Doctoral Dissertation

Identification of chromanone compound derivatives for prohibitin-targeted multiple myeloma treatment

Department of Biomedical Sciences

Graduate School, Chonnam National University

LEE, Young-Eun

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LEE, Young-Eun

Supervised by Professor Shin Myung-Geun

A dissertation submitted in partial fulfilment of the requirements

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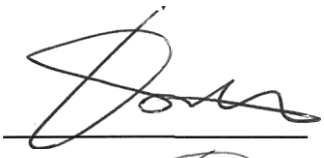
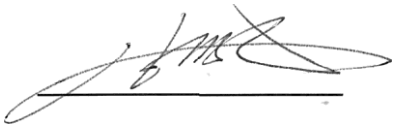
Committee in Charge:

LEE, Je-Jung 

BAEK, Hee-Jo 

KOH, Yang-Seok 

NAM, Taek-Keun 

SHIN, Myung-Geun 

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CONTENTS

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Graduate School, Chonnam National University

(Supervised by Professor Shin, Myung-Geun)

(Abstract)

Background: More than 70% of multiple myeloma (MM) occurs in elderly patients over the age of 65 years, and the incidence of MM has increased significantly in Korea over the past 20 years. Therefore, it is necessary to develop new therapeutic agents that can be safely used in the elderly. Lenalidomide-based therapeutics and proteasome inhibitors, which are currently used as a basis for the treatment of MM in clinical practice, are accompanied by systemic side effects such as pancytopenia, peripheral neuropathy, and severe skin lesions. Therefore, novel therapeutic agents that can overcome serious side effects and resistance to treatment are urgently required.

Materials and methods: A chromanone compound (KBB-NX) and its derivative low molecular weight compound were synthesized by targeting prohibitin (PHB), a companion diagnostic marker for MM such as blood cancer. The synthesized low-molecular-weight synthetic material was tested for safety and effectiveness by cell line-level validation tests, blood stability tests, pharmacokinetic tests using

experimental animals, and toxicity tests. A study was conducted to discover a new lead material for treating MM through an efficacy test using a MM mouse model.

Results: In this study, 41 KBB-NX and related inducers were synthesized and had apoptotic effects on MM cells by blocking the PHB2 protein. When these synthetic substances were added to MM cells, the PHB2 function was inactivated, intracellular reactive oxygen species (ROS) production was increased, and MM cells were killed via ROS-mediated apoptosis. Among the synthetic KBB-NX derivatives, KBB-NX, KBB-NX14, KBB-NX15, KBB-NX21, and KBB-NX26 were considered lead materials because their IC50 was less than 50 μM. In pharmacokinetic studies of KBB-NX14 and KBB-NX21 compounds, their half-life was relatively short (less than 2 hours), so additional structural changes, derivative synthesis, and formulation studies were required. A blood safety evaluation confirmed that KBB-NX and related inducers were relatively stable in the body.

Conclusion: Synthetic KBB-NX and its derivatives combined with PHB2 protein in MM cells to overproduce ROS and induce apoptosis. Among the 33 synthetic compounds, KBB-NX, KBB-NX14, KBB-NX15, and KBB-NX21, and KBB-NX26 were considered new therapeutic agents for MM. The results of this study and the production of synthetic materials provide important clues for the development of new therapeutic agents with guaranteed safety and efficacy in elderly patients with MM.

Keywords: multiple myeloma, prohibitin, KBB-NX, synthetic compounds, therapeutics.

I. Introduction

Multiple myeloma (MM) is a blood cancer in which a large amount of abnormal monoclonal immunoglobulin is produced and accumulated throughout the body due to the tumorigenic proliferation of plasma cells. Neoplastic plasma cells proliferate in the bone marrow and destroy extensive systemic skeletal bones such as the skull, vertebrae, and long bones. Therefore, patients with MM often experience severe bone pain due to bone destruction, and the main symptoms are anemia, hypercalcemia, and acute renal failure.

MM accounts for 1-2% of all cancers and slightly more than 17% of hematologic malignancies. The incidence is higher in men than in women, and by race, the incidence is higher in African Americans (1). In the United States, there are 34,000 new cases of MM each year and 13,000 deaths due to MM (1, 2). In Korea, 1222 cases occurred in 1999, but 2425 cases occurred in 2018, increasing the incidence rate by 98.4% over the past 20 years. During the same period, the average annual percentage change was 3.81%, and the crude incidence rate per million population increased by 82.4% from 25.91 in 1999 to 47.27 in 2018 (3). The median age of patients with MM at diagnosis is 65-74 years, and it occurs in about 2% of patients under 40 years of age (4). According to data from the Central Cancer Registry in 2019, MM accounted for 0.7% of all cancers in Korea in 2017, and by age group, those in their 70s accounted for 33.2%, followed by those in their 60s (30.3%), and those in their 50s (17.2%). Factors that increase the risk of developing MM are unknown, but old age, radiation, and exposure to some chemicals are important.

In general, treatment is performed for symptomatic MM, and follow-up is used for asymptomatic MM. However, in the case of high-risk, asymptomatic MM, treatment is recommended as part of a clinical study. Treatment can be broadly divided into 1) chemotherapy for MM and 2) supportive therapy for symptoms. The first thing to be considered when deciding on chemotherapy is whether autologous hematopoietic stem cell transplantation is possible, considering the patient's age, activity level, and accompanying diseases. In Korea, insurance benefit for transplantation has recently been extended to the age of 69 years. Because thalidomide was reported to be effective for the treatment of MM, lenalidomide, pomalidomide, bortezomib, carfilzomib, ixazomib, thalidomide, and effective drugs such as elotuzumab and daratumumab are continuously being developed and used for treatment. As described above, common side effects of therapeutic agents used to treat MM include pain, general weakness, gastrointestinal side effects, peripheral neuropathy, and cytopenias.

However, despite the introduction of these new drugs, there is an urgent need for a new drug that can significantly improve the survival rate of elderly MM patients with a 5-year survival rate of 50% in those over 65 years of age. Thalidomide-based immunomodulators and bortezomib-based proteosome-suppressing anticancer drugs, widely used as MM therapeutics in clinical practice, destroy or inhibit normal hematopoietic stem cells, thereby reducing their function to pan blood cells. In addition to nephropathy, these drugs cause serious side effects such as peripheral neuritis, embolism/thrombosis, and the onset of secondary primary cancer, so their use is very limited in elderly MM patients. Anticancer drugs currently used as MM treatment in clinical practice have increased drug resistance, so there is an urgent need for a new

MM treatment that can overcome such drug resistance. In this study, in order to overcome the limitations of these MM therapeutics and meet clinical needs, this study synthesized and evaluated chromanone-based synthetic compounds targeting prohibitin2 (PHB2), a companion marker for blood cancer, and evaluated the optimal lead material.

II. Materials and methods

1. Chemistry

The chemical synthesis reaction was monitored by thin layer chromatography using a precoated silica gel 60 F254 plate (Merck, Burlington, MA, USA). Nuclear Magnetic Resonance (NMR) spectroscopy is used to determine the structure and map of organic substances by analyzing the framework between carbon and hydrogen molecules in the analysis of organic compounds. The most popular NMR analysis is ^{1}H-NMR, which analyzes hydrogen atoms through the spin resonance of hydrogen atoms. When ^{1}H-NMR is used, hydrogen atoms present in the solvent in which the substance is to be analyzed are also analyzed. To prevent this when performing ^{1}H-NMR analysis, all hydrogens in the solvent are replaced with deuterated solvent.

1.1.General procedure for synthesis of KBB-NX and derivatives

All solvents and chemicals were used as purchased without further purification. All reported yields are the isolated yields after column chromatography or crystallization. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a JEOL JNM-ECS400 spectrometer at 400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR, respectively. The chemical shift (δ) is reported in ppm relative to tetramethylsilane (TMS) as an internal standard, and CDCl3, DMSO-d6, CD3OD-d4 were used as solvents. Multiplicity of peaks are reported as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), td (triplet of doublets), qd (quartet of doublets), dt (doublet of triplets), dq (doublet of quartets), ddd (doublet of doublets of doublets), and m (multiplet). High resolution mass spectra (HRMS) were obtained by an electric

ionization technique from the Korea Basic Science Institute, Daegu, Korea. Low resolution mass spectra were recorded on an Agilent 1260 Infinity II LC−MS spectrometer. Purity of all tested compounds was ≥ 95%, as estimated by High-performance liquid chromatography (HPLC) analysis. Samples were analyzed on a Waters Agilent HPLC system equipped with a PDA detector and a Waters SB-C18 column (1.8 μm, 2.1 × 50 mm). The mobile phase was used with buffer A (ultrapure H_2O containing 0.1% TFA) and buffer B (chromatographic grade CH_3CN). The flow rate was 0.5 mL/min.

1.2.Compound E

The NMR data performed to verify the molecular structure and chemical composition of the compound were as follows: ^{1}H NMR (400 MHz, Chloroform-d) δ 6.53 (s, 1H), 5.19 (s, 2H), 5.13 (td, J = 7.7, 6.7, 3.6 Hz, 1H), 4.89 (s, 2H), 3.78 (s, 3H), 3.46 (d, J = 4.5 Hz, 6H), 3.29 (d, J = 6.8 Hz), 2H), 2.48 (s, 3H), 1.73 (s, 3H), 1.64 (s, 3H).

1.3.Compound C

NMR data are as follows: ^{1}H NMR (400 MHz, Chloroform-d) δ 6.44 (d, J = 1.9 Hz, 1H), 6.29 (d, J = 1.8 Hz, 1H), 5.13 (d, J = 9.8 Hz, 4H), 3.77 (s, 3H), 3.45 (dd, J = 8.9, 0.6 Hz, 6H), 2.46 (d, J = 0.6 Hz, 3H).

1.4.XN

NMR data are as follows: ^{1}H NMR (400 MHz, Methanol-d4) δ 7.75 (d, J = 14.7 Hz, 1H), 7.62 (d, J = 14.9 Hz, 1H), 7.46 (d, J = 9.6 Hz, 2H), 6.80 (d, J = 7.5 Hz,

2H), 5.97 (s, 1H), 5.17 (s, 1H), 3.85 (s, 3H), 3.20 (d, J = 7.2 Hz, 2H), 1.73 (s, 3H), 1.62(s, 3H).

1.5.Compound 1

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.55 (d, J = 10.5 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 6.11 (s, 1H), 5.38 (dd, J = 12.5, 2.2 Hz, 1H), 5.12 (t, J = 8.3 Hz, 1H), 3.77 (s, 3H), 3.21 (t, J = 6.9 Hz, 2H), 2.92 (dd, J = 16.7, 12.5 Hz, 1H), 2.72 (dd, J = 16.6, 3.2 Hz, 1H), 1.58 (d, J = 316.5 Hz, 6H).

1.6.Compound 2

^{1}H NMR (400 MHz, Chloroform-d) δ 14.66 (s, 1H), 7.78 (d, J = 14.6 Hz, 1H), 7.57 (d, J = 15.0 Hz, 1H), 7.50 (s, 1H), 6.65 (d, J = 2.6 Hz, 1H), 6.49 (dd, J = 3.3, 1.7 Hz, 1H), 6.18 (s, 1H), 5.92 (s, 1H), 3.89 (s, 3H), 3.39 (d, J = 7.2 Hz, 2H), 1.79 (d, J = 21.9 Hz, 6H).

1.7.Compound 3

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.77 (s, 1H), 7.52 (s, 1H), 7.22 (d, J = 12.7 Hz, 1H), 6.72 (s, 1H), 6.64 (d, J = 15.8 Hz, 1H), 6.07 (s, 1H), 3.66 (s, 3H), 2.58 (t, J = 6.8 Hz, 2H), 1.73 (t, J = 6.8 Hz, 2H), 1.20 (s, 6H).

1.8.Compound 4

^{1}H NMR (400 MHz, Chloroform-d) δ 14.68 (s, 1H), 7.84 (d, J = 15.0 Hz, 1H), 7.60 (d, J = 14.8 Hz, 1H), 7.09 (d, J = 3.5 Hz, 1H), 6.72 (d, J = 3.5 Hz, 1H), 6.15 (s, 1H), 5.92 (s, 1H), 3.88 (s, 3H), 3.39 (d, J = 7.2 Hz, 2H), 2.51 (s, 3H), 1.79 (d, J = 21.3 Hz, 6H).

1.9.Compound 5

^{1}H NMR (400 MHz, Chloroform-d) δ 8.46 (d, J = 15.4 Hz, 1H), 8.24 (s, 1H), 7.88 – 7.78 (m, 3H), 7.74 (d, J = 7.2 Hz, 1H), 7.55 – 7.38 (m, 3H), 5.91 (s, 1H), 5.18 (t, J = 6.9 Hz, 1H), 3.80 (s, 3H), 3.24 (d, J = 6.9 Hz, 2H), 1.71 (s, 3H), 1.61 (s, 3H).

1.10. Compound 6

^{1}H NMR (400 MHz, Chloroform-d) δ 14.70 (s, 1H), 9.06 – 8.91 (m, 2H), 8.25 (dd, J = 15.6, 1.7 Hz, 1H), 8.22 – 8.13 (m, 1H), 8.05 (d, J = 7.1 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.45 (dd, J = 8.7, 5.5 Hz, 1H), 6.35 (s, 1H), 5.30 (t, J = 6.7 Hz, 1H), 3.89 (s, 3H), 3.41 (d, J = 7.0 Hz, 2H), 1.79 (d, J = 21.3 Hz, 6H).

1.11. Compound 7

^{1}H NMR (400 MHz, DMSO-d_6) δ 14.44 (s, 1H), 10.65 (s, 1H), 7.91 (d, J = 15.7 Hz, 1H), 7.74 – 7.57 (m, 3H), 7.44 – 7.33 (m, 2H), 7.26 (t, J = 7.5 Hz, 1H), 6.08 (s, 1H), 3.90 (s, 3H), 2.47 – 2.37 (m, 2H), 1.53 – 1.44 (m, 2H), 1.09 (s, 6H).

1.12. Compound 8

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 2H), 7.62 (s, 4H), 7.14 (d, J = 15.2 Hz, 2H), 6.93 (d, J = 15.9 Hz, 2H), 6.08 (s, 2H), 3.57 (s, 6H), 1.66 (t, J = 6.7 Hz, 4H), 1.12 (s, 12H).

1.13. Compound 9

^{1}H NMR (400 MHz, DMSO-d_6) δ 14.20 (s, 1H), 10.66 (s, 1H), 8.64 (s, 1H), 8.54 (d, J = 15.9 Hz, 1H), 8.32 (d, J = 8.6 Hz, 1H), 8.12 (d, J = 8.1 Hz, 2H), 7.69 (d, J

= 15.9 Hz, 1H), 7.65 – 7.49 (m, 5H), 6.07 (s, 1H), 5.19 – 5.11 (m, 1H), 3.78 (s, 3H), 3.16 (d, J = 6.9 Hz, 2H), 1.70 (s, 3H), 1.60 (s, 3H).

