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Activity Report

IFCC Professional Scientific Exchange Programme (PSEP)

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IFCC Professional Scientific Exchange Programme (PSEP)
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First of all I would like to express my deepest gratitude to the IFCC Professional Scientific Exchange Programme (PSEP) for having given me the opportunity to take part in this experience. I was very proud and excited when I received that my application was accepted to attend in the programme in Research in Sustainable Chemistry Center (CIQS) of the National Autonomous of Mexico University (UNAM) and Autonomous of State of México University (UAEMEX).

It has been an important experience to my professional curriculum; I obtained answers to many long-standing questions in Clinical chemistry. I evaluated antimitotic properties of the molecules obtained by organic synthesis in cell lines models and analyze the intracellular signaling mechanism. It also offered me the possibility to meet colleagues from many countries and to discuss with them different subjects in Clinical chemistry and share our respective experience in research.

The CIQS of the UNAM and CICMED of the UAEMEX are institutions engaged in the various processes that require interaction across sectors and sustainable set of institutions involved in the development of drug treatments, insurance, scientific and research potential in Latin America and continue the development of methodologies and organic molecules that represent a solution to the current problems of resistance and decrease in the effectiveness of conventional treatments.

The results of this project are going to contribute to others research and to establishing measures in Health Public. The IFCC's support has been essential in the acquisition of new knowledge and learning certainly.

Best regards,
Cristian Layton
IFCC Professional Scientific Exchange Programme (PSEP) Colombia 2012
National College of Bacteriology (CNB- Colombia)

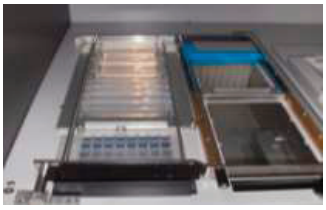
Chronological and Thematic

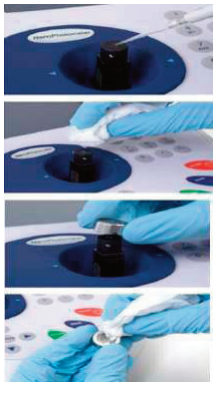
Content

Activities carried out between September and December 2012 in the Laboratory of Molecular Biology Research Center of Medical Science and Research Centre in Sustainable Chemistry, development of advanced research projects, in which I have been supporting and receiving training.

Genotyping of Human Papillomavirus

Performing for nucleic acid extraction method LINEAR ARRAY HPV Genotyping Test in specimens suspected of HPV. Was assembled from samples including positive and negative controls, this method are arranged in columns on a support associated with pump and pressure by addition of absolute ethanol and substances based Buffer concentration gradients and filters genetic material obtained after lysis with proteinase K. Cell samples were quantified by obtaining half of Nanospectrophotometry concentration of 25 ug / ul and purity of 2.5. They are stored at 2-8 ° C for subsequent manipulation by PCR.
Specimens suspected of HPV. Master Mix is prepared by adding 125 ul of magnesium to the MM vial ROCHE Kit. They have 50 ul of the mixture in each reaction tube and 50 ul of sample (obtained by method 29 March), 9700 ROCHE programming thermocycler according to manufacturer's instructions, genotyping by “LINEAR ARRAY HPV Genotyping Test.”





Spectrophotometric Quantification of Nano- and Standard-Volume Samples

The spectrophotometric analysis of nucleic acids, proteins, and bacterial cell cultures is part of the daily routine of the laboratory.
Cell 12 samples were quantified by obtaining half by Nanospectrophotometry concentration of 25 ug / ul and purity of 2.5. They are stored at 2-8 ° C for subsequent manipulation by PCR.
Quantifying Nanospectrophotometry of 20 samples of RNA extracted from the umbilical cord and placenta in the project that evaluates oxidative stress in pregnancy, with a mean concentration of 15 ug / ul and purity of 2.5.

