SYNTHESIS OF CLASS II HMG-COA REDUCTASE INHIBITORS; AS NEW POTENT NOSOCOMIAL ANTIBIOTICS

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INTRODUCTION

Antibiotic resistant pathogenic bacteria such as vancomycin resistant *Enterococcus* (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA) are the most common causes of infections in the U.S. and European hospitals.(1) Current antibiotics used to treat these infections are not specific for the pathogenic bacteria. Furthermore, the antibiotic market is still dominated by antibiotics discovered half a century ago and there is a need for new antibacterial scaffolds.(2) The goal of this project is to design and synthesize a new breed of antibiotics for the selective treatment of resistant Gram positive bacteria. Previous studies report that Gram positive cocci bacteria such as; *Staphylococcus aureus* and *Enterococcus faecalis* use exclusively the mevalonate pathway for the biosynthesis of isopentenyl diphosphate needed to build their bacterial cell wall.(3) In the mevalonate pathway, class II HMG-CoA reductase (II-HMGR) converts HMG-CoA to mevalonate and is one of the main enzymes for bacterial survival.(4) Thus, inhibition of II-HMGR represents a potential antibacterial strategy for nosocomial pathogenic bacteria.

EXPERIMENTAL

A total of 20 ***N*-bsha** (5-(*N*-(4-butylphenyl)sulfamoyl)-2-hydroxybenzoic acid) analogues were synthesized. Enzymatic inhibition of the analogues for *E. faecalis* and *S. aureus* II-HMGR was determined by spectrophotometric assays, monitoring the disappearance of NADPH at 340 nm. The antimicrobial activities of the compounds were initially tested at 100 µM against MRSA and *E. faecalis* using Muller Hinton broth and 96 well-plate. The bacterial cultures were incubated at 37 ^{o}C for 14 and 20 hours, respectively. Appropriate positive and negative controls were used. To determine the minimum inhibitory concentration (MIC) against MRSA and *E. faecalis*, the compounds were incubated with $2x10^5$ bacteria in 96 well-plate at 37 ^{o}C for 18 hours. Concentration range of each compound used was 0.5 µM to 128 µM. An *in-vitro* homogeneous, colorimetric method for determining the number of viable J774A.1 cells (mitochondrial function) using the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega) was used to determine cytotoxicity of the compounds.(5)

RESULTS

Synthesized analogues were obtained in high yields and with 95-100% purity, as determined by HPLC.(6) The synthesized analogues were better II-HMGR inhibitors than ***N*-bsha** and displaced the natural substrate, HMG-CoA.(6) The best ***N*-bsha** analogue inhibitor presented an IC_{50} of 6 µM for *E. faecalis* II-HMGR, in the HMG-CoA to mevalonate direction. Interestingly, 4 ***N*-bsha** analogues showed antibacterial activity against MRSA and *E. faecalis*. The most potent antibacterial ***N*-bsha** analogue showed MIC's of (>32 ≤ 64) µM for MRSA and (>8 ≤ 16) µM for *E. faecalis*. The bioactive analogues resulted not toxic *in vitro* to murine J774A.1 cells, at 50 and 100 µM concentrations. The present study provides a new class of potential antibacterial scaffolds for the selective treatment of MRSA and VRE infections.

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REFERENCES

1. Klevens, R. M., Morrison, M. A., Nadle, J., Petit, S., Gershman, K., Ray, S., Harrison, L. H., Lynfield, R., Dumyati, G., Townes, J. M., Craig, A. S., Zell, E. R., Fosheim, G. E., McDougal, L. K., Carey, R. B., Fridkin, S. K., and Investigators, A. B. M. Jama-Journal of the American Medical Association, 298 (2007).
2. Taubes, G. Science, 321 (2008).
3. Wilding, E. I., Brown, J. R., Bryant, A. P., Chalker, A. F., Holmes, D. J., Ingraham, K. A., Iordanescu, S., Chi, Y. S., Rosenberg, M., and Gwynn, M. N. Journal of Bacteriology, 182 (2000).
4. Wilding, E. I., Kim, D. Y., Bryant, A. P., Gwynn, M. N., Lunsford, R. D., McDevitt, D., Myers, J. E., Rosenberg, M., Sylvester, D., Stauffacher, C. V., and Rodwell, V. W. *Journal of Bacteriology, 182* (2000).
5. Mosmann, T. Journal of Immunological Methods, 65 (1983).
6. Synthesis of Class II HMG-CoA Reductase Inhibitors as New Potent Nosocomial Antibiotics. USA Patent 66199.P1.US