1.14. Compound 10

^{1}H NMR (400 MHz, DMSO-d_6) δ 14.47 (s, 1H), 10.58 (s, 1H), 7.72 (d, J = 15.6 Hz, 1H), 7.58 (d, J = 15.6 Hz, 1H), 7.19 (s, 2H), 6.90 (d, J = 5.3 Hz, 1H), 6.05 (s, 1H), 5.10 (s, 1H), 4.25 (s, 5H), 3.82 (s, 3H), 3.11 (d, J = 8.7 Hz, 2H), 1.66 (s, 3H), 1.57 (s, 3H).

1.15. Compound IX

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.29 (d, J = 12.3 Hz, 2H), 6.79 (d, J = 11.8 Hz, 2H), 6.09 (s, 1H), 5.26 (d, J = 15.5 Hz, 1H), 5.11 (t, J = 7.2 Hz, 1H), 3.78 (s, 3H), 3.18 (d, J = 7.2 Hz, 1H), 2.96 (dd, J = 16.7, 12.7 Hz, 1H), 1.59 (s, 3H), 1.54 (s, 3H).

1.16. Compound 11

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.55 (d, J = 10.5 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 6.11 (s, 1H), 5.38 (d, J = 12.5 Hz, 1H), 5.12 (t, J = 8.3 Hz, 1H), 3.77 (s, 3H), 3.21 (t, J = 7.5 Hz, 2H), 2.92 (dd, J = 16.7, 12.5 Hz, 1H), 2.72 (d, J = 16.6 Hz, 1H), 1.58 (d, J = 16.5 Hz, 6H).

1.17. Compound 12

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.52 (s, 1H), 6.45 (d, J = 3.8 Hz, 1H), 6.44 – 6.36 (m, 1H), 6.09 (s, 1H), 5.44 (dd, J = 14.5, 3.5 Hz, 1H), 5.09 (t, J = 7.3 Hz,

1H), 3.77 (s, 3H), 3.16 (d, J = 7.3 Hz, 2H), 3.13 – 3.04 (m, 1H), 2.00 (d, J = 9.4 Hz, 2H), 1.59 (s, 6H).

1.18. Compound 13

^{1}H NMR (400 MHz, Methanol-d_4) δ 6.87 (d, J = 3.4 Hz, 1H), 6.63 (d, J = 2.4 Hz, 1H), 6.08 (s, 1H), 5.54 (dd, J = 10.1, 3.2 Hz, 1H), 5.13 (t, J = 7.8 Hz, 1H), 3.76 (s, 3H), 3.19 (d, J = 7.2 Hz, 2H), 3.03 – 2.89 (m, 1H), 2.84 (dd, J = 16.6, 3.9 Hz, 1H), 2.44 (s, 3H), 1.60 (d, J = 4.6 Hz, 6H).

1.19. Compound 14

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.87 (dd, J = 4.2, 1.7 Hz, 1H), 8.33 (dd, J = 8.3, 1.7 Hz, 1H), 8.06 (d, J = 7.2 Hz, 1H), 7.92 (d, J = 8.1 Hz, 1H), 7.65 (t, J = 7.3 Hz, 1H), 7.52 (dd, J = 8.3, 4.2 Hz, 1H), 6.51 (dd, J = 12.7, 2.9 Hz, 1H), 6.15 (s, 1H), 5.17 (t, J = 7.2 Hz, 1H), 3.81 (s, 3H), 3.07 (dd, J = 16.7, 3.0 Hz, 1H), 2.88 (dd, J = 16.7, 13.1 Hz, 1H), 1.55 (d, J = 14.9 Hz, 6H).

1.20. Compound 15

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.41 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 6.11 (s, 1H), 5.36 (dd, J = 12.8, 2.6 Hz, 1H), 3.78 (s, 3H), 3.20 (s, 1H), 2.69 (dd, J = 16.8, 2.8 Hz, 1H), 2.62 – 2.48 (m, 2H), 1.60 (td, J = 18.6, 17.1, 6.6 Hz, 2H), 1.23 (d, J = 6.9 Hz, 6H), 1.11 (s, 6H).

1.21. Compound 16

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 8.63 (s, 1H), 8.16 – 8.03 (m, 5H), 7.56 (q, J = 7.4, 7.0 Hz, 4H), 6.71 (d, J = 16.3 Hz, 1H), 6.17 (s, 1H), 3.72 (s, 3H), 1.95 (s, 3H), 1.73 (t, J = 6.7 Hz, 2H), 1.22 (s, 6H).

1.22. Compound 17

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 6.94 (s, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.83 (d, *J* = 8.3 Hz, 1H), 6.11 (s, 1H), 5.31 (dd, *J* = 11.9, 2.9 Hz, 1H), 5.12 – 5.03 (m, 1H), 4.20 (s, 4H), 3.67 (s, 3H), 3.09 (d, *J* = 7.0 Hz, 2H), 2.85 (dd, *J* = 16.4, 12.1 Hz, 1H), 2.56 (dd, *J* = 16.4, 3.1 Hz, 1H), 1.56 (s, 3H), 1.54 (s, 3H).

1.23. Compound 18

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (s, 1H), 7.04 (d, J = 16.0 Hz, 1H), 6.93 (d, J = 15.9 Hz, 2H), 6.79 (d, J = 16.0 Hz, 1H), 6.08 (s, 1H), 6.01 (s, 2H), 3.80 (s, 1H), 3.57 (s, 3H), 2.48 (s, 1H), 1.66 (t, J = 6.7 Hz, 2H), 1.12 (s, 6H).

1.24. Compound 19

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.81 (d, *J* = 15.1 Hz, 2H), 6.92 (d, *J* = 9.1 Hz, 2H), 6.54 (s, 1H), 6.43 (s, 1H), 5.75 (d, *J* = 10.0 Hz, 1H), 3.87 (s, 3H), 2.00 (d, *J* = 9.7 Hz, 1H), 1.47 (s, 6H).

1.25. Compound 20

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.79 (d, *J* = 8.7 Hz, 2H), 6.88 (dd, *J* = 19.4, 9.4 Hz, 3H), 6.53 (s, 1H), 6.41 (s, 1H), 5.79 – 5.69 (m, 1H), 3.86 (s, 3H), 2.00 (q, *J* = 12.2, 10.5 Hz, 1H), 1.47 (s, 6H).

1.26. Compound 21

^{1}H NMR (400 MHz, Chloroform-d) δ 14.05 (s, 1H), 7.84 (d, J = 15.4 Hz, 1H), 7.68 (d, J = 15.6 Hz, 1H), 7.52 (s, 2H), 7.45 (d, J = 8.5 Hz, 2H), 6.02 (d, J = 2.3 Hz, 1H), 5.94 (d, J = 2.3 Hz, 1H), 5.30 (s, 1H), 3.92 (s, 3H).

1.27. Compound 22

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.74 (d, J = 15.0 Hz, 1H), 7.56 (d, J = 15.2 Hz, 1H), 7.11 (d, J = 3.6 Hz, 1H), 6.74 (d, J = 3.5 Hz, 1H), 5.95 (d, J = 2.1 Hz, 1H), 5.89 (d, J = 2.1 Hz, 1H), 3.87 (s, 3H), 2.47 (s, 3H).

1.28. Compound 23

^{1}H NMR (400 MHz, Chloroform-d) δ 14.17 (s, 1H), 7.97 (t, J = 6.1 Hz, 3H), 7.92 – 7.79 (m, 3H), 7.75 (dd, J = 8.7, 1.5 Hz, 1H), 7.55 – 7.46 (m, 2H), 6.03 (d, J = 2.4 Hz, 1H), 5.96 (d, J = 1.9 Hz, 1H), 3.96 (s, 3H).

1.29. Compound 24

^{1}H NMR (400 MHz, Chloroform-d) δ 14.13 (s, 1H), 7.98 (d, J = 15.4 Hz, 1H), 7.66 (d, J = 15.3 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.50 (d, J = 8.3 Hz, 1H), 7.35 (d, J = 8.8 Hz, 1H), 7.22 (s, 1H), 6.99 (s, 1H), 5.99 (dd, J = 25.6, 2.3 Hz, 2H), 3.96 (s, 3H).

1.30. Compound 25

^{1}H NMR (400 MHz, Chloroform-d) δ 14.20 (s, 1H), 7.81 (q, J = 15.5 Hz, 2H), 7.53 (d, J = 8.1 Hz, 2H), 7.27 (s, 3H), 5.98 (dd, J = 29.5, 2.3 Hz, 2H), 5.33 (s, 1H), 3.92 (s, 3H), 2.93 (hept, J = 6.7 Hz, 1H), 1.27 (d, J = 7.2 Hz, 6H).

1.31. Compound 26

^{1}H NMR (400 MHz, Chloroform-d) δ 7.77 (s, 2H), 7.45 (d, J = 8.6 Hz, 2H), 7.29 (t, J = 7.7 Hz, 4H), 7.13 (d, J = 7.8 Hz, 5H), 7.09 (t, J = 7.6 Hz, 2H), 7.01 (d, J = 8.5 Hz, 2H), 6.02 – 5.84 (m, 3H), 5.37 (s, 1H), 3.89 (s, 3H), 3.86 (s, 2H), 2.04 (s, 3H).

1.32. Compound 27

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.72 (s, 2H), 10.73 (s, 2H), 7.89 (d, J = 15.7 Hz, 2H), 7.81 (s, 4H), 7.69 (d, J = 15.7 Hz, 2H), 6.24 (d, J = 14.2 Hz, 2H), 6.04 (s, 2H), 5.94 (s, 2H), 3.90 (s, 6H), 3.42 (s, 2H).

1.33. Compound 28

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.63 (s, 1H), 10.71 (s, 1H), 8.60 (s, 1H), 8.49 (d, J = 15.9 Hz, 1H), 8.29 (d, J = 8.6 Hz, 2H), 8.09 (d, J = 8.2 Hz, 2H), 7.64 – 7.46 (m, 5H), 6.04 – 5.89 (m, 2H), 3.79 (s, 3H).

1.34. Compound 29

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.54 (d, J = 8.5 Hz, 2H), 7.40 (d, J = 7.9 Hz, 2H), 6.05 (dd, J = 23.5, 1.7 Hz, 2H), 5.40 (dd, J = 12.5, 2.9 Hz, 1H), 3.80 (s, 3H), 3.01 – 2.86 (m, 1H), 2.70 (dd, J = 16.7, 3.1 Hz, 1H).

1.35. Compound 30

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.50 (s, 1H), 6.45 (d, J = 3.3 Hz, 1H), 6.43 – 6.35 (m, 1H), 6.05 (d, J = 1.6 Hz, 1H), 5.94 (d, J = 1.5 Hz, 1H), 5.44 (dd, J = 11.7, 3.2 Hz, 2H), 3.78 (s, 3H), 3.09 (dd, J = 16.7, 11.3 Hz, 1H), 2.76 (dd, J = 16.8, 3.7 Hz, 1H).

1.36. Compound 31

^{1}H NMR (400 MHz, Methanol-d_4) δ 6.87 (d, J = 3.5 Hz, 1H), 6.63 (d, J = 2.5 Hz, 1H), 6.05 (d, J = 1.9 Hz, 1H), 5.96 (d, J = 2.0 Hz, 1H), 5.55 (dd, J = 11.5, 3.2 Hz, 1H), 3.78 (s, 3H), 3.04 – 2.91 (m, 1H), 2.82 (dd, J = 16.7, 3.5 Hz, 1H), 2.43 (s, 3H).

1.37. Compound 32

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 (dd, *J* = 23.7, 12.1 Hz, 4H), 7.61 (d, *J* = 11.5 Hz, 1H), 7.54 – 7.45 (m, 2H), 6.06 (d, *J* = 16.6 Hz, 2H), 5.61 (d, *J* = 12.9 Hz, 1H), 3.72 (s, 3H), 3.06 (dd, *J* = 15.7, 13.1 Hz, 1H), 2.69 (d, *J* = 16.5 Hz, 1H).

1.38. Compound 33

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51 (d, J = 7.2 Hz, 1H), 7.45 (d, J = 7.9 Hz, 1H), 7.17 (dt, J = 14.5, 7.2 Hz, 2H), 6.64 (s, 1H), 6.06 – 5.95 (m, 2H), 4.98 (dd, J = 8.4, 3.7 Hz, 1H), 3.75 (s, 3H), 3.31 (d, J = 3.7 Hz, 1H), 3.17 – 3.03 (m, 1H).

1.39. Compound 34

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 8.26 (d, J = 8.9 Hz, 2H), 8.10 (d, J = 8.9 Hz, 2H), 6.76 (s, 1H), 6.36 (d, J = 1.4 Hz, 1H), 6.18 (d, J = 1.5 Hz, 1H), 3.83 (s, 3H), 1.22 – 1.05 (m, 2H).

1.40. Compound 35

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.37 (d, J = 7.9 Hz, 2H), 7.25 (d, J = 7.8 Hz, 2H), 6.04 (dd, J = 23.9, 2.1 Hz, 2H), 5.36 (dd, J = 12.5, 2.8 Hz, 1H), 3.80 (s, 3H), 3.04 – 2.83 (m, 2H), 2.67 (dd, J = 16.4, 2.8 Hz, 1H), 1.23 (d, J = 6.9 Hz, 6H).

1.41. Compound 36

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.75 (s, 1H), 6.73 (s, 1H), 6.02 (d, J = 2.0 Hz, 1H), 5.97 (s, 2H), 5.94 (d, J = 2.0 Hz, 1H), 5.31 (dd, J = 12.7, 2.7 Hz, 1H), 3.80 (s, 3H), 3.70 (s, 3H), 2.98 (dd, J = 16.4, 12.8 Hz, 1H), 2.51 (d, J = 2.8 Hz, 1H).

1.42. Compound 37

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 6.93 (d, J = 2.0 Hz, 1H), 6.88 (dd, J = 8.3, 2.0 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.01 (d, J = 2.1 Hz, 1H), 5.93 (d, J = 2.0 Hz, 1H), 5.29 (dd, J = 12.3, 2.9 Hz, 1H), 4.20 (s, 4H), 3.70 (s, 3H), 2.89 (dd, J = 16.4, 12.3 Hz, 1H), 2.52 (dd, J = 16.4, 3.0 Hz, 1H).

1.43. Compound 38

^{1}H NMR (400 MHz, Chloroform-d) δ 7.53 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 6.13 (d, J = 2.3 Hz, 1H), 6.09 (d, J = 2.3 Hz, 1H), 5.36 (dd, J = 12.9, 3.0 Hz, 1H), 3.88 (s, 3H), 3.81 (s, 3H), 2.95 (dd, J = 16.5, 12.9 Hz, 1H), 2.77 (dd, J = 16.5, 3.1 Hz, 1H).

1.44. Compound 39

^{1}H NMR (400 MHz, Chloroform-d) δ 7.64 (d, J = 8.1 Hz, 2H), 7.61 – 7.57 (m, 2H), 7.53 (d, J = 8.5 Hz, 2H), 7.45 (t, J = 7.5 Hz, 2H), 7.36 (t, J = 7.4 Hz, 1H), 6.17 (d, J = 2.2 Hz, 1H), 6.10 (d, J = 2.2 Hz, 1H), 5.46 (dd, J = 13.1, 2.9 Hz, 1H), 3.90 (s, 3H), 3.82 (s, 3H), 3.07 (dd, J = 16.5, 13.1 Hz, 1H), 2.84 (dd, J = 16.6, 3.0 Hz, 1H).

