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Immunosuppressant effects of novel imidazolyl leflunomide analogues: *In vitro* determination of IFN-gamma in human lymphocyte culture

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Background and Aims: Rheumatoid arthritis is a chronic systemic inflammatory disorder with synovial cell hyperplasia. leflunomide is a new isoxazolic drug that is used in rheumatoid arthritis. It inhibits pyrimidine synthesis in stimulated T-lymphocytes and is an immunomodulatory drug. In this project the immunosuppressive effect of leflunomide novel analogues (analogues: N-(4-trifluoromethylphenyl)-2-methylthio-1-benzylimidazole-5-carboxamide(6a), N-(4-trifluoromethylphenyl)-2-ethylthio-1-benzylimidazole-5-carboxamide(6b), N-(4-fluorobenzyl)-2-methylthio-1-benzylimidazole-5-carboxamide(7a), N-(4-fluorobenzy-l)-2-methylthio-1-benzylimidazole-5-carboxamide(7b)) was evaluated via in vitro method in which Gamma Interferon (IFN-gamma) in human lymphocyte cell cultures was measured. The results were compared with negative control, 0.5% DMSO. Cyclosporine was used as the positive control.
Methods: In order to achieve this purpose human lymphocyte cells were cultured in a 96-well plate (200 µL) and different concentration of analogues and controls were added to each well. Analogue Concentrations were (100- 50- 10- 5- 1 mM) and cyclosporine concentrations were (10-1- 10-2- 10-4 mM). Ficol was used for separation of lymphocytes. Then the plate was incubated at 37˚C in 5% CO2 for 48 hours. The supernatant's absorbance was measured by ELISA reader in 450 nm and 620 nm as measuring and reference wavelength respectively. IFN- gamma concentration in each well was calculated using standard concentration of it.
Results: All compounds in some concentrations show significant differences compared to the negative control and were weaker than positive control. Compounds 6a, 6b were found to be more active than compounds 7a, 7b.
Conclusions: These analogues have imidazole ring that could be matched with dihydroorotate dehydrogenase (DHODH) enzyme so that these can be a good inhibitor for this enzyme. It seems that replacement of a linker CH2 between trifluoromethylphenyl ring and imidazolecarboxamido moiety, compuonds7, reduces activity significantly. Compuonds 6 have more similarity to the parent compound leflunomide.

Keywords: Leflunomide; Gamma Interferon; ELISA; Imidazolyl analogues