8205-15.0675)
p value	0.144$^{\alpha}$	0.005$^{\alpha}$	0.004$^{\alpha}$	0.002$^{\alpha}$	0.004$^{\alpha}$

min: minimum max:maximum, $^{\alpha}$ Kruskal-Wallis Test. Descriptive statistics are presented as median (min-max).

100.7702); at 5 µM, it was 58.9517 (56.4719, 71.6888); at 10 µM, it was 46.1018 (44.4110, 63.2350); at 15 µM, it was 44.0728 (35.6190, 53.9921); at 25 µM, it was 43.9601 (33.2519, 44.8619); at 50 µM, it was 38.0988 (33.3646, 38.6624); at 75 µM, it was 31.8993 (27.2778, 39.0000); and at 100 µM, it was 33.3646 (29.8703, 35.84444). The lowest vitality percentage was observed at the 75 µM dose. Statistically significant differences between groups were found in the following comparisons: 0 µM vs 25 µM (p=0.024), 0 µM vs 50 µM (p=0.009), 0 µM vs 75 µM (p=0.002), 0 µM vs 100 µM (p=0.002), 1 µM vs 25 µM (p=0.035), 1 µM vs 50 µM (p=0.015), 1 µM vs 75 µM (p=0.003), 1 µM vs 100 µM (p=0.004), 5 µM vs 75 µM (p=0.027), and 5 µM vs 100 µM (p=0.029) (Table 2).

A significant difference in vitality percentage was observed between the doses in the H929 cell line (p=0.004) (Figure 4c). The median vitality percentage at 0 µM was 103.0819 (102.3519, 103.4063), at 1 µM was 87.3479 (81.3463, 90.9975), at 5 µM was 41.0381 (36.9018, 44.4444), at 10 µM was 28.7915 (24.4120, 29.4403), at 15 µM was 22.4655 (18.8158, 25.7907), at 25 µM was 17.7615 (17.2749, 19.0592), at 50 µM was 17.2749 (13.2197, 21.5733), at 75 µM was 17.7615 (14.7607, 18.4914), and at 100 µM was 19.7891 (14.4898, 20.5190). The lowest vitality percentage was observed at the 50 µM dose. Statistically significant differences between groups were found in the following comparisons: 0 µM vs 25 µM (p=0.008), 0 µM vs 50 µM (p=0.006), 0 µM vs 75 µM (p=0.005), 0 µM vs 100 µM (p=0.012), 1 µM vs 25 µM (p=0.01), 1 µM vs 50 µM (p=0.008), 1 µM vs 75 µM (p=0.006), 1 µM vs 100 µM (p=0.016), 5 µM vs 25 µM (p=0.037), 5 µM vs 50 µM (p=0.031), and 5 µM vs 75 µM (p=0.023) (Table 2).

A significant difference in vitality percentage was observed between the doses in the U266 cell line (p=0.002) (Figure 4d). The median vitality percentage at the 0 µM dose was 99.4889 (99.3832, 99.9118). At 1 µM, it was 97.1623 (83.3127, 103.9295), at 5 µM, 59.3127 (55.8237, 61.6387), at 10 µM, 34.0440 (31.7180, 34.8898), at 15 µM, 32.6696 (30.6607, 32.8810), at 25 µM, 29.4977 (27.2775, 29.6035), at 50 µM, 28.1233 (27.9111, 28.1233), at 75 µM, 28.4405 (28.2290, 28.9691), and at 100 µM, 27.2775 (27.2775, 27.9118). The lowest vitality percentage was observed at 100 µM. Statistically significant differences between groups included 0 µM vs 25 µM (p=0.01), 0 µM vs 50 µM (p=0.003), 0 µM vs

