

A study on targeted myristoylated 27 kDa HIV-1 Nef in HEK 293 cells: Cloning and expression

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HIV continues to be a major global public health issue, there were approximately 34 million people living with HIV in 2011. However, the development of anti-viral has blunted the AIDS epidemic in the Western world but globally the epidemic has not been curtailed. Nef is one of these accessory genes that are only present within HIV and SIV genome and thought to play a key role in the progression to AIDS, given its central role in HIV pathogenesis, Nef considered as a potential anti-viral target for preventing or at least delaying pathogenesis. The biologically active 27 kDa myristoylated Nef protein expressed from HEK 293 cells is a protein model to be used for significantly specific antibody production to lower the pathogenicity of HIV infection. To express the this protein model, pQBI-6His a mammalian expression vector constructed with base pair 5504 to express 627 bp HIV-1 Nef under CMV promoter. Cultivated HEK 293 was transfected by 4 ug/ml of successful clone of pQBI-Nef-6H is and stable transfection selected in 0.7 mg/ml of G418 antibiotic. It shows that targeted 27 kDa HIV-1 Nef was not successfully expressed in HEK 293 cells either in transient transfection or stable transfection when transfected. However, non-targeted HIV-1 Nef was detected in western blot by anti-Nef (anti mouse) manufactured by Thermo scientific. It suggesting that, the Nef protein that was detected not identically synthesis through post-translation modification though it was expressed in the cytoplasm of HEK 293 cells. The ability of not expressing the targeted myristoylated 27 kDa nef protein was to various unpreventable factors due to time limitation and lacking of skills in the field cloning and cell culture.

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Novel phthalimide analogs as anti-angiogenic and anti-cancer agents

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Phthalimide moiety is one of thalidomide metabolites and might be the effective part of thalidomide toward many diseases. The mode of action of thalidomide in cancer therapy mainly depends on its immunomodulatory and anti-angiogenic activity, therefore, the current study focused on the efficacy of the newly synthesized phthalimide derivatives as immunostimulatory/immunosuppressive agents against immune cells, and their growth inhibitory effect against various cancer cell lines, as well as their anti-angiogenic activity. A facile synthetic approach of novel phthalimide dithiocarbamate and dithioate analogs 4a-k, 5a-e and 5g-k was achieved by the reaction of N-chloromethyl or N-bromoethylphthalimide with carbon disulfide (CS₂) and different amines. Phthalimide derivatives 5e and 5i exhibited the highest cytotoxic activities against MCF-7 and Hep-G2 cells. Both derivatives 5e and 5i inhibited nitric oxide (NO), tumor necrosis factor- α (TNF- α), vessel endothelial growth factor (VEGF) and its receptor (VEGFR). Derivative 5e showed immunosuppressive activity through its inhibition of immune cell functions and proliferation. Taken together, our study improved that some of the newly synthesized phthalimide derivatives may act as anti-angiogenic and anti-cancer agents.

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