Real Time PCR

Installation of real-time PCR technique; Capillaries were arranged with the working mixture and analyzing the samples to stand in the LightCycler thermal cycler is programmed according to ROCHE

and standardization of the technique. Assembly was performed to measure the expression of other genes analyzed.
Installation of real-time PCR technique to quantifying the expression of genes associated with pesticide resistance in plants isolated from the Toluca . This type of PCR was performed on the Roche LightCycler thermocycler.
File review and development of database in SPSS 17.0 for descriptive analysis relating the level of cardiovascular risk, body mass index and HPV infection.
To determine the PRRSV virus that affects pigs, as an integral part of the public service provided by the CICMED. Participate as an observer in the standardization of the technique for extraction of RNA in serum samples from pigs.
8 samples were quantified by obtaining half of Nanospectrophotometry concentration and purity acceptable .. They are stored at 2-8 ° C for subsequent manipulation by PCR.
Database in SPSS 17.0 for descriptive analysis, statistical analysis nonparametric tests.
Realize explanation of the program to be used to determine the PRRSV virus that affects pigs, as an integral part of the public service provided by the CICMED and bring forward the Veterinary Medical Heydeck Sandra Lopez. Participate as an observer in the standardization of the technique for extraction of RNA in serum samples from pigs.
8 samples were quantified by obtaining half of Nanoespectrofotometría concentration and purity acceptable .. They are stored at 2-8 ° C for subsequent manipulation by PCR.
Database in SPSS 17.0 for descriptive analysis relating the level of cardiovascular risk, body mass index and HPV infection. Statistical analysis nonparametric tests.

“Advanced training in technical evaluation of molecules with antimitotic properties obtained by organic synthesis in cell culture models”

ABSTRACT

TETRAHIDROFURANOSIL-1, 2, 3-TRIAZOLES are important organic compounds with different applications in pharmacological and industrial sector (1). "Click Chemistry" was developed in parallel with the interest within the pharmaceutical, materials, and other industries in capabilities for generating large libraries of compounds for screening in research (2). "Click Chemistry" has been associated with thermodynamically-favored reactions and cycloaddition reactions. 1, 2, 3-TRIAZOLES capacity has been employed in compounds synthesis with anti HIV activity and antimicrobial affect. (3) The clinical application of this compounds include anti-fungal activity, it can contribute to the actual resistances levels to antifungal agents. Actually, fungal infection has been got high resistance levels; it has reduced the life expectancy in immunocompromised patients.

Key Word: Click Chemistry, Triazoles, antimitotic effect.

INTRODUCTION

"Click Chemistry" is a modular construct to add molecules to different supports and use its pharmacokinetic in medicinal

chemistry. (4) Is a subarea of synthetic chemistry optimized small materials and simple reactions, (5) "Click" has proven to be a powerful tool in the preparation of "building blocks" and are ideals in cycloaddition reactions involving heteroatoms. (6, 7) The process characteristics include simple reaction conditions; oxygen, water, materials, reagents and simple product isolation. The purification process may by nonchromatographic methods; crystallization (process of formation of solid crystals precipitating from a solution) or distillation (method of separating mixtures based on differences in volatilities of components in a boiling liquid mixture). (8) The actual cycloaddition step is reliable for different types of reactions. The azide group is by far the more convenient of the 1, 3-dipolar components and ease of introduction and reduction to primary amino group. (8)

Invasive fungal infections in immunocompromised patients have increased in recent years (10). There are various variables than increase the infection possibility; fundamentally affected with AIDS, patients undergoing cancer chemotherapy and treated with steroids. (11) The Fungal resistance emerging intrinsic and antifungal classics resistance in vitro is an important factor in the HIV-infected patients with Candida albicans. (12) This is greatly the widespread use of fluconazole in the treatment and Yeast infection prevention has increased the detection of these with decreased susceptibility to fluconazole (13). The new antifungals, amongst the Triazoles group (2) maybe an improvement of the clinical efficacy and a reduction in parallel toxicity.
Azides and alkynes are essentially inert to most organic and biological conditions, including highly functionalized biological molecules, molecular oxygen, water and most common reaction conditions in organic synthesis.(2) The kinetics stability of alkynes and azides is directly responsible for its cycloaddition process is slow; but has been demonstrated that the use of salts of copper (I) catalyzes this class of reactions can reduce the reaction times obtained exclusively substituted regioisomers at positions 1.4
Additionally, the azide-alkyne cycloaddition catalyzed by copper (Cu-AAC) is a simple method for the synthesis of 1, 2, 3-triazoles regioselective 1, 4-disubstituted; this process allows the formation of a thermally and hydrolytically stable “Chemical link” between two different molecules. The study of azide-alkyne cycloaddition catalyzed by copper has pharmacological properties as potential antiviral agents, which is an important expectation in the search for applications for this type of compounds (2).

