Poster Presentation – 01

Studies on the Synthesis of Some Novel Benzamide Compounds As HDAC Inhibitor

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Introduction and Aim: Cancer is a multistage disease consisting of genetic and epigenetic factors. One of the most important epigenetic rearrangements, histone acetylation is catalyzed by histone acetyltransferase (HAT) and histone deacetylase (HDAC), which function in opposition to each other. Studies have shown that HDAC inhibitors significantly control tumor incidence and metastasis. In this study, benzamide compounds were synthesized considering their HDAC inhibitory properties.

Materials and Methods: Aminoalkylcarboxylic acid was synthesized by adding 1,1'-CDI, DBU, TEA to arylmethanol solution. The solution was acidified with HCl (pH=5) to precipitate a white solid which was collected by filtration, and purified by column chromatography to give aminoalkylcarboxylic acids. Substituted benzamide compounds were synthesized by reacting carboxylic acids with DMF, oxalyl chloride, imidazole and o-substituted phenylamine and then purified by column chromatography.

Results: The structure of the synthesized compounds was elucidated by elementary analysis, ^{1}H NMR, ^{13}C NMR and mass spectral data. All spectral data were in accordance with assumed structures.

Conclusion: The structures of the synthesized compounds have been elucidated; activity studies are continued.

Keywords: anticancer, benzamide, epigenetic, HDAC inhibitors,

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Poster Presentation – 02

High-passage LNCaP cells show castration resistance

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Introduction and Aim: Androgen deprivation is the primary treatment for prostate cancer (PCa). However, patients eventually develop castration resistance (CR), and become unresponsive to the treatment after a few years of medication. Therefore, deciphering the mechanisms of castration resistance and development of cell culture protocols for CR-PCa cells are of high importance. Here, we aimed to show the CR potential of LNCaP cells with high passage numbers ($LNCaP_{HP}$).

Materials and Methods: LNCaP cells were first cultured in RPMI1640 medium supplemented with 10% FBS and 1%pen/strep at 37°C and 5% CO_2. In order to obtain CR, LNCaP cells were passaged once a week until P67 in RPMI1640 medium w/o phenol red, supplemented with Charcoal Stripped Fetal Bovine Serum (CSS) and 1%pen/strep. Cell viability was determined using CVDK8 kit. PSA levels of high and low passage cells were compared using ELISA kit. Viability was analyzed in CSS-treated and untreated cells upon R1881 application. Expression levels of AR and CR-associated AKR1C3 and AR-V7 genes were determined via qRT-PCR of total RNA samples from P28, P67 and CSS-applied $LNCaP_{HP}$ cells.

Results: $LNCaP_{HP}$ cells showed decreased expression of AR and increased expression of ARV7 and AKR1C3 genes. All the gene expressions were increased in CSS-applied $LNCaP_{HP}$ cells. Although PSA levels were undetectable in $LNCaP_{LP}$ cells, the levels were consistent with CR in $LNCaP_{HP}$.

Conclusions: Our results imply that $LNCaP_{HP}$ cells show increased expressions in CR-related genes and PSA levels, therefore, can be used in in vitro assays to decipher the mechanisms of CR.

Keywords: Castration resistance, prostate cancer, LNCaP, gene expression, androgen receptor

Poster Presentation – 03

TNF-α-mediated effects significantly contribute to TGF-β1-induced epithelial mesenchymal transition in prostate cancer metastasis

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Introduction and Aim: TGF-β1 plays a major role on epithelial mesenchymal transition (EMT). Many factors in the tumor microenvironment contribute to EMT leading metastasis and inflammation-induced alterations are known to involve both in carcinogenesis and metastasis. NKX3.1 is a prostate epithelium-specific marker and a tumor suppressor protein, which has previously shown to undergo TNF-α-induced proteasomal degradation in inflammatory microenvironment. Recent studied revealed its regulatory role in maintenance of stemness raising new questions on the role of NKX3.1 in transition to mesenchymal phenotype. Therefore, we aimed to investigate NKX3.1-related alterations during both TNF-α- and TGF-β1-mediated cellular events in EMT including expressional changes of the EMT markers and viability and anoikis resistance of prostate cancer cells.

Materials and Methods: LNCaP cells cultured with conditioned media (CM) derived from macrophages for 8 days in order to mimic the chronic inflammatory tumor microenvironment of the prostate. Various concentrations of the mentioned cytokines were used in combination in order to distinguish the EMT-related cellular effects of TNF-α and TGF-β1. Cellular alterations such as expressional changes of EMT markers, cell viability and anoikis resistance were investigated.

