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Syntheses and Studies of Multifunctional Inhibitors for Enzymes in Mevalonate Pathway

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Abstract

The mevalonate pathway is an important biosynthetic pathway, which plays a key role in multiple cellular processes for synthesizing cholesterol, prenylated proteins, isopentenyl tRNA, dolichol, hormones, ubiquinone, heme A, and N-glycosylation. These end-products are vital for diverse cellular processes including cell growth and differentiation, signal transduction pathways, and mitochondrial electron transport. Manipulation of this pathway results in alteration of malignant cell growth and survival in cell culture and animal models, with promising potential interesting therapeutic targets for many other areas of ongoing research, including oncology, autoimmune disorders, atherosclerosis, Alzheimer disease, and cardiovascular disease.

The mevalonate pathway has been exploited in the design of cholesterol-lowering drugs by inhibiting the enzyme HMG-CoA reductase with statin drugs as a means of reducing the risk of cardiovascular disease. Statin is not satisfactory in treatment of cardiovascular disease due to significant side effects, such as headaches, nausea, fever, extreme muscle pain, serious liver problems, and even deaths. Therefore, it is important to design and develop other cholesterol-lowering inhibitors targeting other enzymes instead of HMG-CoA reductase for the inhibition of cholesterol biosynthesis. Several enzymes in mevalonate pathway, such as mevalonate kinase, phosphomevalonate kinase, mevalonate 5-diphosphate decarboxylase, farnesyl pyrophosphate synthase, and squalene synthase, have also been suggested as important regulatory enzymes in the biosynthesis of cholesterol and cell growth and differentiation.

The objective of the present study is to design, synthesize, and study multifunctional inhibitors for simultaneous inactivation of more than one enzyme in the mevalonate pathway, which may block effectively the formation of cholesterol and downstream products and regulate cell growth and differentiation for the treatment of heart disease and cancer. In the present study, various types of potential multifunctional inhibitors based on bisphosphonates were designed and synthesized to inhibit mevalonate kinase, phosphomevalonate kinase, mevalonate 5-diphosphate decarboxylase, and farnesyl pyrophosphate synthase.

The results showed that these compounds exhibited potent inhibition on the four enzymes simultaneously and the IC_{50} values are at several nanomole to micromole range. One bisphosphonate compound with geranyl and mevalonate group exhibits potent inhibition for rat mevalonate kinase, phosphomevalonate kinase, mevalonate 5-diphosphate decarboxylase, and farnesyl pyrophosphate synthase with IC_{50} values of 2.7 μM, 4.2 μM, 0.8 μM, and 0.029 μM, respectively. This inhibitor also strongly inactivate the corresponding enzymes from *S. pneumoniae* with low IC_{50} values of 0.9 μM, 6.5μM, 1.1 μM, and 0.015 μM, respectively. The results show that the bisphosphonate moiety and the mevalonate analogs all make important contribution for the inhibition of the four enzymes. It is usually difficult to inhibit efficiently one enzyme completely with a single inhibitory drug, which potentially result in toxic side effects. The inhibition of more than one enzyme in a metabolic pathway using a combined drug treatment might be more effective than inhibition of only one individual enzyme.

To predict the structure and orientation of the multifunctional inhibitors in the binding cavity of these four enzymes, the binding interactions between small molecule inhibitor and enzyme were also investigated by using the computer molecular docking simulation. Molecular docking analysis illustrated that these compounds bind to the active site of

the enzyme in a favorable position via hydrogen bonds, metal ions, hydrophobic and van der Waals interactions with the amino acid residues. These results indicated that the active compounds are likely to be competitive inhibitors. Characterization of enzyme kinetics is in good agreement with the predicted binding mode based on simulation study.

Meanwhile, a convenient colorimetric high throughput assay method was developed for the measurement of FPPS activity and inhibitor screening instead of traditional radioactive assay that is inconvenient, expensive, time-consuming, and inaccurate due to product inhibition. This novel sensitive high-throughput screening assay is based on the amount of free phosphate generated in a reaction. The resulting Pi reacted with molybdate to form a colorless Keggin type phosphomolybdate ($PMo_{12}MoO_{40}^{3-}$) complex, which was then reduced to give a phosphomolybdate-blue ($PMo_{12}MoO_{40}^{7-}$) complex with absorbance at 830 nm. The sensitivity of this assay method was found to be much higher than that of the traditional radioactive assay method. This FPPS assay method can be used in high-throughput screening procedures for rapid screening of large numbers of compounds under similar test conditions.