1.45. Compound 40

^{1}H NMR (400 MHz, Chloroform-d) δ 7.53 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 6.13 (d, J = 2.3 Hz, 1H), 6.09 (d, J = 2.3 Hz, 1H), 5.36 (dd, J = 12.9, 3.0 Hz, 1H), 3.88 (s, 3H), 3.81 (s, 3H), 2.95 (dd, J = 16.5, 12.9 Hz, 1H), 2.77 (dd, J = 16.5, 3.1 Hz, 1H).

1.46. Compound 41

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 7.55 (s, 1H), 6.37 (s, 1H), 6.31 (s, 1H), 5.88 (d, J = 1.9 Hz, 2H), 5.85 (s, 2H), 3.69 (d, J = 8.5 Hz, 6H), 2.98 – 2.82 (m, 1H).

1.47. Compound 42

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.62 – 6.58 (m, 2H), 6.55 (dd, J = 8.2, 2.0 Hz, 2H), 5.88 – 5.83 (m, 2H), 4.11 (s, 4H), 3.70 (s, 3H), 2.85 (q, J = 13.9 Hz, 2H).

2. Cell culture and reagents

Human multiple myeloma (MM) cell lines IM9 (#CCL-159) and U266 (#CCL-155), obtained from the American Type Culture Collection (Manassas, VA, USA) were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium supplemented with 10% heat-inactivated fetal bovine serum (FBS; Atlas Biologicals, Fort Collins, CO, USA) containing 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco, Carlsbad, CA, USA) and maintained at 37°C in humidified 5% CO_2.

Xanthohumol (KBB-NX) compounds and derivatives were synthesized and used by KBlueBio Inc. (Hwasun, Korea). Lenalidomide (LEN), 2,7-dichlorofluorecin diacetate (DCF-DA), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cytarabine (Ara-C) was purchased from Selleckchem (Houston, TX, USA). RPMI 1640 medium and D-PBS were purchased from Welgene (Daegu, Korea). A fluorescein isothiocyanate (FITC) Annexin V apoptosis detection kit and PI/Rnase staining buffers were manufactured by BD Biosciences (San Jose, CA, USA).

3. In vitro efficacy evaluation

3.1.Cell viability analysis

KBB-NX and derivatives were prepared as a stock solution of 100 mM in DMSO, stored at −20°C, and diluted in medium. The water-soluble tetrazolium salt (WST) assay was performed using the EZ-cytox cell viability assay kit (DoGenBio, Seoul, Korea) according to the manufacturer's instructions. MM cells (5×10^5 cells per well)

were plated in 96-well plates. After 24 h, cells were treated with DMSO control or KBB-NX (25, 50, or 100 μM). After another 24, 48, and 72 h, 10 μL of EZ-cytox reagent was added to each well and incubated for 40 min at 37°C. The absorbance of the samples was measured at 450 nm using a VERSA Max Micro plate reader (Molecular Devices, San Jose, CA, USA). The assay was performed in triplicate.

3.2.Apoptosis analysis

MM cells (3×10^6 cells per well) were seeded into 6-well plates and 24 h later, treated with KBB-NX, KBB-NX14, KBB-NX21, and KBB-NX26 (20, 50, 100 μM) for 48 h. Cells were stained with FITC-conjugated annexin-V and propidium iodide (PI) at room temperature (RT) for 15 min. Apoptotic cells were analyzed using a BD FACS Calibur Flow cytometer (BD Biosciences).

Apoptosis-related proteins were analyzed by the Proteome Profiler Human Array (R&D Systems, Minneapolis, MN, USA). After treatment, cell lysates (200 μg) were extracted and applied per array set comprised of two nitrocellulose membranes with spotted capture antibodies. The pixel density of spots was quantified using HLI mage++ software (Western Vision). Optical spot densities were normalized against respective reference array spots and the against control.

3.3.Cell cycle analysis

MM cells (3×10^6 cells per well) were seeded into 6-well plates and 24 h later, treated with KBB-NX14 (50 μM) for 48 h. Cells were fixed in cold 70% ethanol and stored at −20°C for 24 h. After staining with PI/Rnase staining solution (BD Biosciences) for

15 min at RT, the histogram distribution of DNA content was analyzed using a BD FACS Calibur Flow cytometer (Becton Dickinson).

3.4.Reactive oxygen species (ROS) analysis

MM cells were treated with KBB-NX21 (20, 50, 100 μM) for 48 h and then exposed to 20 μM DCF-DA (Sigma-Aldrich) by blocking light at 37°C under 5% CO_2 for 30 min. After washing with D-PBS, analysis was performed using a FACS Calibur Flow cytometer (Becton Dickinson).

3.5.Western blot analysis

MM cells (1×10^7 cells per well) were seeded into T25 flasks and 24 h later, they were treated with LEN, KBB-NX14, and KBB-NX21 (50 μM) for 48 h. After collecting the treated cells, they were lysed using RIPA buffer (Bio-Rad), and then protein was qualified by the Bradford assay. Proteins were separated by size using sodium dodecyl sulfate polyacrylamide gel electrophoresis. Separated protein was transferred to a polyvinylidene fluoride membrane and treated with a blocking solution (5% nonfat dry milk with 1× TBST) for 1 h. After blocking, PHB2 (#E1Z5A; cell signaling technology) and β-actin (#13E5; cell signaling technology) primary antibodies were added and gently stirred overnight at 4°C. Then, horseradish peroxidase (HRP)-linked anti-rabbit IgG secondary antibody was added and the reaction was agitated at RT for 1 h. Protein bands were visualized by a chemiluminescence method using a kit. The intensity of each protein band was measured using an AI600 chemiluminescence imager (GE Healthcare, Little Chalfont, UK).

4. Colony forming unit assay for hematopoietic stem/progenitor cells

Isolated peripheral blood stem cells (PBSC) were resuspended at a concentration of 1×10^6 cells per mL in RPMI 1640 media supplemented with 2% FBS and penicillin/streptomycin. Colony forming unit (CFU) assays were performed using methylcellulose semisolid medium. The StemSpanSFEM (Stemcell Technologies, Vancouver, Canada). Briefly, an aliquot (0.3 mL) of resuspended PBSC was added to 3 mL of methylcellulose based medium (Methocult Optimum H4043, Stemcell Technologies) before plating 1.1 mL in duplicate 30 mm Petri dishes. CFUs were counted manually after 14 days of incubation at 37°C in 5% CO_2.

5. In vivo efficacy evaluation of synthetic KBB-NX and derivatives

5.1. Development of MM xenograft model

NOD/SCID IL-2Rγ^{null} (NSG) mice were purchased from Jackson Laboratory (Bar Harbor, MA, USA). To establish a human MM xenograft model, RPMI8226-GFP-FLuc cells (1×10^7 per mouse) were intravenously injected into 6- to 11-week-old male and female NSG mice. Tumor growth was monitored by bioluminescence imaging using the Night Owl System (Berthold Technologies, Bad Wildbad, Germany). The animals were acclimatized for 1 week in an animal facility at Chonnam National University. All animal procedures were performed according to the guidelines of the Institutional Animal Care and Use Committee and were approved by the Animal Ethics Committee (Approval No. CNU IACUC-H-2020-36 and CNU IACUC-H-2022-1).

5.2.Assessment of MM xenograft model

To assess the anti-MM effects of LEN and KBB-NX21 in the MM xenograft model, NSG mice (n=4 per group) were divided into the following treatment groups. Experiment set: no treatment (PBS control), LEN (5 mg/kg/day), and KBB-NX21 (5 mg/kg/day). Mice were treated with LEN or KBB-NX21 (5 mg/kg/day) by intravenous injection for 3 days a week and repeated for 3 weeks with a 1-day interval each week. Tumor growth was monitored weekly by bioluminescence imaging in the dorsal view; 10 min before imaging, mice were intraperitoneally injected with D-Luciferin (150 mg/kg/mouse, Perkin Elmer, USA). The Night Owl System (Berthold Technologies) was used for imaging.

6. Toxicity assessment

For the initial toxicity evaluation, KBB-NX14 and KBB-NX21 among the 33 types of KBB-NX derived compounds obtained were intravenously injected into the femoral vein of rats at a dose of 5 mg/kg, and blood was collected 3 h later. Hepatotoxicity (ALT), nephrotoxicity (BUN, creatinine), and cardiotoxicity (CK-MB, myoglobin) markers were evaluated from blood samples.

7. Pharmacokinetic study

In order to proceed with the initial pharmacokinetic study, a catheter was inserted into the femoral vein of the rat, and KBB-NX14 and KBB-NX21 were intravenously administered at a dose of 5 mg/kg. After administration, blood was

collected at 5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 6 h, 10 h, and 24 h to measure the blood concentration.

8. Metabolic stability assessment

A liver microsomal stability test was performed to evaluate the metabolic stability of the KBB-NX series of synthetic compounds. The activity of the enzyme system was confirmed by the % remaining value of buspirone, the reference compound obtained after the reaction. The specific test method was performed as follows. After incubating the microsome diluted with potassium phosphate buffer at 37°C for 5 min, the drug and NADPH were reacted at 37°C for 30 min. To terminate the reaction, cold acetonitrile containing an internal standard was added to remove the protein. After centrifugation (4000 rpm, 4°C, 15 min), 100 μL of the supernatant was analyzed by LC-MS/MS.

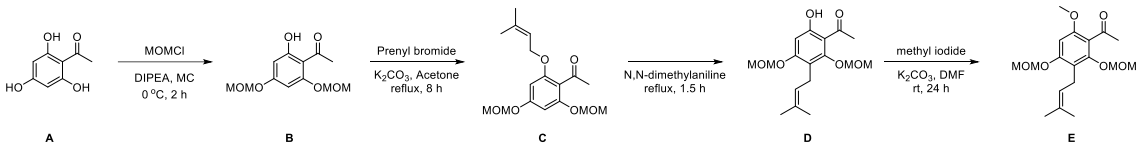
III. Results

Synthesis of PHB2 protein targeting KBB-NX and derivatives

KBB-NX and its derivatives were synthesized using the following step-by-step reaction scheme (Fig. 1).

[Synthesis of compound E1]

The reaction scheme and specific synthesis method of compound E synthesis are as follows.



(i) Synthesis of compound B

Dissolve 25.0 g (0.135 mol) of 2,4,6-Trihydroxyacetophenone (compound A) and 87.129 g (0.674 mol) of N,N-Diisopropylethylamine (DIPEA) in 1,100 mL of MC. After the solution was cooled to 0℃, 43.418 g (0.539 mol) of chloromethyl methyl ether (MOMCl) was slowly added and stirred at RT for 2 h. The obtained product was separated and purified by silica gel column chromatography (ethyl acetate (EA):hexane (Hex)=1:4 volume ratio) to obtain 29.6 g of 1-(2-hydroxy-4,6-bis(methoxymethoxy)phenyl)ethan-1-one (compound B) (yield 85.7%).

(ii) Synthesis of compound C

After dissolving 29.6 g (0.116 mol) of the obtained compound B in 1,200 mL of acetone, 28.69 g (0.193 mol) of prenylbromide and 63.857 g (0.462 mol) of K_2CO_3 are added. The inside of the flask is substituted with Ar and heated to reflux for 8 h. The obtained product was separated and purified by silica gel column

chromatography (EA:Hex=1:4) to obtain 36.4 g of 1-(2,4-bis(methoxymethoxy)-6-((3-methylbut-2-en-1-yl)oxy)phenyl)ethan-1-one (compound C) (yield 97.1%).

(iii) Synthesis of compound D

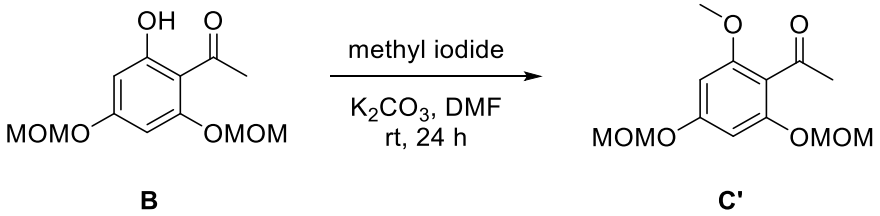
To 36.4 g (0.112 mol) of the obtained compound C, 205.4 g (1.694 mol) of N,N-dinethylaniline was added to replace the inside of the flask with Ar. Stir at 220℃ for 1.5 h. Extraction was performed using 10% HCl aqueous solution and EA, and water was removed using $MgSO_4$. The obtained product was separated and purified by silica gel column chromatography (EA:Hex=1:4) to obtain 25.2 g of 1-(6-hydroxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)ethan-1-one (compound D) (yield 69.1%).

(iv) Synthesis of compound E

Methyl iodide 27.6 g (0.194 mol) and K_2CO_3 21.474 g (0.155 mol) were added to the obtained compound D 25.2 g (77.689 mmol) in 240 mL of DMF, and the inside of the flask was substituted with Ar. Then, the mixture is stirred at RT for 24 h. The obtained product is extracted using water and EA, and moisture is removed using $MgSO_4$. The obtained product was separated and purified by silica gel column chromatography (EA:Hex=1:4) to obtain 24.605 g of 1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)ethan-1-one (compound E) (yield 93.6%).

[Synthesis of compound C']

The reaction scheme and specific synthesis method of compound C synthesis are as follows.



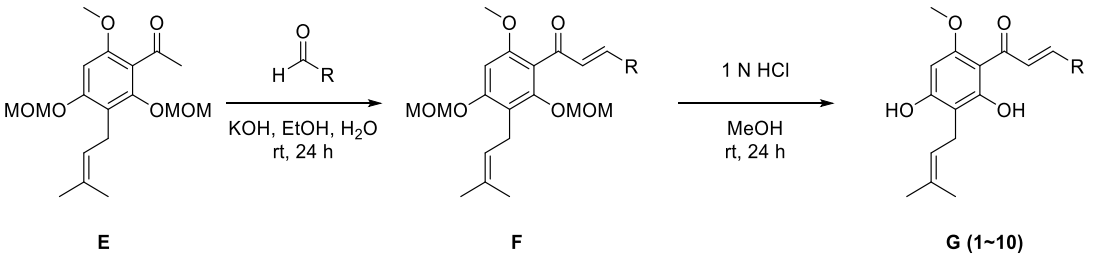
B **C'**

(i) Synthesis of compound C'

To the obtained compound B 10.9 g (42.537 mmol), 18.1 g (0.128 mol) of methyl iodide and 17.636 g (0.128 mol) of K_2CO_3 were added in 131 mL of DMF, and the inside of the flask was substituted with Ar. Then, the mixture is stirred at RT for 24 h. The obtained product is extracted using water and EA, and moisture is removed using $MgSO_4$. The obtained product was separated and purified by silica gel column chromatography (EA:Hex=1:4) to obtain 8.930 g of 1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)ethan-1-one (compound C') (yield 77.7%).

[Synthesis of compounds 1-10]

The reaction scheme and specific synthesis method of compounds 1-10.