MATERIALS AND METHODS

Molecules synthesis
The four triazoles tested, were provided under “Click Chemistry”

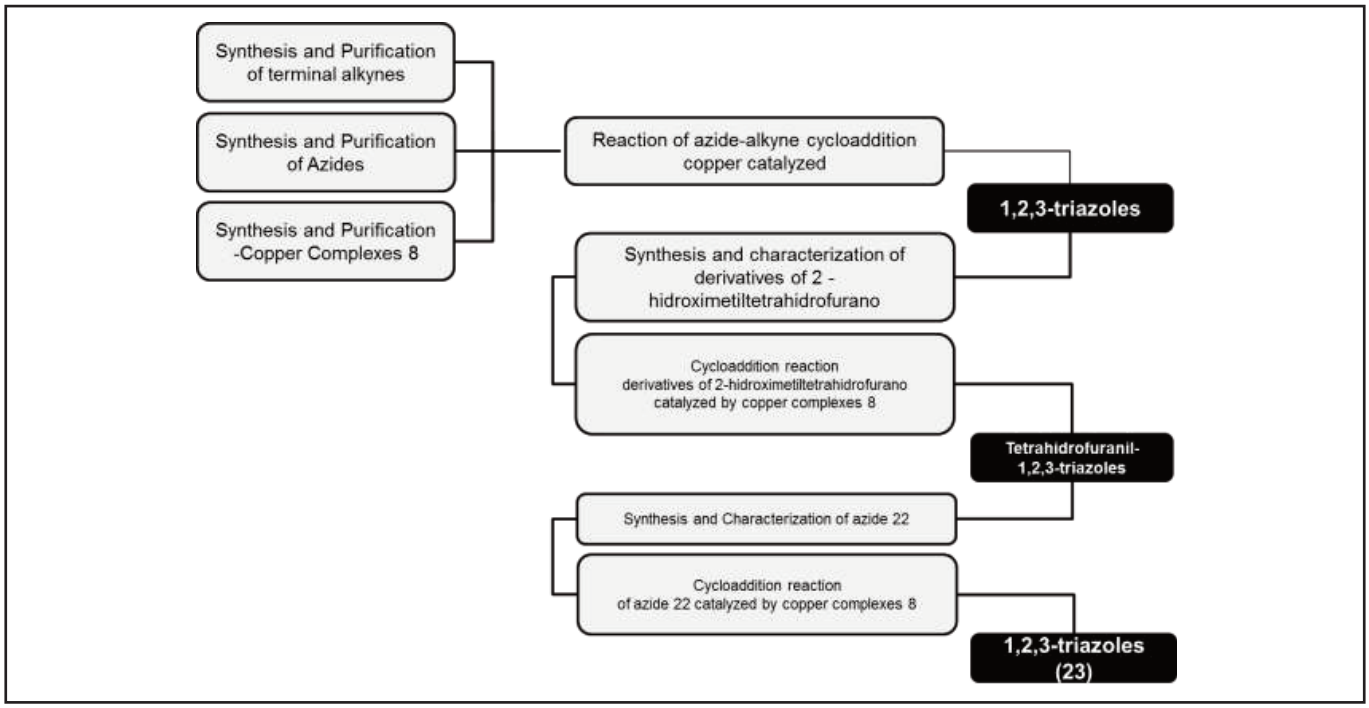


Figure 1
Model of TETRAHIDROFURANOSIL-1, 2, 3-TRIAZOLES synthesis. (Modified) (2)