Results: Overexpression of NKX3.1 resulted in significant alterations of EMT markers. Increase in fibronectin expression observed in both TNF-α- and TGF-β1-mediated inflammatory conditions was reverted by NKX3.1. Increased protein levels of Vimentin, Twist-1, Snail-1, Snail-2, and Zeb-1 in cytokine induction was detected to enhanced by NKX3.1 ectopic expression suggesting that NKX3.1 expression in inflammatory microenvironment results in induction of mesenchymal markers.

Conclusion: NKX3.1 contributes to positive regulation of mesenchymal phenotype.

Keywords: prostate cancer, stemness, epithelial mesenchymal transition

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Poster Presentation – 04

Studies on the Synthesis of Some Novel Imidazopyridine Compounds as HDAC Inhibitors

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Introduction and Aim: Epigenetic mechanisms control a variety of aspects of cancer biology, including primary tumor development and invasion by histone modification. Gene expression is regulated by the balance between histone acetyltransferase (HAT) and histone deacetylase (HDAC). HDAC inhibitors diminish angiogenesis, alter immunological response, and cause differentiation and cell death in cancer cells therefore they are considered as promising antineoplastic agents. In this study, imidazopyridine compounds were designed and synthesized considering their HDAC inhibitory properties.

Materials and Methods: Designed products were obtained by adding 1,1'-CDI, **o-substituted phenylamine and trifluoroacetic acid onto imidazopyridine** methanol solution in THF **and purified by column chromatography.**

Results: The structure of the synthesized compounds was elucidated by ^{1}H NMR, ^{13}C NMR and mass spectral data. All spectral data were in accordance with assumed structures.

Conclusion: The structures of the synthesized compounds have been elucidated; activity studies are ongoing.

Keywords: epigenetic, histon modification, HDAC inhibitors

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Poster Presentation – 05

3D Prostate Cancer Model to Represent Ephitelial Mesenchymal Transition

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Introduction and Aim: Use of 3D cell culture model instead of the conventional 2D has been shown to have many advantages such as better representing the in vivo cellular interactions and drug responses in a time and cost saving way enabling more reliable investigation of the molecular mechanisms of cancer and development of new therapeutics. Therefore, our study aims to develop a 3D cell culture model to represent the epithelial-mesenchymal transition (EMT) of the prostate cells induced by the inflammatory tumour microenvironment.

Materials and Methods: Prostate spheroids were formed using poly-HEMA coated U-bottom 96-well plates. Spheroid diameter was followed under microscope for 7 days in order to ensure the spheroid stability and viability experiments were performed for 2D and 3D cell culture models under inflammatory conditions. Alterations in inflammation-mediated EMT factors were studied by western blotting.

Results: EMT markers in spheroids were observed to change distinctly in comparison to 2D-cultured cells upon inflammatory conditions based on the results showing expressional changes in NKX3.1, fibronectin, E-cadherin, vimentin, Snail-1 and Twist-1. Although protein levels of NKX3.1, fibronectin, and Snail-1 differed in 2D- and 3D-cultured cells, expressional changes upon inflammation remained in the same way. However, E-cadherin, vimentin, and Twist-1 were shown to be enhanced in 3D spheroids where they suppressed in 2D cells suggesting that 3D spheroids are necessary to mimic inflammation-induced EMT of prostate cells.

Conclusion: We optimized a 3D EMT model of prostate cells, which can serve as a useful tool for prostate cancer research and anticancer drug development studies.

Keywords: Prostate spheroids, epithelial mesenchymal transition, 3D cell culture, prostate cancer

This study was supported by Scientific and Technological Research Council of Turkey (222S863) and Ege University (27410).

Poster Presentation – 06

Investigation of the Protective Effect of Resveratrol on Some Enzyme Activities on Toluene Toxicity in Rats

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Introduction and Aim: Toluene is hydrocarbon and has toxic effects. Toxicity from exposure to toluene and the carcinogenicity of are a concern. About 80% of which is metabolized in the liver, triggers the production of ROS by creating oxidative stress in the body and leads to the activation of the apoptotic signaling mechanism. Against this toxic effect, in addition to the antioxidants produced by our body, the antioxidants we consume with food also provide a protective effect. Resveratrol, is a well-known antioxidant and anticancer phytochemical used in cancer research. The protective and anticancer effects of resveratrol on some biochemical parameters in brain tissues of rats were evaluated.

