An efficient Erythropoietin (EPO)-expression system in mammalian cells is required for massive production for therapeutic use. Ammonium ion is a major problem in the production of valuable recombinant proteins in cultured animal cells. Therefore, it is of importance to devise a system by which a high productivity of human therapeutic recombinant protein can be maintained or enhanced under low ammonium concentration. To reduce the ammonium ion accumulated in EPO producing Chinese Hamster Ovary (CHO) cells, IBE, we introduced the first two genes of the urea cycle, carbamoyl phosphate synthetase (CPSI) and ornithine transcarbamoylase (OTC), into IBE using a stable transfection method. Transfectants expressing CPSI and OTC, were selected and confirmed by RT-PCR. The CO5 cell line, IBE expressing CPSI and OTC had 12.6%, 21.6%, and 24% higher cell growth and 15%, 26%, and 33% lower ammonia concentrations in the media per cell than the parental cell line, IBE, at the time of the cells reaching a high density, when the media were changed at 1-, 2-, and 3-day intervals, respectively. In addition, CO5 cells showed 2-2.5 times higher productivity of EPO than IBE cells. Comparisons of the glycosylation of EPO purified from both cell lines, IBE and CO5 revealed that EPO produced from CO5 cells contained a more acidic proportion of isoforms with approximately 15% higher sialic acid contents per EPO than that produced from IBE cells in spite of the higher EPO production in CO5 cells. These results suggest that the improvement of higher ammonia removal activity in CHO cells from the introduction of the first two enzymes of the urea cycle led to enhance recombinant human EPO productivity with higher cell viability as well as increased sialylation of EPO due to the reduction of the ammonia concentrations in culture media.

[PD1-1] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Molecular modeling study of indeno[1,2-c]isoquinolines and 3-arylisoquinolines using CoMFA

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• The potent antitumor activities of 3-arylisoquinolines promoted us to explore the structure-activity relationship of these compounds. A series of 3-arylisoquinoline derivatives were evaluated for antitumor cytotoxicity against human lung tumor cell (A 549). For the next stage, we decided to prepare the constrained form of 3-arylisoquinolines as indeno[1,2-c]isoquinolines. As a result, diverse spectrum against human tumor cell lines was obtained. In order to study structure-activity relationship (SAR) of these compounds the comparative molecular field analysis (CoMFA) was carried out. CoMFA has been a useful technique in defining important 3-dimentional (3-D) properties and postulated pharmacophore model. In order to carry out conformational search of these compounds, we used the X-ray crystallographic structure of 7,8-dimethoxy-3-phenyliosquinolin-(2H)-one as well as a grid search. Finally, we could get good Cross-Validated R2 (Q2) values with pharmacophore models. A facile synthesis of indeno[1,2-c]isoquinolines with a 3D-QSAR study will be presented.

[PD1-2] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Formal synthesis of core unit of apicularen A and its synthetic derivatives

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Over the past few years, a variety of macrocyclic salicylate natural products have been isolated from both terrestrial and marine sources based on their ability to induce a particular phenotype in mammalian cells. Extracts of the myxobacterium Chondromyces showed high cytotoxicity against cultivated mammalian cells and bio-guided fractionation revealed the cytotoxicity was due to one main metabolite identified as the novel macrolide apicularen A. Beginning to understand the molecular basis for these distinct activities will require structure-function correlation studies and the development of synthetic chemistry in this area. Apicularen A possesses a structure characterized by a salicylic acid residue, a macrolide ring bridged by an oxygen atom in such a way as to