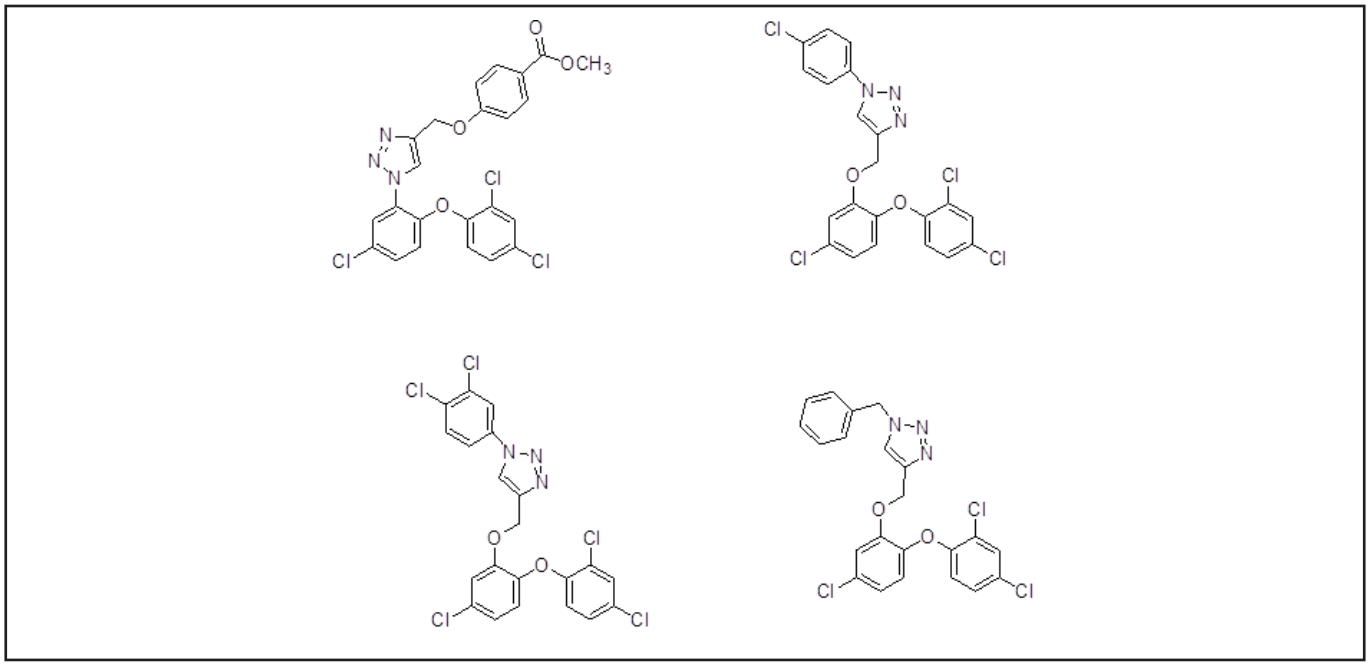


Figure 2
TETRAHIDROFURANOSIL-1, 2, 3-TRIAZOLES synthetized by "clic" chemistry by reactivity of azides and alkynes front various copper salts and complexes of copper (I). A. B.C.D. (2)

Method for yeast microdilution

TETRAHIDROFURANOSIL-1, 2, 3-TRIAZOLES synthetized by "clic" chemistry were probed in RPMI agar with six different fungi species; Candida albicans, Candida glabratta, Candida parapsilopsis, Rhodotorula.

1. Prepare a solution, weighing the powder sufficient for obtain a concentration at least 100 times the highest concentration of antifungal tested (14).
2. Fluconazole: A solution of a concentration of 1280 g /ml. The diluent employed was water sterile distilled. For preparation of fluconazole dilutions we followed the method of double serial dilutions additive. The preparing dilutions differs according to whether soluble or insoluble antifungal in water (15). Dilution of fluconazole (soluble in water) was prepared at a dilution series concentration 10 times higher than the desired final concentration, using as diluent RPMI 1640.