E **F** **G (1~10)**

(i) Synthesis of compound F0

1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)ethan-1-one (compound E) 500.0 mg (1.478 mmol) and 4-(methoxymethoxy)benzaldehyde 270.1 mg (1.625 mmol) is dissolved in 8.6 mL of ethanol. After the solution is cooled to 0℃, KOH 4.145 g (73.877 mmol) and H_2O 5.3 mL are added, and the inside of the flask is substituted with Ar. Then, the mixture was stirred at RT for 24 h. The resulting compound was extracted with EA, separated and purified by silica gel column chromatography (EA:Hex=1:3) to obtain 443.7 mg of (E)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (compound F0) (yield 61.7%).

(ii) Synthesis of XN

After dissolving the obtained compound F0 of 443.7 mg (0.912 mmol) in 110 mL of methanol, 12.1 mL of 6N HCl aqueous solution is added. The inside of the flask is substituted with Ar and stirred at RT for 24 h. After the obtained product was extracted with EA, separated and purified by silica gel column chromatography (EA:Hex=1:2) to obtain 321.0 mg of (E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (XN) (yield 99.3%).

(iii) Synthesis of compound F1

Synthesis was carried out with reference to (i) Synthesis of compound F0 of 328.1 mg (1.773 mmol) of 4-bromobenzaldehyde to obtain 654.5 mg of (E)-3-(4-bromophenyl)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound F1) (yield 87.6%).

(iv) Synthesis of compound 1

654.5 mg (1.295 mmol) of the obtained compound F1 was synthesized with reference to (ii) Synthesis of XN of 91.7 mg of (E)-3-(4-bromophenyl)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound 1) (yield 17.0%).

(v) Synthesis of compound F2

328.1 mg (1.773 mmol) of furan-2-carbaldehyde was synthesized with reference to (i) Synthesis of compound F0 to obtain 502.2 mg of (E)-3-(furan-2-yl)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound F2) (yield 81.6%).

(vi) Synthesis of compound 2

The obtained compound F2 502.2 mg (1.206 mmol) was synthesized with reference to (ii) Synthesis of XN to obtain 325.1 mg of (E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(furan-2-yl)prop-2-en-1-one (compound 2) (yield 82.1%).

(vii) Synthesis of compound F3

Furan-3-carbaldehyde 68.15 mg (0.709 mmol) was synthesized with reference to (i) Synthesis of compound F0 to obtain 47.2 mg of (E)-3-(furan-3-yl)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound F3) (yield 19.2%).

(viii) Synthesis of compound 3

47.2 mg (0.113 mmol) of the obtained compound F3 was synthesized by referring to (ii) Synthesis of XN to obtain 18.2 mg of (E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(furan-3-yl)prop-2-en-1-one (compound 3) (yield 48.9%).

(ix) Synthesis of compound F4

5-methylthiophene-2-carbaldehyde 134.23 mg (1.064 mmol) was synthesized by referring to (i) Synthesis of compound F0 to obtain 294.8 mg of (E)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-3-(5-methylthiophen-2-yl)prop-2-en-1-one (compound F4) (yield 74.5%).

(x) Synthesis of compound 4

The obtained compound F4 294.8 mg (0.660 mmol) was synthesized by referring to (ii) Synthesis of XN to obtain 38.3 mg of (E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(5-methylthiophen-2-yl)prop-2-en-1-one (compound 4) (yield 16.2%).

(xi) Synthesis of compound F5

1-naphthaldehyde 110.77 mg (0.709 mmol) was synthesized by referring to (i) Synthesis of compound F0 to obtain 276.1 mg of (E)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-3-(naphthalen-1-yl)prop-2-en-1-one (compound F5) (yield 98.0%).

(xii) Synthesis of compound 5

276.1 mg (0.579 mmol) of the obtained compound F5 was synthesized by referring to (ii) Synthesis of XN to obtain 15.7 mg of (E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(naphthalen-1-yl)prop-2-en-1-one (compound 5) (yield 7.0%).

(xiii) Synthesis of compound F6

Quinoline-8-carbaldehyde 255.4 mg (1.625 mmol) was synthesized by referring to (i) Synthesis of compound F0 to obtain 590.1 mg of (E)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-3-(quinolin-8-yl)prop-2-en-1-one (compound F6) (yield 83.6%).

(xiv) Synthesis of compound 6

The obtained compound F6 590.1 mg (1.236 mmol) was synthesized with reference to (ii) Synthesis of XN to obtain 156.8 mg of (E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(quinolin-8-yl)prop-2-en-1-one (compound 6) (yield 32.6%).

(xv) Synthesis of compound F7

Benzofuran-2-carbaldehyde 111.4 mg (0.762 mmol) was synthesized by referring to (i) Synthesis of compound F0 to obtain 256.8 mg of (E)-3-(benzofuran-2-yl)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound F7) (yield 86.6%).

(xvi) Synthesis of compound 7

The obtained compound F7 256.8 mg (0.550 mmol) was synthesized with reference to (ii) Synthesis of XN to obtain 74.4 mg of (E)-3-(benzofuran-2-yl)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound 7) (yield 35.7 %).

(xvii) Synthesis of compound F8

Terephthalaldehyde 89.18 mg (0.665 mmol) was synthesized with reference to (i) Synthesis of compound F0 to obtain 501.0 mg of (2E,2'E)-3,3'-(1,4-phenylene)bis(1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one) (compound F8) (yield 43.8%).

(xviii) Synthesis of compound 8

The obtained compound F8 101.8 mg (0.131 mmol) was synthesized with reference to (ii) Synthesis of XN to obtain 45.1 mg of (2E,2'E)-3,3'-(1,4-phenylene)bis(1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one) (compound 8) (yield 60.4%).

(xix) Synthesis of compound F9

Anthracene-9-carbaldehyde 319.97 mg (1.551 mmol) was synthesized with reference to (i) Synthesis of compound F0 to obtain 713.1 mg of (E)-3-(anthracen-9-yl)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound F9) (yield 91.6%).

(xx) Synthesis of compound 9

The obtained compound F9 713.1 mg (1.354 mmol) was synthesized with reference to (ii) Synthesis of XN to obtain 153.1 mg of (E)-3-(anthracen-9-yl)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound 9) (yield 25.8%).

(xxi) Synthesis of compound F10

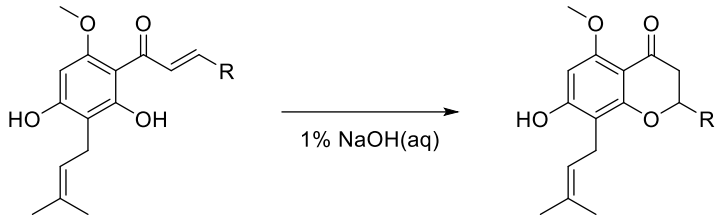
2,3-dihydrobenzo[b][1,4]dioxine-6-carbaldehyde 254.68 mg (1.551 mmol) was synthesized with reference to (i) Synthesis of compound F0 to obtain 685.4 mg of (E)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound F10) (yield 95.7%).

(xxii) Synthesis of compound 10

The obtained compound F 10685.4 mg (1.415 mmol) was synthesized with reference to (ii) Synthesis of XN to obtain 147.5 mg of (E)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound 10) (yield 26.3%).

[Synthesis of compounds 11-18]

The reaction scheme and specific synthesis method of compounds 11-18.



G **H (11-18)**

(i) Synthesis of IX

After adding 112 mL of 1% NaOH aqueous solution to (E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (XN), 200.0 mg (0.135 mmol), the mixture is stirred at RT for 2 hr. The obtained product was extracted with EA under acidic conditions using 1N HCl aqueous solution, and then separated and purified by silica gel column chromatography (EA:Hex=1:1) to obtain 183.1 mg of 7-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one (IX) (yield 91.6%).

(ii) Synthesis of compound 11

Compound 1 64.7 mg (0.155 mmol) was synthesized with reference to (i) Synthesis of IX to obtain 55.4 mg of 2-(4-bromophenyl)-7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one (compound 11) (yield 85.6%).

(iii) Synthesis of compound 12

Compound 2 108.1 mg (0.329 mmol) was synthesized with reference to (i) Synthesis of IX to obtain 100.3 mg of 2-(furan-2-yl)-7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one (compound 12) (yield 92.8%).

(iv) Synthesis of compound 13

Compound 4 36.0 mg (0.100 mmol) was synthesized with reference to (i) Synthesis of IX to obtain 25.1 mg of 7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)-2-(5-methylthiophen-2-yl)chroman-4-one (compound 13) (yield 69.7%).

(v) Synthesis of compound 14

110.4 mg (0.283 mmol) of compound 6 was synthesized by referring to (i) Synthesis of IX to obtain 21.5 mg of 7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)-2-(quinolin-8-yl)chroman-4-one (compound 14) (yield 19.5%).

(vi) Synthesis of compound 15

(E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-isopropylphenyl)prop-2-en-1-one 110.6 mg (0.291 mmol) was synthesized by referring to (i) Synthesis of IX to obtain 65.3 mg of 7-hydroxy-2-(4-isopropylphenyl)-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one (compound 15) (yield 59.0%).(vii) Synthesis of compound 16

Compound 950.0 mg (0.114 mmol) was synthesized with reference to (i) Synthesis of IX to obtain 40.6 mg of 2-(anthracen-9-yl)-7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one (compound 16) (yield 81.2%).

(viii) Synthesis of compound 17

Compound 1050.0 mg (0.126 mmol) was synthesized with reference to (i) Synthesis of IX to obtain 48.1 mg of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one (compound 17) (yield 96.2%).

(ix) Synthesis of compound 18

(E)-1-(6-methoxy-2,4-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-3-(7-methoxybenzo[d][1,3] Dioxol-5-yl)prop-2-en-1-one816.4 mg (1.631 mmol) was synthesized with reference (i) Synthesis of compound F0 to obtain 16.6 mg of 7-hydroxy-5-methoxy-2-(7-methoxybenzo[d][1,3]dioxol-5-yl)-8-(3-methylbut-2-en-1-yl)chroman-4-one (compound 18) (yield 2.5%).

[Synthesis of compounds 19-20]

The reaction scheme and specific synthesis method of compounds 19-20.

H **I (19~20)**

(i) Synthesis of compound 19

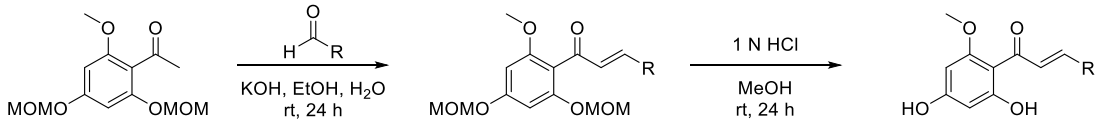
7-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one (IX) 50.0 mg (0.141mmol) and 2,3- Dissolve 96.08 mg (0.423 mmol) of dichloro-5,6-dicyano-p-benzoquinone (DDQ) in 1.69 mL of 1,4-dioxane. The solution was stirred under reflux for 18 h. The obtained product was separated and purified by silica gel column chromatography (MC:MeOH=20:1 volume ratio) to obtain 10.1 mg of 7-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8-(3-methylbut-2-en-1-yl)-4H-chromen-4-one (compound 19) (yield 20.3%).

(ii) Synthesis of compound 20

Compound 12 120.2 mg (0.336 mmol) was synthesized with reference (i) Synthesis of compound 19 to obtain 17.2 mg of 2-(furan-2-yl)-7-hydroxy-5-methoxy-8-(3-methylbut-2)-en-1-yl)-4H-chromen-4-one (compound 20) (yield 14.4%).

[Synthesis of compounds 21-28]

The reaction scheme and specific synthesis method of compounds 21-28.



C' D' E' (21-28)

(i) Synthesis of compound D'0

1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)ethan-1-one (Compound C') 500 mg (1.850 mmol) was synthesized with reference to (i) Synthesis of compound F0

to obtain 355.9 mg of (E)-3-(4-hydroxyphenyl)-1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)prop-2-en-1-one (compound D'0) (yield 43.4%).

(ii) Synthesis of Helichrysetin

(E)-1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (compound D') 235.9 mg (0.564 mmol) was synthesized with reference to (ii) Synthesis of XN to obtain 110.1 mg of (E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (Helichrysetin) (yield 68.2%).

(iii) Synthesis of compound D'1

4-bromobenzaldehyde 410.7 mg (2.220 mmol) was synthesized with reference (i) Synthesis of compound D'0 of 718.3 mg of (E)-3-(4-bromophenyl)-1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)prop-2-en-1-one (compound D'1) (yield 88.8%).

(iv) Synthesis of compound 21

The obtained compound D' 1718.3 mg (1.643 mmol) was synthesized with reference to (ii) Synthesis of Helichrysetin to obtain 458.5 mg of ((E)-3-(4-bromophenyl)-1-(2,4-dihydroxy-6-) methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (compound 21) (yield 79.9%).

(v) Synthesis of compound D'2

5-methylthiophene-2-carbaldehyde 303.4 mg (2.405 mmol) was synthesized by referring to (i) Synthesis of compound D'0 to obtain 265.2 mg of (E)-1-(2-methoxy-

4,6-bis(methoxymethoxy)phenyl)-3-(5-methylthiophen-2-yl)prop-2-en-1-one (compound D'2) (yield 37.9%).

(vi) Synthesis of compound 22

The obtained compound D' 2265.2 mg (0.701 mmol) was synthesized with reference to (ii) Synthesis of Helichrysetin to obtain 198.2 mg of (E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(5-methylthiophen-2-yl)prop-2-en-1-one (compound 22) (yield 97.4%).

(vii) Synthesis of compound D'3

The synthesis of 1-naphthaldehyde 346.7 mg (2.220 mmol) was carried out with reference to (i) Synthesis of compound D'0 to obtain 693.4 mg of (E)-1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)-3-(naphthalen-2-yl)prop-2-en-1-one (compound D'3) (yield 91.8%).

(viii) Synthesis of compound 23

The obtained compound D' 3693.4 mg (1.698 mmol) was synthesized with reference to (ii) Synthesis of Helichrysetin to obtain 523.4 mg of (E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(naphthalen-2-yl) prop-2-en-1-one (compound 23) (yield 96.2%).

(ix) Synthesis of compound D'4

By referring to (i) Synthesis of compound D'0, benzofuran-2-carbaldehyde 324.43 mg (2.220 mmol) was synthesized to obtain 536.8 mg of (E)-3-(benzofuran-2-

yl)-1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)prop-2-en-1-one (compound D'4) (yield 72.8%).

(x) Synthesis of compound 24

536.8 mg (1.347 mmol) of the obtained compound D'4 was synthesized with reference to (ii) Synthesis of Helichrysetin to obtain 410.9 mg of (E)-3-(benzofuran-2-yl)-1-(2,4-dihydroxy-6-methoxyphenyl)prop-2-en-1-one (compound 24) (yield 98.3%).

(xi) Synthesis of compound D'5

4-isopropylbenzaldehyde 329.0 mg (2.220 mmol) was synthesized with reference to (i) Synthesis of compound D'0 to obtain 709.0 mg of (E)-3-(4-isopropylphenyl)-1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)prop-2-en-1-one (compound D'5) (yield 95.7%).