Materials and Methods: 36 male Wistar-albino rats were used. They were divided into 2 groups as control and experimental groups. The control groups were divided into two groups, namely physiological saline and ethanol. The experimental group, on the other hand, was grouped into 4 groups according to toluene and toluene+resveratrol administration as 5 mg/kg, 10 mg/kg and 20 mg/kg resveratrol doses. Brain tissues were prepared for analysis by homogenization, and biochemical parameters were analyzed spectrophotometrically in an autoanalyzer by enzyme kinetics.

Results: AST and ALT enzyme levels, which are increased in liver and muscle damage and metabolic disorders, as well as in tissues such as brain, kidney and heart, showed a significant increase compared to ALP and GGT enzyme levels. Dosage-dependent resveratrol administration showed that a 20 mg/kg dose was effective.

Conclusion: Aminotransferase elevation in brain tissues may be related to cancer and volatile substance use.

Keywords: Toluene, Carcinogen, Apoptosis, Resveratrol

Poster Presentation – 07

Anti-proliferative Effect of Dacomitinib and Retinoic Acid on Triple Negative Breast Cancer

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Introduction and Aim: Breast cancer is the most common diagnosed cancer with the leading cause of cancer-related deaths in women worldwide. Almost 15% of all breast cancer is diagnosed as triple negative breast cancer (TNBC). TNBC shows poor prognosis and high side effects of chemotherapy is seen frequently. Thus, combination therapy is generally preferred to overcome toxicity and drug resistance. Dacomitinib is an irreversible pan-HER inhibitor. Combination strategies of EGFR inhibitors show promise to overcome intrinsic resistance to these inhibitors. All-trans retinoic acid (ATRA), active metabolite of vitamin A, is a promising agent for treating breast cancer. Its anti-tumor effect is predominantly due to an inhibitory effect on growth of tumor. Therefore, in our study we investigated the anti-proliferative effects of combination therapy of Dacomitinib and ATRA on MDAMB231 cells.

Materials and Methods: The cytotoxic effects of Dacomitinib and ATRA on MDA-MB-231 cells were determined using the MTS assay. Colony formation and scratch assay was performed to investigate the clonogenic potential and migration ability of MDA-MB-231 cells, respectively.

Results: Effective combination treatment dosage was found to be 1.5 µM of Dacomitinib and 5 µM of ATRA on MDA-MB-231 cells at 48h. Combination treatment of Dacomitinib and ATRA on MDA-MB-231 cells significantly reduced the migration and colony formation compared to control group.

Conclusion: Our study showed that combination therapy of Dacomitinib and ATRA has high anti-proliferative potential in MDA-MB-231 cells. These findings suggest that this combination therapy holds promise as anticancer agent and to be used in breast cancer therapy.

Keywords: triple negative breast cancer, Dacomitinib**,** all-trans retinoic acid.

Poster Presentation – 08

Evaluation of the Antiproliferative Effect of Safranal in C-4 I Cervical Cancer Cell Line

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Introduction and Aim: Safranal is a monoterpene aldehyde responsible for the aroma of Crocus sativus. Many studies have shown the antioxidant activity of safranal besides some pharmacological properties, including its anti-inflammatory effect. This study aimed to determine the cytotoxic effects of safranal on C-4 I, cervical cancer cell line.

Materials and Methods: To determine the cytotoxic effect of safranal on the C-4 I cell line, cells were incubated for certain times (2-72 hours) and concentrations (25-800µM). After incubation, the viability of cells and the anti-proliferation effect of safranal were determined respectively by MTT and LDH assays. In addition, Morphological changes occurring during incubation in cells were observed under inverted and light microscopes using Giemsa staining.

Results: According to the results, compared to Control group, the % viability of treated cells was decreased depending on concentration and the incubation time, and safranal significantly inhibited the growth of C-4 I cells ($p<0.05$). Some morphological changes such as nuclear condensation, and apoptotic and pyknotic cells were examined under light microscopy with Giemsa staining.

Conclusion: Based on the results obtained from this study, we can be said that safranal has an antiproliferative effect against cervical cancer-derived C-4 I cell lines.

Keywords: C-4 I, Safranal, Cervical cancer, Cytotoxicity, Antiproliferative