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

Kang Sung Kyung°, Le Quynh Manh, Cho Won-Jea
College of Pharmacy, Chonnam National University, Kwangju 500-757, South Korea, .

• 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

Lee Min Jung°, Kim Ji Duck, Zee Ok Pyo, Jung Young Hoon, Ahn Jong-Woong
College of pharmacy, Sungkyunkwan university, College of ocean science and technology, Korea Maritime University

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

form a tetrahydropyran system, and a 10-membered ring lactone bearing a side chain with a doubly unsaturated acylenamine moiety. Here we report a formal synthesis of apicularen core unit and its synthetic derivatives. In retrosynthetic analysis, we equated the introduction of an acetate unit to a two-step procedure involving allylation followed by ozonolysis. The reiterations of the allylation-ozonolysis sequence were to be performed in the designated order. These steps not only would "mimic" nature's polyacetate biosynthetic pathway to apicularen A, but also would have the potential of yielding the correct stereochemistry at each chiral center of the target molecule through the judicious choice of appropriate reagents and conditions. After fifth allylation, exposure of intermediate to NaH for 2h followed by addition of water furnished the macrolide lactone. Then terminal homoallyl group was ozonolyzed followed by semicarbazonation and derivatizations. Another derivatizations was carried out as follows, the terminal homoallyl group was homologated by hydroboration-oxidation, then the alcohol obtained was oxidized by TPAP to give the corresponding aldehyde, which was also semicarbazonated and derivatized.

[PD1-3] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Synthesis and COX-2 Inhibitory Properties of Luotonin A Homologues

Park Jae Gyu°, Kim Dong Hyun, Rahaman A. F. M. Motiur, Chang Hyeun Wook, Lee Eung Seok, Jahng Yurngdong
Yeungnam University

Luotonin A was isolated from Peganum nigellastrum Bunge (Zygophyllaceae) which was named Luo-Tuo-Hao in China and used as a Chinese traditional medicine for the treatment of rheumatism, abscess, and inflammation. The basic fractions of P. nigellastrum showed antitumor activtity, and the origin of such an activity was recently revealed by identifying its constituent luotonin A which inhibited the growth of leukemia P-388 cells (IC_{50} = 1.8 μg/mL). Such an intriguing properties of luotonin A led developments of efficient methods for total synthesis. Although Luo-Tuo-Hao has been used more likely for the treatment of inflammation-related symptoms, no efforts towards to explore antinflammatory activity of P. nigellastrum itself as well as its components have been pursued as yet. Our continuing interests in the conformational effects on biological activity as well as search for anti-inflammatory agents spurred us to design a series of lutonin A related compounds in which the dihedral angles between planar 4(3H)-quinazolinone and quinoline rings could be controlled in a regular fashion by a methylene bridge connecting N3 of 4(3H)-quinazolinone and C2 of quinoline. We herein described the synthesis and properties of homologous series of luotonin A.

[PD1-4] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Protective effects of synthetic of 3-Alkoxy-6-allylthiopyridazine against aflatoxin B_1-induced hepatotoxicity

Shin Hea-Soon°, Kang Joo-Yeon, Park Myung-Sook, Kwon Soon-Kyoung
College of Pharmacy, Duksung Women's University

3-Alkoxy-6-allylthiopyridazine derivatives showed the strongest protective effect against oxidative stress and their anticancer effect determined on the growth of SK-Hep-1 hepatocellular carcinomar cells. The allythio group as a pharmacologically active group was introduced into pyridazine nucleus and a substituent such as halogen or alkoxy was also introduced into paraposition of allylthio group. Five kinds of 3-alkoxy-6-allylthiopyridazine derivatives were synthesized and their chemoprotective activities examined in rats exposed to aflatoxin B_1-toxicant. Rats were pretreated with five 3-alkoxy-6-allylthiopyridazine derivatives at daily oral doses of 50 mg/kg for 10consecutive days, and during this period with one or three repeated doses of the potent hepatotoxin, aflatoxin B_1. The hepatoprotective effects of the 3-alkoxy-6-allylthiopyridazine derivatives against aflatoxin B_1 administration were showed the significantly normal as compared with control in body and liver weights. Aspartate aminotransferase and alanine aminotransferase levels were markedly elevated after aflatoxin B_1 administration, and pretreatment with 3-alkoxy-6-allylthiopyridazine derivatives, before aflatoxin B_1 administration, resulted in decreased levels of these enzymes. In addition, the 3-alkoxy-6-allylthiopyridazine derivatives,