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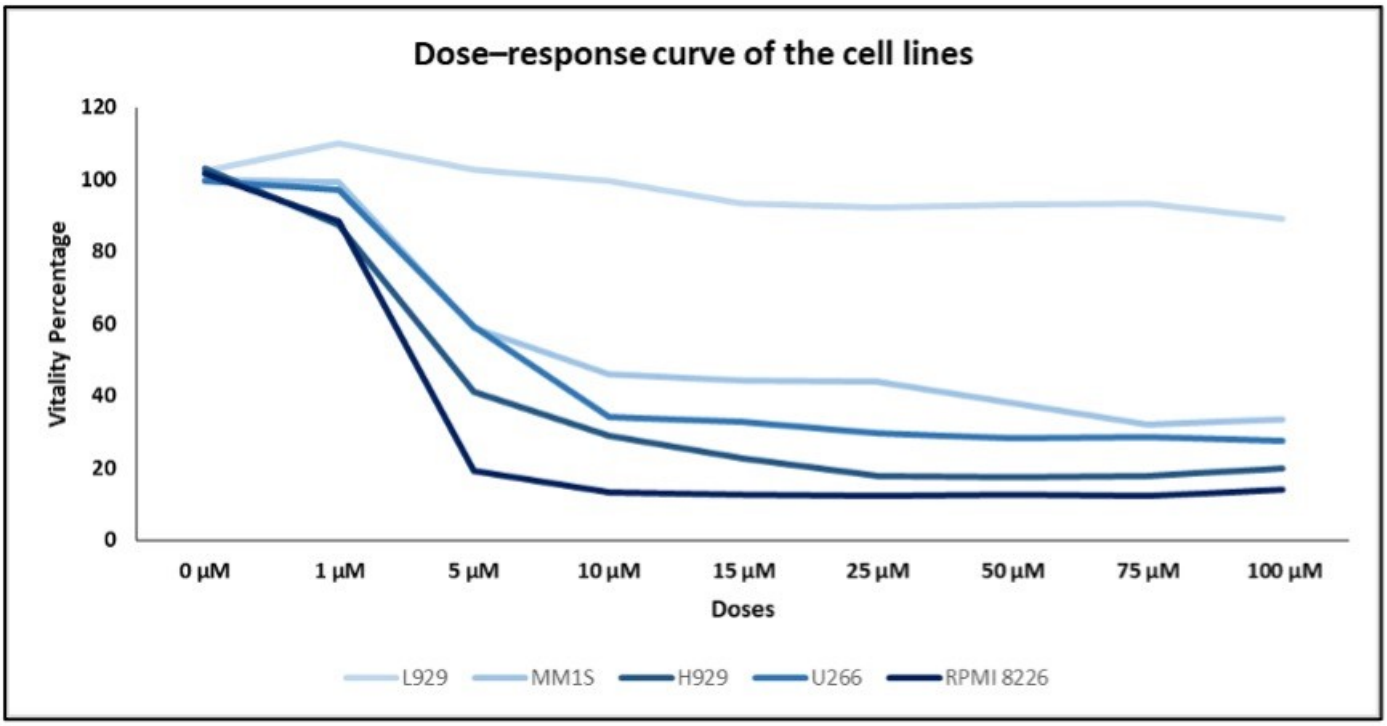

Figure 3. Dose-response curves in all cell lines at all doses.