(xii) Synthesis of compound 25

276.1 mg (0.579 mmol) of the obtained compound D'5 was synthesized with reference to (ii) Synthesis of Helichrysetin to obtain 265.1 mg of (E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(4-isopropylphenyl)prop-2-en-1-one (compound 25) (yield 54.8%).

(xiii) Synthesis of compound D'6

4-(diphenylamino)benzaldehyde 287.59 mg (1.052 mmol) was synthesized with reference to (i) Synthesis of compound D'0 to obtain 254.5 mg of (E)-3-(4-

(diphenylamino)phenyl)-1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)prop-2-en-1-one (compound D'6) (yield 46.9%).

(xiv) Synthesis of compound 26

The obtained compound D'6 254.5 mg (0.484 mmol) was synthesized with reference to (ii) Synthesis of Helichrysetin to obtain 72.0 mg of (E)-3-(4-(diphenylamino)phenyl)-1-(4-hydroxy-2-methoxy-6-(methoxymethoxy)phenyl)prop-2-en-1-one (compound 26) (yield 34.0%).

(xv) Synthesis of compound D'7

121.59 mg (0.906 mmol) of terephthalaldehyde was synthesized with reference to (i) Synthesis of compound D'0 to obtain 618.0 mg of (2E,2'E)-3,3'-(1,4-phenylene)bis(1-(2)-methoxy-4,6-bis(methoxymethoxy)phenyl)prop-2-en-1-one) (compound D'7) (yield 52.6%).

(xvi) Synthesis of compound 27

The obtained compound D' 7200.0 mg (0.313 mmol) was synthesized with reference to (ii) Synthesis of Helichrysetin to obtain 34.5 mg of (2E,2'E)-3,3'-(1,4-phenylene)bis(1-(4-hydroxy-2-methoxy-6-(methoxymethoxy)phenyl)prop-2-en-1-one) (compound 27) (yield 23.8%).

(xvii) Synthesis of compound D'8

Anthracene-9-carbaldehyde 400.61 mg (1.942 mmol) was synthesized with reference to (i) Synthesis of compound D'0 to obtain 768.1 mg of (E)-3-(anthracen-9-

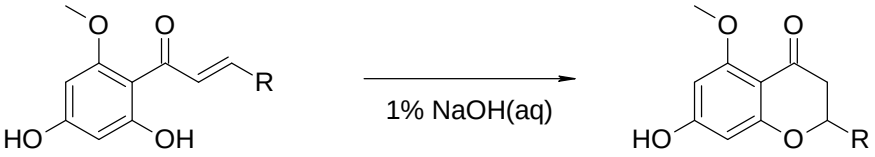
yl)-1-(2-methoxy-4),6-bis(methoxymethoxy)phenyl)prop-2-en-1-one (compound D'8) (yield 90.6%).

(xviii) Synthesis of compound 28

The obtained compound D'8 768.1 mg (1.675 mmol) was synthesized with reference to (ii) Synthesis of Helichrysetin to obtain 65.0 mg of (E)-3-(anthracen-9-yl)-1-(2,4-dihydroxy-6)-methoxyphenyl)prop-2-en-1-one (compound 28) (yield 10.5%).

[Synthesis of compounds 29-37]

The reaction scheme and specific synthesis method of compounds 29-37.



E' **F' (29~37)**

(i) Synthesis of compound 29

The obtained compound 21 357.5 mg (1.024 mmol) was synthesized by referring to (i) Synthesis of IX to obtain 246.2 mg of 2-(4-bromophenyl)-7-hydroxy-5-methoxychroman-4-one (compound 29) (yield 68.9%).

(ii) Synthesis of compound 30

(E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(furan-2-yl)prop-2-en-1-one 276.4 mg (1.062 mmol) was synthesized with reference to (ii) Synthesis of compound

11 to obtain 59.1 mg of 2-(furan-2-yl)-7-hydroxy-5-methoxychroman-4-one (compound 30) (yield 21.4%).

(iii) Synthesis of compound 31

The obtained compound 22153.6 mg (0.529 mmol) was synthesized by referring to (i) Synthesis of IX to obtain 44.8 mg of 7-hydroxy-5-methoxy-2-(5-methylthiophen-2-yl)chroman-4-one (compound 31) (yield 29.2%).

(iv) Synthesis of compound 32

The obtained compound 23438.4 mg (1.369 mmol) was synthesized by referring to (i) Synthesis of IX to obtain 210.6 mg of 7-hydroxy-5-methoxy-2-(naphthalen-2-yl)chroman-4-one (compound 32) (yield 48.0%).

(v) Synthesis of compound 33

The obtained compound 24 325.9 mg (1.050 mmol) was synthesized referring to (i) Synthesis of IX to obtain 121.4 mg of 2-(benzofuran-2-yl)-7-hydroxy-5-methoxychroman-4-one (compound 33) (yield 37.3%).

(vi) Synthesis of compound 34

(E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one 45.5 mg (0.144 mmol) of (i) Synthesis of IX to obtain 16.3 mg of 7-hydroxy-5-methoxy-2-(4-nitrophenyl)chroman-4-one (compound 34) (yield 32.2%).

(vii) Synthesis of compound 35

The obtained compound 25194.6 mg (0.623 mmol) was synthesized referring to (i) Synthesis of IX to obtain 185.6 mg of 7-hydroxy-2-(4-isopropylphenyl)-5-methoxychroman-4-one (compound 35) (yield 95.4%).

(viii) Synthesis of compound 36

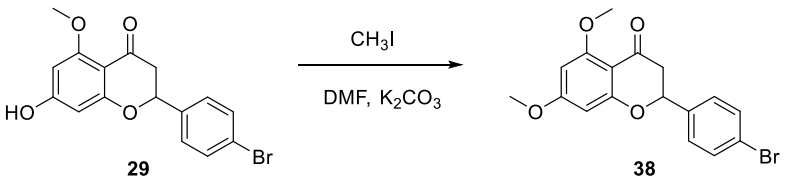
(E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(7-methoxybenzo[d][1,3]dioxol-5-yl)prop-2-en-1-one 76.7 mg (0.231 mmol) was synthesized with reference to (i) Synthesis of IX to obtain 13.7 mg of 7-hydroxy-5-methoxy-2-(7-methoxybenzo[d][1,3]dioxol-5-yl)chroman-4-one (compound 36) (yield 17.9%).

(ix) Synthesis of compound 37

(E)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1-(2,4-dihydroxy-6-methoxyphenyl)prop-2-en-1-one 158.2 mg (0.482 mmol) was synthesized with reference to (i) Synthesis of IX to obtain 84.7 mg of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-7-hydroxy-5-methoxychroman-4-one (compound 37) (yield 53.5%).

[Synthesis of compound 38]

The reaction scheme and specific synthesis method of compound 38.

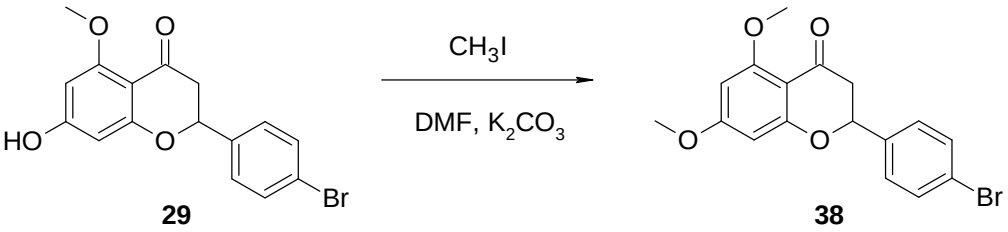


(i) Synthesis of compound 38

29246.5 mg (0.706 mmol) of the obtained compound and K_2CO_3 (292.69 mg, 2.118 mmol, 3.0 eq) were placed in 10 mL RBF and dissolved with DMF (2.2 mL). After replacing the inside of RBF with Ar, methyl iodide (300.6 mg, 2.118 mmol, 0.132 mL) was added and stirred at RT for 24 h. The obtained product is extracted using water and EA, and moisture is removed using $MgSO_4$. The obtained product was separated and purified by silica gel column chromatography (EA:Hex=1:4), and 243.1 mg of 2-(4-bromophenyl)-5,7-dimethoxychroman-4-one (compound 38) (yield 94.8%).

[Synthesis of compounds 39-40]

The reaction scheme and specific synthesis method of compounds 39-40.



(i) Synthesis of compound 39

3850.0 mg (0.143 mmol) of the obtained compound and phenylboronic acid (34.92 mg, 0.286 mmol, 2.0 eq), $Pd(PPh_3)_4$ (8.27 mg, 0.007 mmol, 0.05 eq) and K_2CO_3 (59.37 mg, 0.430 mmol, 3.0 eq) Put in 10 mL RBF, and add Toluene (3.0 mL), EtOH (0.85 mL), H_2O (0.3 mL). After that, the inside of the RBF is replaced with Ar and stirred for 12 h under reflux condition. The obtained product is extracted using water

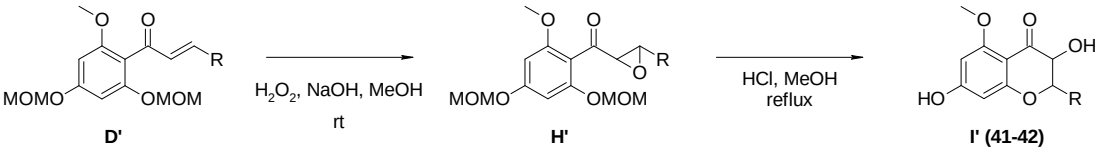
and EA, and moisture is removed using $MgSO_4$. The obtained product was separated and purified by silica gel column chromatography (EA:Hex=1:4) to obtain 33.3 mg of 2-([1,1'-biphenyl]-4-yl)-5,7-dimethoxychroman-4-one (compound 39) (yield 64.5%).

(ii) Synthesis of compound 40

42.24 mg (0.344 mol, 2.0 eq) of pyridin-4-ylboronic acid was synthesized with reference to (i) Synthesis of compound 39 to obtain 9.9 mg of 5,7-dimethoxy-2-(4-(pyridin-4-yl)phenyl)chroman-4-one (compound 40) (yield 15.9%).

[Synthesis of compounds 41-42]

The reaction scheme and specific synthesis method of compounds 41-42.



(i) Synthesis of compound H'1

After adding (E)-1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)-3-(7-methoxybenzo[d][1,3]dioxol-5-yl)prop-2-en-1-one 200.0 mg (0.463 mmol) to RBF, the inside of RBF is substituted with Ar. After dissolution in MeOH (7.0 mL), 5N NaOH aqueous solution (4.6 mL) and 30% H_2O_2 aqueous solution (0.15 mL, 3.0 eq) are added, and the mixture is stirred at RT for 3 h. Thereafter, MeOH is removed through vacuum drying and extracted using EA. Moisture was removed using $MgSO_4$, and the solvent was removed by vacuum drying (2-methoxy-4,6-

bis(methoxymethoxy)phenyl)(3-(7-methoxybenzo[d][1,3]dioxol-5-yl)oxiran-2-yl)methanone (compound H'1) 190.6 mg is obtained (yield 91.9%).

(ii) Synthesis of compound 41

After dissolving the obtained compound H' 1190.6 mg (0.425 mmol) in 17.2 mL of methanol, 5.7 mL of 6N HCl aqueous solution is added. The inside of the flask was substituted with Ar and stirred at RT for 24 h. The obtained product was extracted with EA, and then separated and purified by silica gel column chromatography (EA:Hex=1:2) to obtain 45.1 mg of 3,7-dihydroxy-5-methoxy-2-(7-methoxybenzo[d][1,3]dioxol-5-yl)chroman-4-one (compound 41) (yield 30.5 %).

(iii) Synthesis of compound H'2

(E)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)prop-2-en-1-one 200.0 mg (0.480 mmol) was synthesized with reference to (i) Synthesis of compound H'1 to obtain 182.2 mg of (3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)oxiran-2-yl)(2-methoxy-4,6-bis(methoxymethoxy)phenyl)methanone (compound H'2) (yield 87.7%).

(iv) Synthesis of compound 42

The obtained compound H'2 182.2 mg (0.421 mmol) was synthesized with reference to (ii) Synthesis of compound 41 to obtain 97.2 mg of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-3,7-dihydroxy-5-methoxychroman-4-one (compound 42) (yield 67.0%).

In vitro killing effect of KBB-NX and its derivatives on MM cells

MM cells were used to investigate the cell killing effect of synthetic KBB-NX and 41 derivatives. This study evaluated the IC50 of each synthetic compound using the MTT method, and found KBB-NX, KBB-14, KBB-15, KBB-21, and KBB-NX26 had an IC50 of less than 50 μM in each cell line (Table 2 and Fig. 2). Based on the above results, the ability to induce apoptosis was evaluated for the primary selected compounds. For all five synthetic compounds, an apoptotic effect was observed in a concentration- and time-dependent manner. Compounds KBB-14, KBB-15, KBB-21, and KBB-26 induced more than 80% apoptosis after 48 h incubation in all of the tested MM cells (Fig. 3).

Cytotoxicity test results of KBB-NX compounds against normal hematopoietic stem cells

As a result of the cytotoxicity test on normal hematopoietic stem cells of synthetic KBB-NX-based compounds, a methylcellulose culture system was used. As positive controls, LEN (25 μM, 50 μM) and Cytarabine (50 μM) were used. As a result of the administration of the positive control substances, the number of red blood cell, white blood cell, and megakaryocytic (platelet) cell colonies was significantly reduced. However, when KBB-NX-based compounds (14 and 21) were administered, it was confirmed that even at a concentration of 50 μM, there was no disturbance in the formation of blood cell colonies (Fig. 4).

Therapeutic effect of synthetic xanthohumol and derivative MM using a mouse model

The therapeutic effect of KBB-NX21 was tested using a MM mouse model. A xenograft model was developed by injecting 1×10^7/mL of MM cells containing a fluorescent gene into the tail vein of a complex immunodeficient mouse. KBB-NX21 and LEN, a positive control, were administered into the tail vein at a dose of 5 mg/kg. The drug was administered 3 times a week at 2-day intervals, and 3 cycles were performed for 3 weeks. To determine the effect of the drug treatment, this cycle was performed at 15, 22, 31, 43, 50, 57, and 64 days after transplantation using a bioluminescence imaging device. All mice with MM that were not administered the drug died on day 57 after transplantation. In the group administered KBB-NX21 and LEN, mice survived up to 64 days after transplantation. Serious damage by the administration of therapeutic agents was not observed by cardiac, liver, and kidney toxicity tests verified using blood samples (Fig. 5).