The bisphosphonates can induce cancer cell apoptosis by inhibiting enzymes in mevalonate pathway, preventing the generation of isoprenoid moieties and thereby impairing the isoprenylation of small GTP proteins. So the bisphosphonate compounds were also evaluated against cancer cell lines using MTT assay methods with high-throughput screening. Some multifunctional inhibitors also inhibited effectively cancer cell growth and induced apoptosis. One bisphosphonate compound with geranyl and mevalonate group showed a significant anti-proliferative activity on Hela cells with IC_{50} of about 20 μM. In addition, Hela cancer cells treated with our fluorescent inhibitors were examined by confocal fluorescence microscopy. Many fluorescent particles appear

in both cytoplasm and nucleus. The particle density is higher in the cytoplasm than that in the nucleus, indicating that the fluorescent inhibitors can get into cell although they are highly charged compounds.

In conclusion, a series of multifunctional inhibitors based on bisphosphonate analogs were designed and synthesized, which show potent inhibition for mevalonate kinase, phosphomevalonate kinase, mevalonate 5-diphosphate decarboxylase, and farnesyl pyrophosphate synthase from both rat and *S. pneumoniae*. An efficient colorimetric high-throughput enzyme assay method for FPPS was successfully established, which provided a convenient screening method for inhibitors of FPPS. The confirmation of multi-targeted inhibition and establishment of SAR analysis with concurrent aid of computational simulation helped to unravel the identified multifunctional compounds as a new class of multifunctional enzyme inhibitors. Cell survival assay with new bisphosphonate analogs demonstrated their potential usage for cancer treatment. Therefore, inactivation of these enzymes involved in mevalonate pathway provides an alternative potentially useful method for treating cardiovascular disease, cancer, and bacteria infection.

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
AD	Alzheimer’s disease
ADP	Adenosine diphosphate
APP	Amyloid precursor protein
bHLH	Basic helix-loop-helix
ApoB-100	Apolipoprotein B-100
CVD	Cardiovascular disease
DMAPP	Dimethylallyl diphosphate
eNOS	endothelial nitric oxide synthase
FPP	Farnesyl pyrophosphate
FPPS	Farnesyl pyrophosphate synthase
FTase	Farnesyl transferase
FTIs	Farnesyl transferase inhibitors
GPP	Geranyl pyrophosphate
GGPP	Geranylgeranyl pyrophosphate
GGTases	Geranylgeranyl transferase
GGTIs	Geranyl geranyltransferase inhibitors
HDL	High-density lipoprotein
HIV-1	Human immunodeficiency virus 1
HMG-COA	3-Hydroxy-3-methylglutaryl-coenzyme
IPP	Isopentenyl diphosphate
IPTG	Isopropyl-beta-D-thiogalactopyranoside
KM	Michaelis-Menten Constant
LBMD	Low bone mineral density

LDL	Low-density lipoprotein
MDD	Mevalonate 5-diphosphate decarboxylase
MVA	Mevalonate, or mevalonic acid
MVAP	Mevalonate 5-phosphate
MVAPP	Mevalonate 5-diphosphate
MVK	Mevalonate kinase
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide
NADH (reduced)	Nicotinamide adenine dinucleotide
N-BPs	Nitrogen-containing bisphosphonates
NMR	Nuclear magnetic resonance
PCR	Polymerase chain reaction
PDB	Protein data bank (http://www.rcsb.org/pdb/)
PKB	Protein kinase B
PMK	Phosphomevalonate kinase
PSQPP	Presqualene diphosphate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SMCs	Smooth muscle cells
SREBPs	Sterol regulatory element binding proteins
Stp	*Streptococcus pneumoniae*
THF	Tetrahydrofuran
THP	Tetrahydropyranoxy group
TG	Triglyceride
TLC	Thin layer chromatography
VLDL	Very low density lipoproteins

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