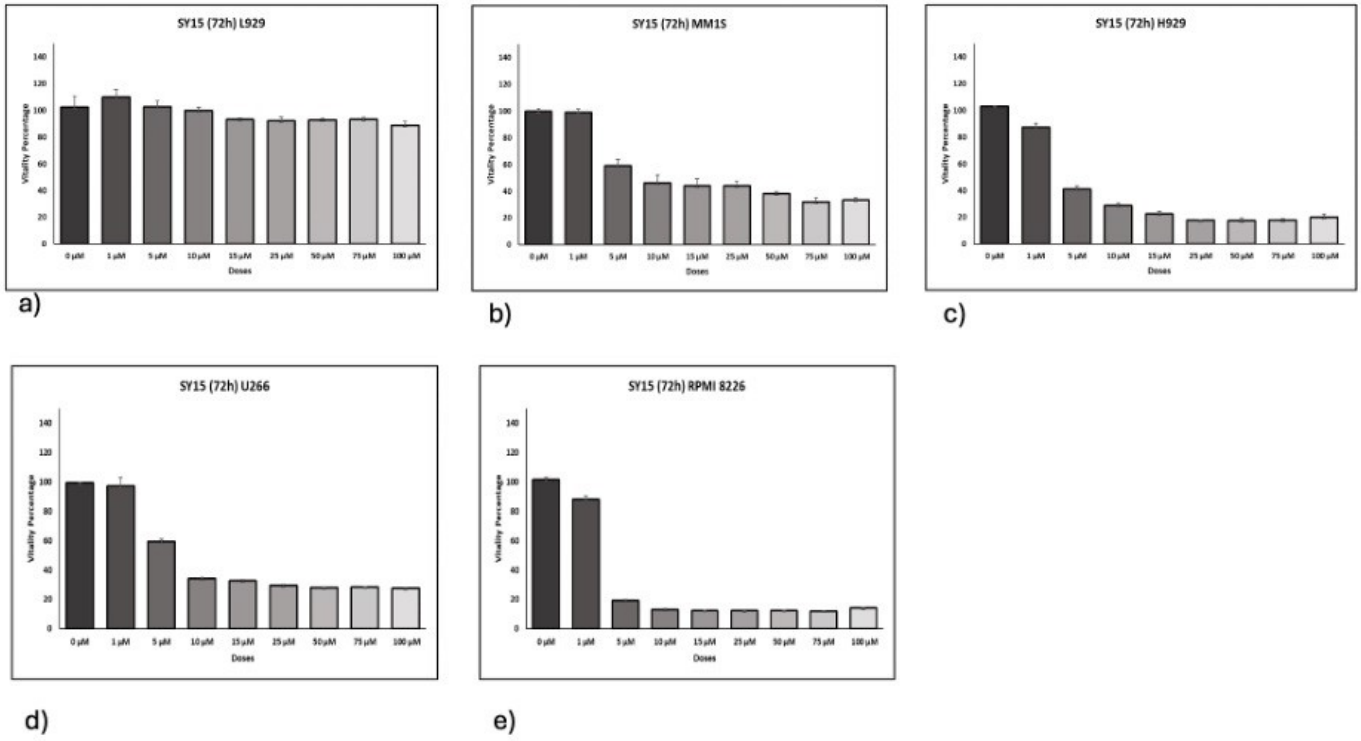

Figure 4. a) Percentage of viability between doses in L929 cell line; b) Percentage of viability between doses in MM1S cell line; c) Percentage of viability between doses in H929 cell line; d) Percentage of viability between doses in U266 cell line; e) Percentage of viability between doses in RPMI8226 cell line.

75 µM (p=0.013), 0 µM vs 100 µM (p<0.001), 1 µM vs 25 µM (p=0.016), 1 µM vs 50 µM (p=0.005), 1 µM vs 75 µM (p=0.021), 1 µM vs 100 µM (p=0.001), 5 µM vs 50 µM (p=0.029), 5 µM vs 100 µM (p=0.008), and 10 µM vs 100 µM (p=0.037) (Table 2).

A significant difference in vitality percentage was observed between the doses in the RPMI 8226 cell line (p=0.004; Figure 4e). The median vitality percentage at the 0 µM dose

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was 101.5240 (96.6401, 101.8358), at 1 μM was 88.2230 (84.6899, 93.4187), at 5 μM was 19.1201 (18.9123, 21.1984), at 10 μM was 13.0931 (11.1188, 13.9244), at 15 μM was 12.4696 (12.3657, 12.9892), at 25 μM was 12.3657 (12.1579, 12.3657), at 50 μM was 12.4696 (12.0540, 12.5736), at 75 μM was 12.0540 (11.8462, 12.2618), and at 100 μM was 14.0284 (13.8205, 15.0675). The lowest vitality percentage was observed at the 75 μM dose. Statistically significant differences between groups were noted for the following comparisons: 0 μM vs 10 μM (p=0.016), 0 μM vs 15 μM (p=0.017), 0 μM vs 25 μM (p=0.003), 0 μM vs 50 μM (p=0.007), 0 μM vs 75 μM (p<0.001), 1 μM vs 25 μM (p=0.013), 1 μM vs 50 μM (p=0.027), 1 μM vs 75 μM (p=0.003), 5 μM vs 25 μM (p=0.045), and 5 μM vs 75 μM (p=0.013) (Table 2).

DISCUSSION

The treatment of multiple myeloma (MM) involves using a combination of drugs, each producing different responses. These include corticosteroids, alkylating agents, anthracyclines, proteasome inhibitors (PIs), immunomodulatory drugs (IMIDs), histone deacetylase inhibitors (HDAC inhibitors), monoclonal antibodies (mAbs), and nuclear export inhibitors [17]. For a long time, especially, the alkylating agent melphalan and the corticosteroid prednisone were the main cytotoxic drugs used in treatment [18]. Later, high-dose melphalan chemotherapy followed by autologous stem cell transplantation began to be used, and this method was shown to extend survival in younger patients compared to conventional chemotherapy [19]. Various combinations of melphalan and prednisone became the most common treatment for elderly patients. Subsequently, thalidomide, bortezomib, and lenalidomide were added to treatment protocols for these patients [20]. One of the most important drugs in MM treatment, bortezomib, was the first proteasome inhibitor deemed suitable for treating relapsed and refractory MM patients [21, 22]. The introduction of this PI has become the most effective treatment against multiple myeloma by preventing pro-apoptotic protein degradation and promoting tumor cell death. Using these drugs in different formats has improved the average survival of patients with multiple myeloma [23]. However, adverse events have been noted in MM patients who develop resistance to both first-generation IMIDs and proteasome inhibitors [24].