Mode of action and mechanism of synthetic xanthohumol and derivatives

KBB-NX active substances bind to intracellular PHB2 protein (mainly located in the inner membrane of mitochondria) to incapacitate the PHB2 protein function, thereby increasing the generation of ROS in the cell and causing mitochondrial function mutations. KBB-NX, a synthetic low-molecular compound, and its derivatives inhibited the expression of PHB2 protein in the cell, thereby lowering the function and stability of mitochondria, generating an excess of active oxygen in the

cell. The over-produced ROS in the cell then phosphorylates JNK and ERK proteins and enters the apoptosis pathway, stopping them in subG1 of the cell cycle, eventually leading to cell death. The PHB2 antagonist KBB-NX and its derivatives increase intracellular ROS as described above, thereby reducing the metabolic stress of cells. As a result, the pathological compensatory mechanism induces intracellular mitochondrial hyperproliferation, and excessive ROS is generated in the cell, promoting ROS-mediated apoptosis. KBB-NX synthetic active substances were used to treat MM cell lines and the decrease in PHB2 protein levels was confirmed by western blot. More detailed results of PHB2 protein reduced-ROS increased-mediated apoptosis by treatment with the KBB-NX series of active substances were clearly shown in an analysis using an apoptosis protein array (Fig. 6).

Pharmacokinetic and in vivo toxicity study

Among the KBB-NX synthetic derivatives, KBB-NX14 and KBB-NX21 compounds, which have distinct apoptotic effects on MM cells, were used for the initial pharmacokinetic studies. A catheter was inserted into the femoral vein of a rat as shown in the figure below for KBB-NX14 and KBB-NX21. After the intravenous administration of 5 mg/kg, blood was drawn at 5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 6 h, 10 h, and 24 h after administration, then an initial pharmacokinetic test was performed. Both compounds have a relatively short blood half-life of less than 2 hours, so structural changes, additional derivative synthesis, or half-life extension formulation technology are needed to increase blood circulation. The total systemic

exposure of the administered drug was derived as AUC (h⋆ng/mL), the half-life of drug elimination was described as $t_{1/2}$ (h), and the clearance was described as CL (L/h) as parameters. The AUC, t1/2, and CL of KBB-NX14 were 699 ± 134, 1.40 ± 0.48, and 1.83 ± 0.32, respectively. For KBB-NX21, they were 1520 ± 192, 0.94 ± 0.23, and 0.83 ± 0.11 for each parameter (Fig. 7).

Results of metabolic stability

Metabolic stability tests were performed on KBB-NX14 and KBB-NX21 synthetic compounds (Table 3). The metabolic stability of KBB-NX14 and KBB-NX21 compounds in humans was 64.2±4.32 and 61.1±1.13, respectively, and half-lives of 30-60 min demonstrated they were relatively stable compounds.

IV. Discussion

The *prohibitin* (*PHB*) gene is evolutionarily conserved and distributed in plants, microorganisms, and mammals. In humans, the *PHB* gene is located on the long arm of chromosome 17 (17q21). The intrinsic function of PHB is as a negative regulator of cell differentiation and thus it has a tumor suppressor function. More specific functions of PHB include mitochondrial function and morphology maintenance, and it is involved in the regulation of transcriptional processes. PHB is classified into type 1 (PHB1) and type 2 (PHB2) (5). PHB1 is a receptor for the chikungunya virus (6) and dengue virus 2 (7). PHB2 is related to the pathophysiology of aging and various diseases in addition to various cell physiological functions by acting as a receptor for mitophages by attracting them to the inner mitochondrial membrane (8). In a previous study (9), PHB, especially the PHB2 protein, was highly expressed in blood cancer cells, involved in various pathophysiological pathways, and shown to be a marker for the diagnosis of blood cancer.

This study has researched the pathophysiology of PHB-related tumor molecules and found that PHB protein overexpressed in hematological cancer cells was involved in the development of hematological cancer via the wnt-β catenin tumorigenic pathway (10). In addition, overexpressed PHB protein is involved in the development and progression of blood cancer by binding to oncogenes related to tumorigenesis (11). Furthermore, genetic mutations in the promoter region of the *PHB* gene are frequently observed in hematologic cancer cells, and this genetic mutation is associated with the development of hematologic cancers and related molecular pathophysiology.

As the first step in the development of a new anticancer drug targeting the PHB protein overexpressed in blood cancer cells in our previous research, this study synthesized and tested a low-molecular compound that alkylates the PHB protein. When the PHB protein alkylation compound was added to leukemia cell culture medium, the leukemia cells died in a concentration and time-dependent manner (12). Although these alkylated compounds had to be specifically attached to the PHB protein to induce alkylation, the specificity was not high due to the characteristics of the alkylated compound, and side effects were predicted if used as drugs. Therefore, in this study, a substance that selectively binds to the PHB2 protein and neutralizes the PHB2 protein was developed. It was reported that KBB-NX extracted from hops binds specifically to PHB2 and has an apoptotic effect on MM cells. Thirty-three compounds were synthesized and various MM cell killing effects were analyzed, and five compounds were presented as novel MM therapeutic compounds (Table 2).

KBB-NX and its derivatives synthesized in this study had various MM killing effects. KBB-NX, KBB-NX14, KBB-NX15, KBB-NX21, and KBB-NX26 had an IC50 of less than 50 μM, providing important clues for the development of new therapeutic agents for MM in the future. The MM death mechanism involved apoptosis by direct binding to the PHB2 protein and loss of the PHB2 protein function, thereby causing an increase in intracellular ROS.

In this study of a new treatment for MM, natural xanthohumol, a model material, had therapeutic effects on various tumors. KBB-NX kills tumor cells via multiple signaling pathways including Akt, AMPK, ERK, IGFBP2, NF-κB, and STAT3 (13). KBB-NX kills tumor cells by modulating Notch1, caspases, MMPs, Bcl-2, cyclin D1, Akt,

AMPK, ERK, IGFBP2, NF-κB, STAT3, oxidative stress markers, tumor-suppressor proteins, and miRNAs (13).

Metabolic stability can affect pharmacokinetic parameters such as drug clearance, half-life, and oral bioavailability. Furthermore, it is regarded as an important characteristic that a drug candidate should possess. The in vitro liver microsome system is used to predict the degree of drug metabolism in the liver, a major organ where drug metabolism occurs (14). The evaluation criteria for the liver microsomal stability test are as follows. If the results are 90% or more, it is a very stable compound with a half-life of 3 h or more, and in the case of a compound with 70-90%, it is considered a stable compound with a half-life of about 1-3 h. If the resulting value is 50-70%, it is considered a relatively stable compound with a half-life of 30-60 min and if the result is 50% or less, the compound has a very short half-life of less than 30 min when administered in the body, and is considered unstable with no value as a human drug. KBB-NX14 and KBB-NX21, which were discovered as preliminary materials for the treatment of MM in this study, had microsomal stability of 60% indicating their potential for drug development.

In this study, total KBB-NX (free plus conjugate form) was used to measure the concentration of KBB-NX-based compounds in the blood by pharmacokinetic testing. The KBB-NX synthetic compound synthesized in this study is structurally identical to KBB-NX, a natural substance extracted from hops, and the pharmacokinetic test conditions of the KBB-NX natural substance that were performed previously were used (15). According to the previous study report, the free form plasma concentration of KBB-NX does not accurately reflect the concentration

of active metabolites in tissues. Conjugated forms are known to produce biological effects. Therefore, in this study, in the pharmacokinetic test of KBB-NX and its derivatives, which are structurally identical to KBB-NX, the concentrations of the free and conjugated forms were measured in the blood. The blood half-life ($t_{1/2}$, h) and blood loss rate (CLobs, L/h) of the KBB-NX14 synthetic compound were 1.40 ± 0.48 and 1.83 ± 0.32, respectively, and for KBB-NX21, they were 0.94 ± 0.23 and 0.83 ± 0.11, respectively (Fig. 7). The initial concentration of KBB-NX21 was higher than that of KBB-NX14, but subsequent blood loss was faster. Considering the blood half-life, KBB-NX14 had a longer blood duration, that is, circulation time. Both synthetic compounds have a relatively short half-life in the blood of less than 2 h, so when these compounds are used as therapeutic agents, it will be necessary to increase the circulation time in the blood by structural changes and half-life extension formulation technology.

Proteasome inhibitors, LEN-based immunomodulators, and anti-CD38 monoclonal antibody therapeutics currently used in clinical trials for MM treatment have changed the MM treatment landscape, but most patients receiving treatment have a relapsed/refractory disease status. As a result, the prognosis of patients is extremely poor, so a new, safe treatment for MM is needed. Here, this study showed that synthetic KBB-NX and its derivatives might be therapeutic agents that can satisfy the needs of MM treatment.

V. Conclusions

An optimal lead compound for MM treatment was derived using its own synthesis platform for PHB2 blocking KBB-NX, which has been verified as a target for the treatment of intractable blood cancers such as MM. It will provide an important foundation for the development of innovative new drugs for MM.

VI. References

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Prohibitin 타깃 다발골수종 치료 크로마논 화합물 유효물질 발굴

이 영 은

전남대학교대학원 의과학과

(지도교수: 신명근)

(초록)

배경: 다발골수종 70% 이상이 65세 이상의 고령 환자에서 발생하며, 지난 20년 동안 한국에서 다발골수종의 발병률은 현저하게 증가했다. 따라서, 고령자에게 안전하게 사용할 수 있는 새로운 치료제의 발굴이 필요하다. 현재 임상에서 다발골수종 치료의 기초로 사용되는 레날리도마이드 기반 치료제 및 프로테아좀 억제제는 범혈구감소증, 말초신경병증, 중증 피부 병변 등의 전신 부작용을 동반한다. 따라서, 이들 치료제에 대한 심각한 부작용, 치료 내성 또는 내성을 극복할 수 있는 새로운 치료제의 개발이 필요하다.

방법: 혈액암 등 다발골수종에 대한 동반진단표지자로 자체발굴한 prohibitin, (PHB)을 표적으로 KBB-NX 와 이의 유도체 저분자화합물을 합성했다. 합성된 저분자합성물질은 세포주 수준의 유효성 검증시험, 혈액안정성시험, 실험동물을 이용한 약동학 시험, 독성시험을 통해 합성 후보물질의 안전성과 유효성을

확인하였다. 다발골수종 마우스 모델을 이용하여 효능시험을 통하여 새로운 다발골수종 치료 선도물질을 발굴하는 연구를 진행하였다.

결과: 본 연구에서는 PHB2 단백질을 차단하여 다발골수종 세포에 세포사멸 효과를 나타내는 41 종의 KBB-NX 와 관련 유도물질을 합성하였다. 이러한 합성물질을 다발골수종 세포에 처리하면 PHB2 기능이 비활성화되어 세포 내 활성 산소종 (Reactive Oxygen Species, ROS) 생성이 증가하여 ROS 매개 세포고사 기전이 작동되어 다발골수종 세포를 사멸시켰다. 합성 KBB-NX 유도체 중 KBB-NX, KBB-NX14, KBB-NX15, KBB-NX21 및 KBB-NX26 은 IC50 이 50 μM 이하여서 선도물질로 발굴되었다. KBB-NX14 및 KBB-NX21 화합물 약동학 시험에서 반감기가 2 시간이내로 비교적 짧아 추가적인 구조변경, 유도체 합성 및 제형 연구가 필요했다. 혈액내 안전성평가 결과에서 KBB-NX 와 관련 유도물질은 체내에서 비교적 안정함을 확인하였다.

결론: 합성 KBB-NX 와 유도체는 다발골수종 세포에서 PHB2 단백과 결합하여 ROS 가 과잉 생산되어 세포고사를 유도하였다. 41 개의 합성화합물 중 KBB-NX, KBB-NX14, KBB-NX15, KBB-NX21 및 KBB-NX26 은 다발골수종 새로운 치료제 선도물질로 발굴되었다. 본 연구결과와 합성물질은 고령의 다발골수종 환자에서 안전성과 효능이 담보된 새로운 치료제 개발의 중요한 단서를 제공하였다.

Keywords: 다발골수종, prohibitin, KBB-NX, 합성화합물, 치료제.

Tables and Figures

Table 1. List of synthesized KBB-NX and its derivatives

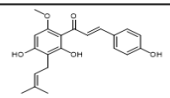
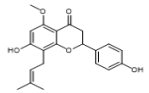
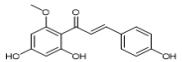
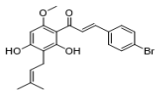
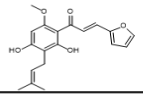
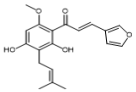
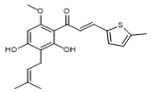
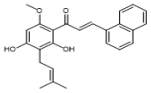
Entry	Structure	IUPAC name	IC50 (μM)	
			IM9	U266
Xanthohumol (XN) KBB-NX		(E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one	30	32
Isoxanthohumol (IX) KBB-NX1		7-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one	≥ 100	≥ 100
Helichrysetin KBB-NX2		(E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one	33	≥ 100
1 KBB-NX26		(E)-3-(4-bromophenyl)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one	48	36
2 KBB-NX9		(E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(furan-2-yl)prop-2-en-1-one	71	≥ 100
3 KBB-NX13		(E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(furan-3-yl)prop-2-en-1-one	≥ 100	≥ 100
4 KBB-NX11		(E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(5-methylthiophen-2-yl)prop-2-en-1-one	≥ 100	≥ 100
5 KBB-NX7		(E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(naphthalen-1-yl)prop-2-en-1-one	≥ 100	≥ 100

Table 1. Continued

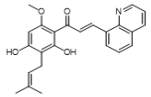
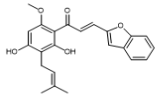
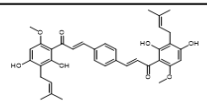
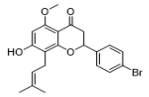
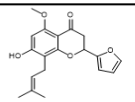
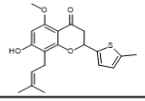
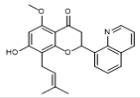
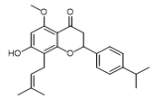
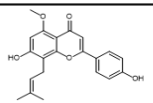
6 KBB-NX14		(E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(quinolin-8-yl)prop-2-en-1-one	27	25
7 KBB-NX8		(E)-3-(benzofuran-2-yl)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one	≥ 100	≥ 100
8 KBB-NX15		(2E,2'E)-3,3'-(1,4-phenylene)bis(1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one)	42	32
11 KBB-NX27		2-(4-bromophenyl)-7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one	82	54
12 KBB-NX3		2-(furan-2-yl)-7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one	≥ 100	≥ 100
13 KBB-NX4		7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)-2-(5-methylthiophen-2-yl)chroman-4-one	≥ 100	≥ 100
14 KBB-NX5		7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)-2-(quinolin-8-yl)chroman-4-one	≥ 100	≥ 100
15 KBB-NX16		7-hydroxy-2-(4-isopropylphenyl)-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one	81	≥ 100
19 KBB-NX6		7-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8-(3-methylbut-2-en-1-yl)-4H-chromen-4-one	≥ 100	≥ 100

Table 1. Continued

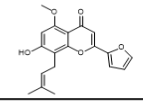
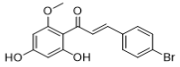
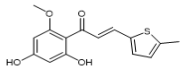
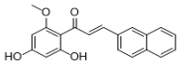
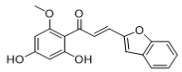
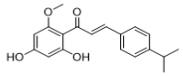
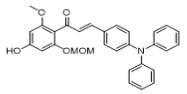
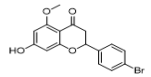
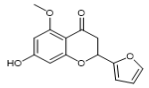
20 KBB-NX10		2-(furan-2-yl)-7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)-4H-chromen-4-one	≥ 100	≥ 100
21 KBB-NX28		E)-3-(4-bromophenyl)-1-(2,4-dihydroxy-6-methoxyphenyl)prop-2-en-1-one	43	42
22 KBB-NX19		(E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(5-methylthiophen-2-yl)prop-2-en-1-one	67	79
23 KBB-NX21		(E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(naphthalen-2-yl)prop-2-en-1-one	22	37
24 KBB-NX23		(E)-3-(benzofuran-2-yl)-1-(2,4-dihydroxy-6-methoxyphenyl)prop-2-en-1-one	33	65
25 KBB-NX30		(E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(4-isopropylphenyl)prop-2-en-1-one	60	56
26 KBB-NX33		(E)-3-(4-(diphenylamino)phenyl)-1-(4-hydroxy-2-methoxy-6-(methoxymethoxy)phenyl)prop-2-en-1-one	76	76
29 KBB-NX29		2-(4-bromophenyl)-7-hydroxy-5-methoxychroman-4-one	≥ 100	≥ 100
30 KBB-NX18		2-(furan-2-yl)-7-hydroxy-5-methoxychroman-4-one	≥ 100	≥ 100

Table 1. Continued

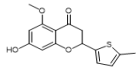
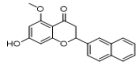
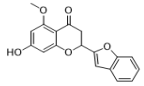
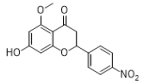
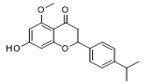
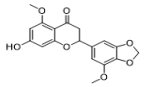
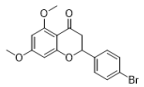
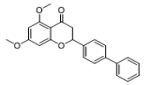
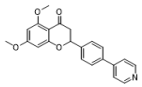
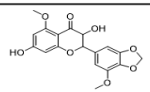
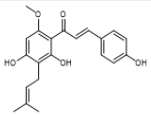
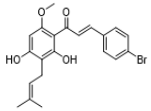
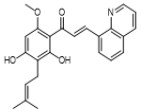
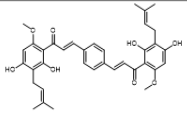
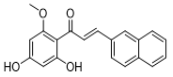
31 KBB-NX20		7-hydroxy-5-methoxy-2-(5-methylthiophen-2-yl)chroman-4-one	≥ 100	≥ 100
32 KBB-NX22		7-hydroxy-5-methoxy-2-(naphthalen-2-yl)chroman-4-one	≥ 100	≥ 100
33 KBB-NX24		2-(benzofuran-2-yl)-7-hydroxy-5-methoxychroman-4-one	≥ 100	≥ 100
34 KBB-NX25		7-hydroxy-5-methoxy-2-(4-nitrophenyl)chroman-4-one	≥ 100	≥ 100
35 KBB-NX31		7-hydroxy-2-(4-isopropylphenyl)-5-methoxychroman-4-one	83	58
36 KBB-NX36		7-hydroxy-5-methoxy-2-(7-methoxybenzo[d][1,3]dioxol-5-yl)chroman-4-one	≥ 100	≥ 100
38 KBB-NX32		2-(4-bromophenyl)-5,7-dimethoxychroman-4-one	76	≥ 100
39 KBB-NX34		2-([1,1'-biphenyl]-4-yl)-5,7-dimethoxychroman-4-one	≥ 100	≥ 100
40 KBB-NX35		5,7-dimethoxy-2-(4-(pyridin-4-yl)phenyl)chroman-4-one	≥ 100	80
41 KBB-NX37		3,7-dihydroxy-5-methoxy-2-(7-methoxybenzo[d][1,3]dioxol-5-yl)chroman-4-one	≥ 100	≥ 100

Table 2. Selected synthetic compound of KBB-NX derivatives as a novel targeted treatment for MM

Entry	Structure	IUPAC name	IC50 (μM)	
			IM9	U266
Xanthohumol (XN) KBB-NX		(E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one	30	32
1 KBB-NX26		(E)-3-(4-bromophenyl)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one	48	36
6 KBB-NX14		E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(quinolin-8-yl)prop-2-en-1-one	27	25
8 KBB-NX15		(2E,2'E)-3,3'-(1,4-phenylene)bis(1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one)	42	32
23 KBB-NX21		(E)-1-(2,4-dihydroxy-6-methoxyphenyl)-3-(naphthalen-2-yl)prop-2-en-1-one	22	37

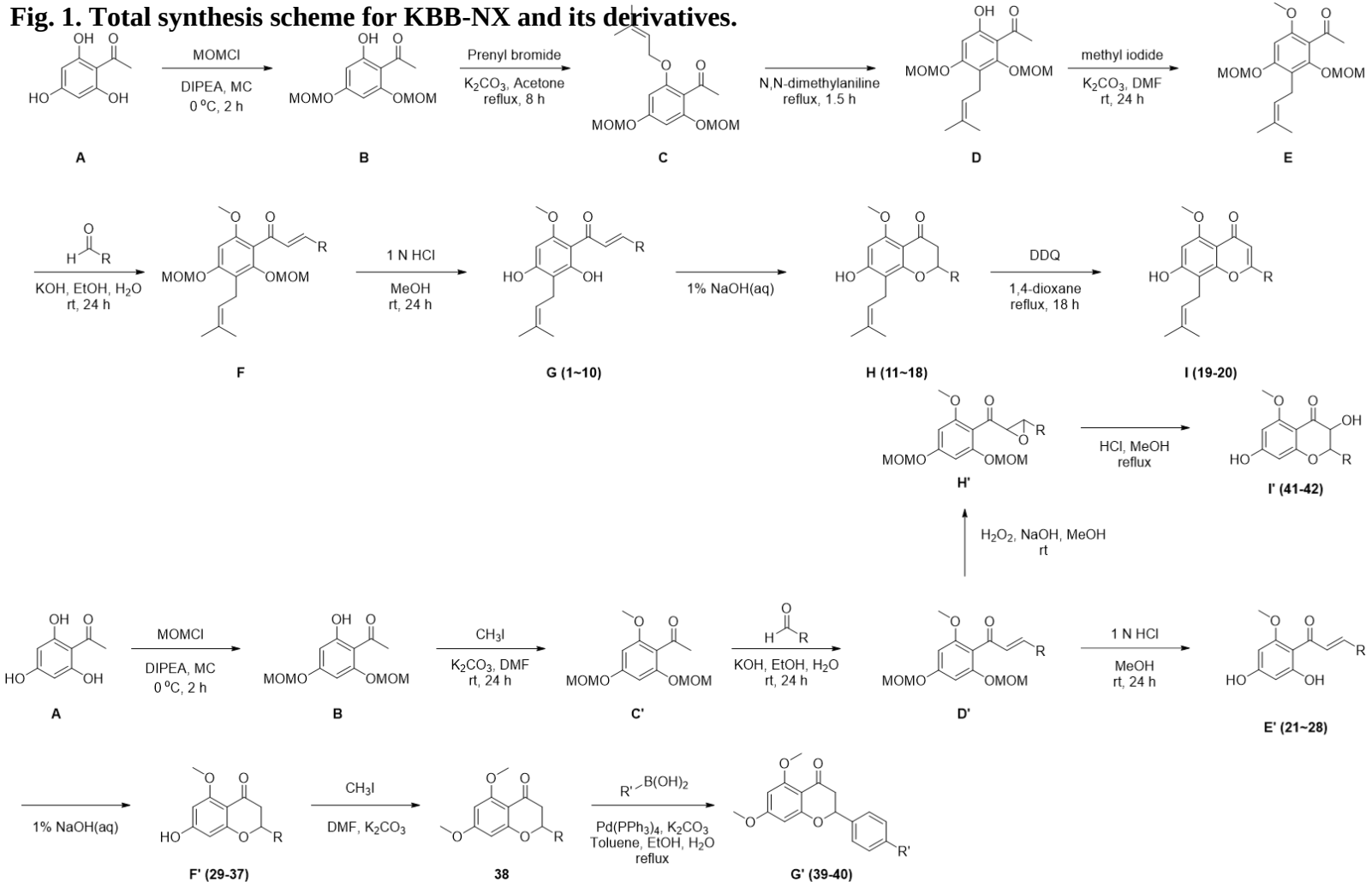
IC50, half maximal inhibitory concentration.

Table 3. Selected synthetic compound of KBB-NX derivatives as a novel targeted treatment for MM

Compound	Mouse(%)	Rat(%)	Human(%)
KBB-NX14	54.4±2.59	67.3±1.41	64.2±4.32
KBB-NX21	24.6±0.62	49.5±3.21	61.1±1.13
Buspirone	0.0±0.00	1.7±0.16	4.4±0.14

%, of remaining after 30 min (mean ± SD, n=3).

Fig. 1. Total synthesis scheme for KBB-NX and its derivatives.



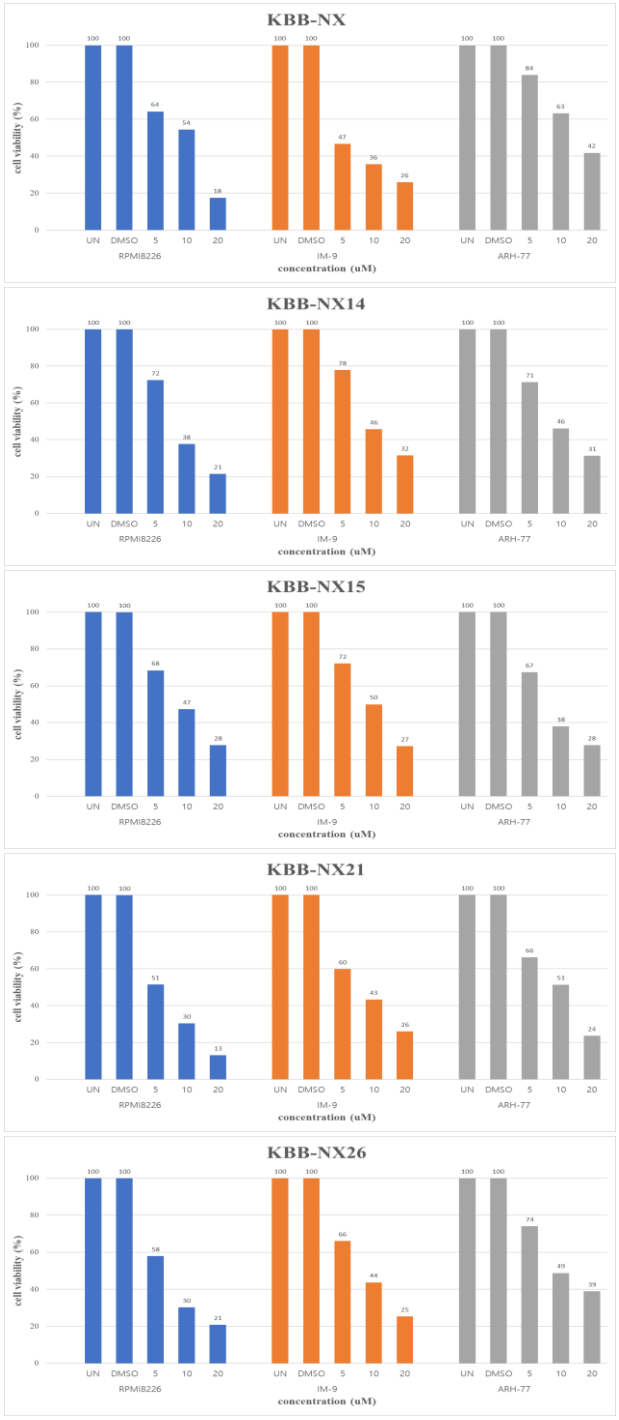


Fig. 2. Killing effect of synthetic KBB-NX and its derivatives on MM cells. An MTT assay was performed to verify the apoptosis effect of KBB-NX and inducing compounds on MM cells. The figure showed remarkable anti-proliferative effect of KBB-NX and KBB-NX synthetic derivatives with an less than 50 μM of IC50.

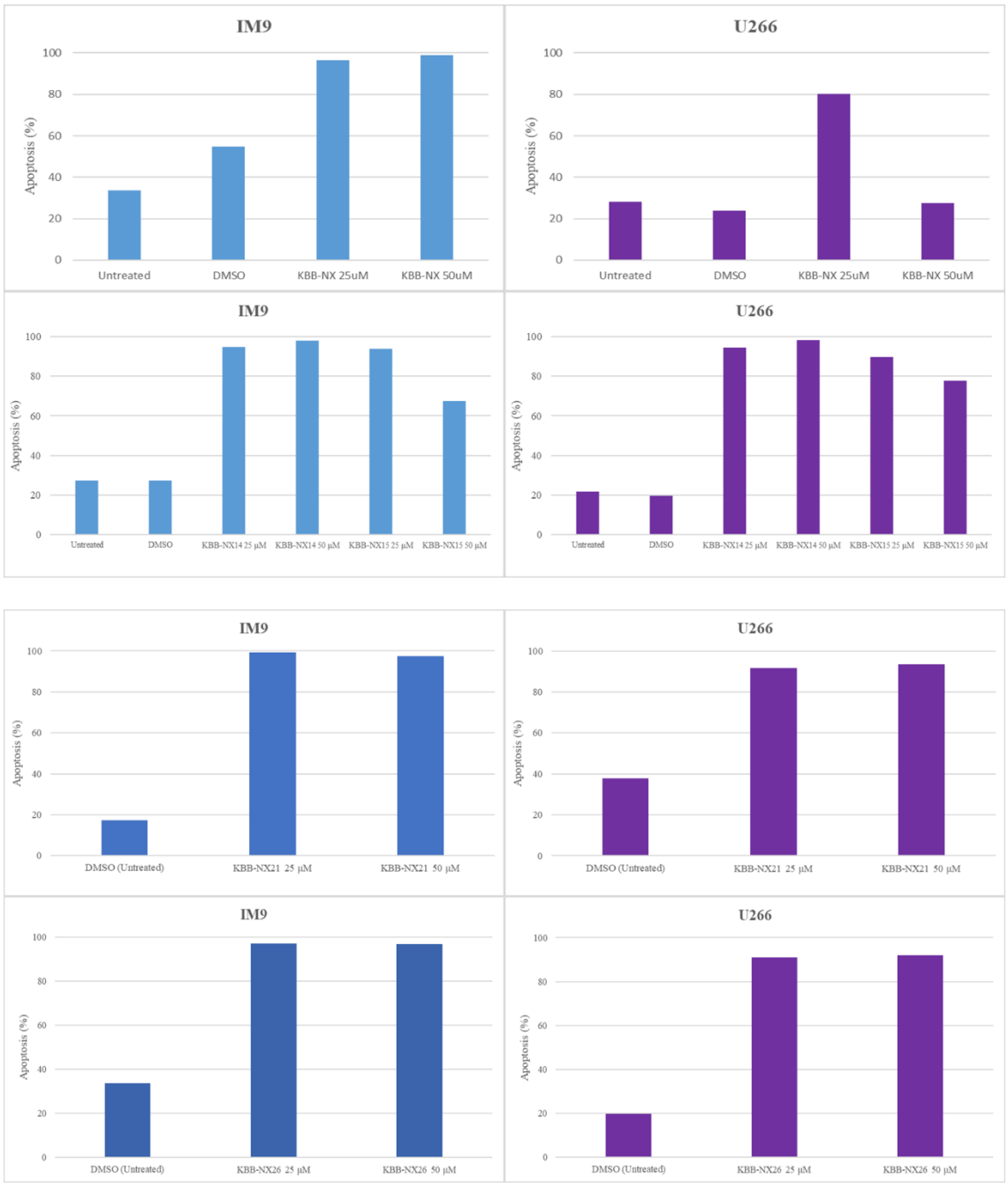


Fig. 3. Apoptosis-inducing effect of synthetic KBB-NX and its derivatives on MM cells. These are the results of quantitative measurement of apoptosis using flow cytometry for KBB-NX and KBB-NX14 compounds, which showed an IC50 of less than 50 μM.