Among the recently discovered anticancer drugs, various benzimidazole derivatives have received particular attention. The benzamide derivative MS-247, synthesized by Yamori and colleagues, showed antitumor activity in 39 cancer cell lines and many organ tissue cells. It binds to AT-rich regions in the minor groove of DNA, inhibits DNA synthesis, creates interstrand crosslinks (ICLs), blocks the cell cycle in the G2/M phase, and induces apoptosis [25]. In a recent study, a new benzamide derivative called VKNG-2 was shown to enhance the effectiveness of chemotherapeutic drugs in colon cancer cell lines by inhibiting the ABCG2 transporter [26]. In another study, a novel benzamide derivative containing benzamidophenyl and phenylacetamidophenyl scaffolds, known as 13f, exhibited strong anticancer activity and a potent PARP-1 inhibitory effect against human colorectal cancer HCT116 and DLD-1 cells. This compound effectively inhibited colony formation and migration in HCT116 cells [27]. In a separate study, N-(9H-purin-6-yl) benzamide derivatives were reported to induce apoptosis and reduce cell proliferation in cancer cell lines, showing cytotoxic and antitumor activity within the range of 3–39 μM [28]. In another recent study, the effect of an imidazole derivative on A549 lung cancer cells was investigated. The compound was found to exhibit anticancer activity by inhibiting cell proliferation and accelerating apoptosis [29]. A new series of 2-amino-1,4-naphthoquinone-benzamide derivatives, labeled 5a–n, was tested across three different cancer cell lines. Results showed that these compounds were most effective in the MDA-MB-231 cell line, less effective in the SUIT-2 cell line, and more effective than the positive control cisplatin in the HT-29 cell line [30]. Following the combined treatment of the benzamide derivative XT5 and imatinib in K562 cell lines, an increase in cytotoxicity was observed, along with Annexin V binding and caspase 3/7 activation. Expression levels of pro-apoptotic genes also increased in K562R and K562S cell lines treated with XT5. While XT2B did not form hydrogen bonds, XT5 showed hydrogen bond interactions with the basic amino acids of the BCR-ABL kinase receptor [31].

Recently synthesized (E)-N-phenyl-4-(pyridine-acylhydrazone) benzamide derivatives were tested for their antiproliferative activity against U266 and RPMI 8226 cell lines using the MTT assay. Compound 8b showed excellent antiproliferative activity against RPMI 8226 cells, had lower toxicity than imatinib, significantly halted the cell cycle in the G0/G1 phase, and induced cell death in RPMI 8226 cells by increasing mitochondrial ROS release, thus producing an antitumor effect [32].

CONCLUSION

Despite the potential of benzamide derivatives to serve as highly effective antitumor drug candidates across various cancer types, few studies have been conducted on MM cell lines. This research contributes to the development of new, potent anticancer agents with high selectivity and low toxicity. The SY-15 molecule exhibits several distinct features that set it apart from other benzamide derivatives and traditional HDAC inhibitors. Notably, it demonstrates significant cytotoxic activity in multiple MM cell lines, including those sensitive to bortezomib. Structurally, SY-15 incorporates a benzothiazole moiety linked to its benzamide core, which may enhance binding affinity through hydrophobic interactions, pi-pi stacking, and hydrogen bonding. This structural characteristic could help explain its selectivity and potency. Additionally, while most classical benzamide derivatives mainly target HDAC enzymes, SY-15 may exert antitumor effects not

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only by inhibiting HDACs but also by modulating alternative apoptotic pathways specific to multiple myeloma cells. Its low cytotoxicity in normal fibroblast cells indicates that SY-15 has a more favorable therapeutic index compared to other existing HDAC inhibitors. This study lays a solid foundation for further evaluation and target identification of SY-15. Future research will focus on testing the efficacy of the benzamide-derived compound in bortezomib-resistant MM cell lines. This will be a highly exciting development, as it will be the first study to target benzamide derivatives in resistant MM cell lines. Detailed efficacy studies of this drug alone or in combination with bortezomib in resistant cell lines could yield better results and potentially offer a new treatment option as an effective anticancer drug for MM therapy, especially in resistant patients.

Ethics Committee Approval: Since this study was conducted on cell lines (in vitro), ethical committee approval was not required.

Informed Consent: In this study, no patient or volunteer was involved, so an informed consent form was not required.

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