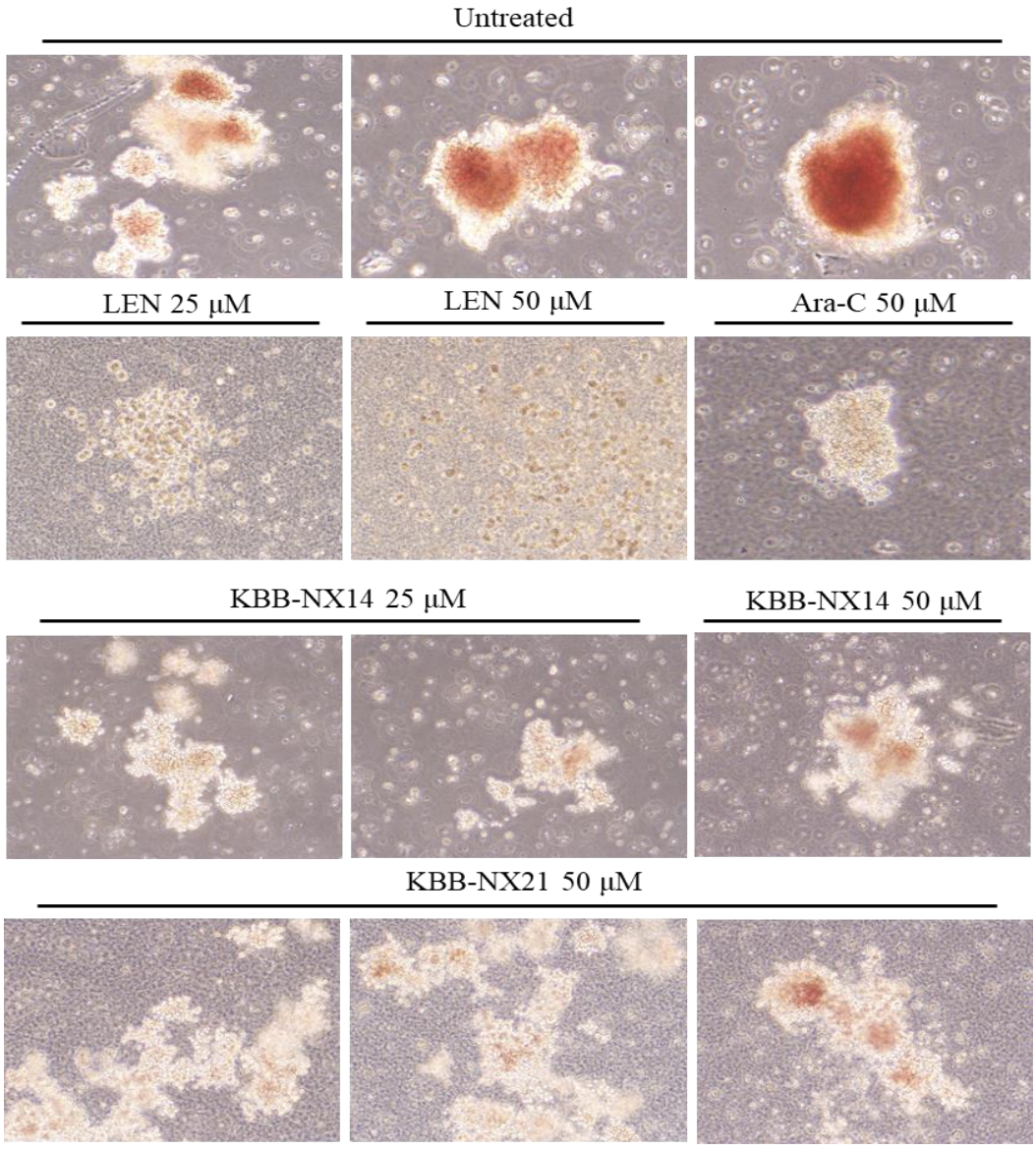


Fig. 4. Cytotoxic effect of synthetic KBB-NX and its derivatives on normal hematopoietic stem cells. To verify the effect of synthetic compounds on the differentiation capacity of normal hematopoietic stem cells, cytotoxicity was evaluated by the co-culture of normal hematopoietic stem cells and synthetic compounds in methylcellulose culture medium for 2 weeks. This confirmed that KBB-NX14 and KBB-NX21 had no toxic effect on the formation of erythroid, leukocyte and megakaryotic (platelet) colonies of normal hematopoietic stem cells.

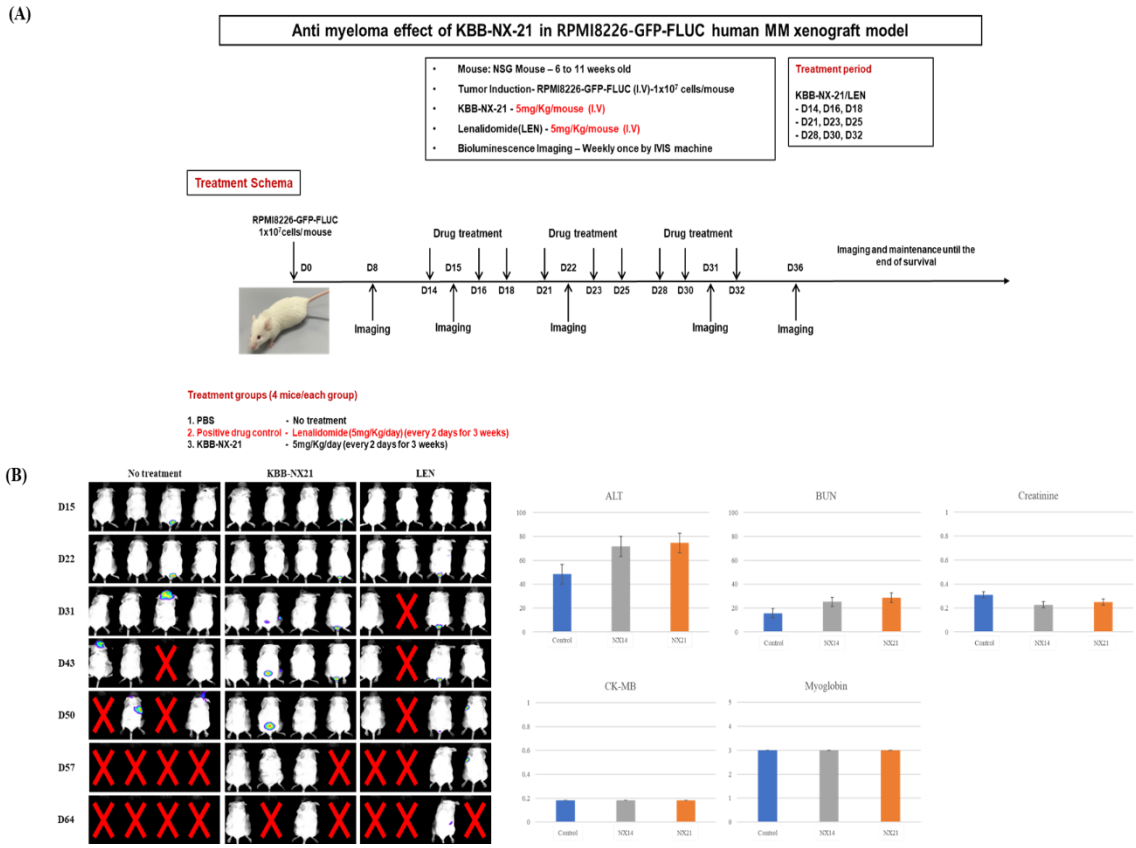


Fig. 5. Effects of KBB-NX compound treatment using the MM mouse model. (A) Protocol of in vivo efficacy evaluation. Synthetic chromanone compound derivatives and LEN as a positive control were used in this protocol. (B) In vivo efficacy of KBB-NX compound derivative. The results showed almost equal in vivo efficacy in the MM mouse model. Toxicity tests revealed minimal damage to the liver, heart, and kidney.

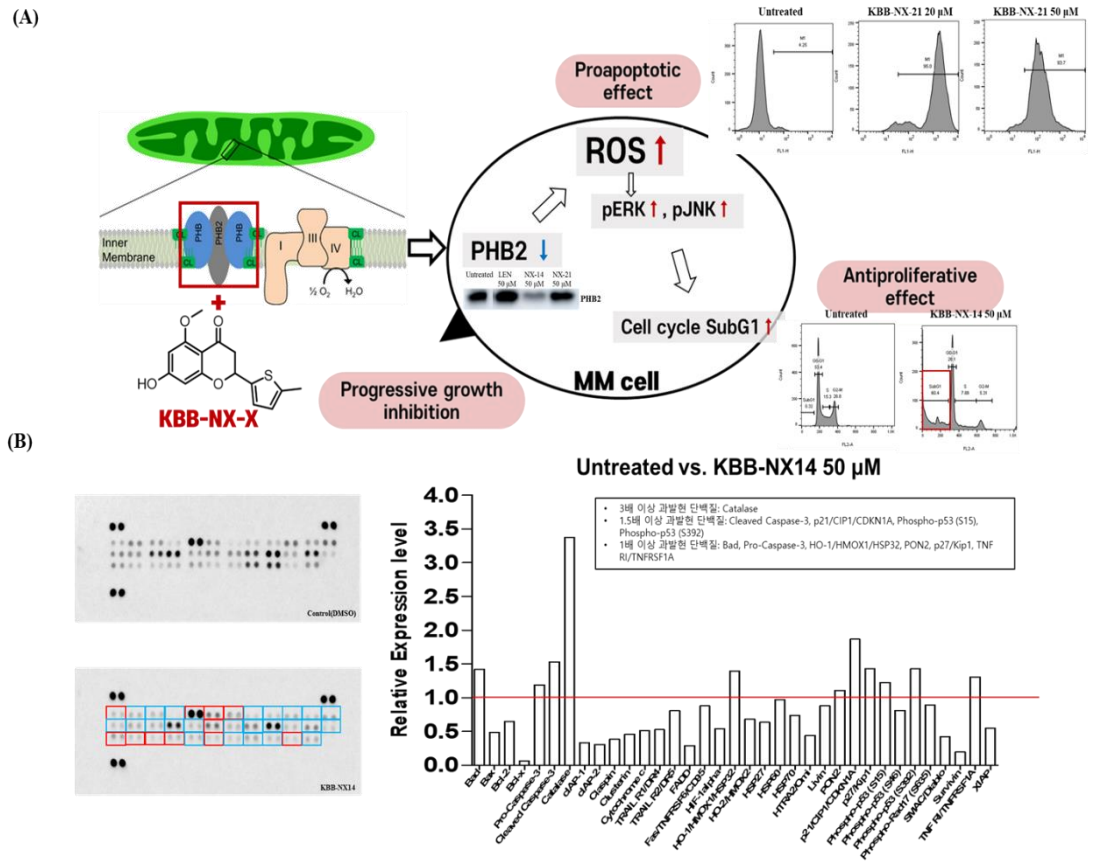
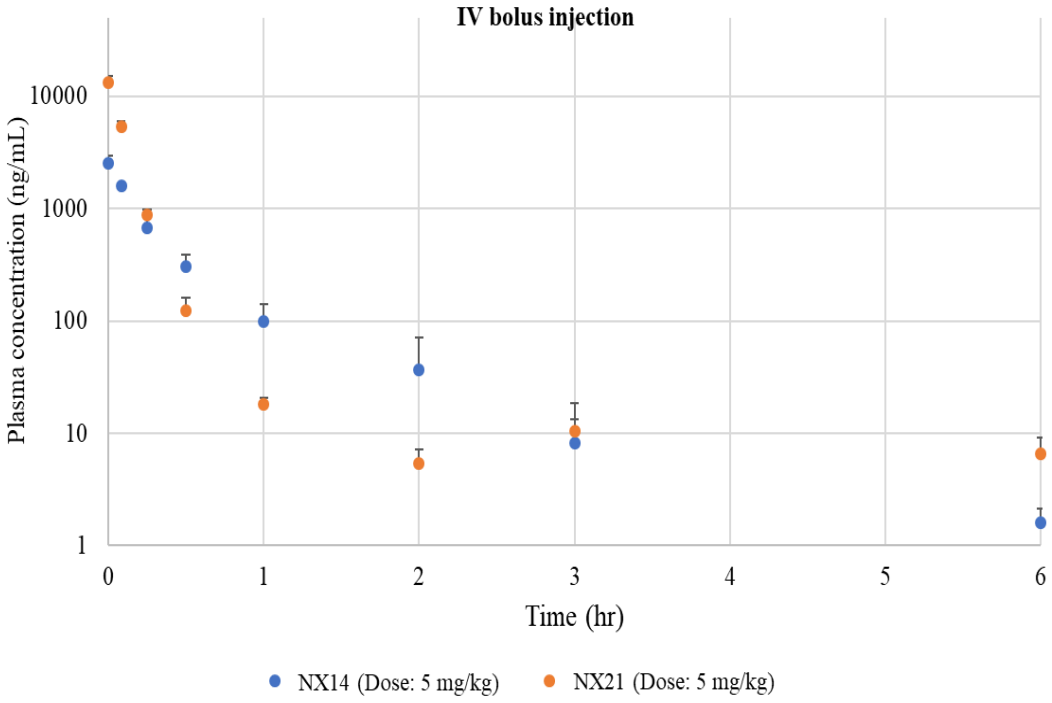


Fig. 6. Mode of action and apoptotic pathway for KBB-NX derivatives to kill MM cells. (A) Development of effective substances for the treatment of MM using a PHB-targeted chromanone compound with an apoptosis mechanism by the excessive production of intracellular ROS. Subsequently, pERK and pJNK were mainly involved in the apoptosis pathway. (B) Apoptotic pathway of KBB-NX14 for MM cells using an apoptosis protein assay. The ROS-induced apoptosis pathway was activated through the pERK- and pJNK-related pathways.



	AUC (hr*ng/mL)	$T_{1/2}$ (hr)	CL_{obs} (L/hr)
NX14	699 ± 134	1.40 ± 0.48	1.83 ± 0.32
NX21	1520 ± 192	0.94 ± 0.23	0.83 ± 0.11

Fig. 7. Initial pharmacokinetic study results of KBB-NX14 and NX21. Plasma concentration-time profiles of synthetic KBB-NX derivatives in the rat plasma after repeated intravenous injection of KBB-NX14 and KBB-NX21 for 3 h. The results showed a relatively short half-life and clearance time, indicating further formulations are required for these compounds.