1. Subsequently a 1/5 dilution added to all tubes 4 ml of RPMI, so that the concentration of antifungal vas twice the desired final concentration (128 µg/ml to 0.25 µg/ml). The final concentrations obtained in the plate after inoculation was between 64 and 0.12 µg/ml.
2. Dilution of Triazoles (insoluble in water): From the stock solution was prepared at a dilution series concentration 100 times the desired final concentration, using as DMSO diluent. In the each tube were transferred to another tube with RPMI (4.9 ml), whereupon the concentration of antifungal obtained is twice the desired final concentration (32 µg/ml – 0.06 µg/ml) and DMSO, 2%.
3. Filling plates: From each tube filling proceeded to sterile microtiter plates of 96 wells with 100 µl of solution, fluconazole or Triazoles; The tube contents were taken two 100 µl per well and successively filled the wells in column No. 2 (2A - 2H). The contents of the tube No. 3 wells were filled column No. 3 (3A - 3H). The contents of the tube were filled in No. 4 wells of column No. 4 (4A - 4H), and so on to column No. 11, column wells were filled in No. 12 in the case of the fluconazole dilution with 100 µl of RPMI (growth control) and dilution of Triazoles with 100 µl. of the dilution resulting from 4.9 ml of RPMI and 100 µl of DMSO (2% DMSO). The well in column No. 1 was filled with 200 µl of RPMI (Sterility control). Once filled plates were closed and frozen at -70 ° C until use.
4. Preparation of inoculum: before preparing the inoculum sowed among Saboraud dextrose agar (SDA) for 24 hours at 30 ° C. In the case of Candida spp strains used in our study, the inoculum prepared as follows: a sterile loop of culture were taken about five colonies ≥1 mm and 24 hours of growth on SDA plate, were suspended in a tube with sterile distilled water. Stirring well and adjusted to an optical density 0.5 McFarland, adding the required amount of sterile distilled water; this was performed with the aid of a spectrophotometer (wavelength 530 nm).
5. Inoculation of plates: were taken from the freezer and left at room temperature until completely thawed.
6. Purity control culture: Incubation of the plates: The plates were incubated at 35 ° C for 48 hours. Previously wrapped in tinfoil (16).

7. Reading and interpreting the results. Visual readout was performed using an inverted mirror. For azoles, such as fluconazole and triazoles, the MIC is defined as the lowest antifungal concentration that produces a ≥ 50% reduction of yeast growth as compared with the free control growth of drug. (17, 18, 19).

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Complement activities: Congress and Conferences

- 10th International Congress on Adolescent Health, University of the State of Mexico, SIEA, CICMED, Deusto University, National Institute of Psychiatry and School of Behavioral Sciences, Toluca, Mexico, November 5,6 and 7, 2012
- 1st Symposium Student and Clinical Laboratory Professionals Mexico State, University of the State of Mexico, Professionals Association of Clinical Laboratory of the State of Mexico, AC and the National Federation of Clinical Chemists, CONAQUIC, AC, Toluca, Mexico, November 11, 2012.
- 2nd Contest of Biochemistry Research, State Autonomous University of Mexico, School of Medicine, Toluca, Mexico, November 16, 2012.
- Keynote - Cellular Reprogramming: induced pluripotent stem cells (iPS) Introducing LXI work with current issues in the clinical laboratory, Professionals Association of Clinical Laboratory of the State of Mexico, Mexico, October 25, 2012

Support activities carried out under the project:

"SOCS-3, JAK-2/STAT3, leptin and adiponectin detection in breast cancer "#
Activities of search and literature review; Reviewing inserts for determination of leptin and adiponectin through kits: "Human Adiponectin ELISA" and "96 Test ELISA Human Leptin" commercial household Genway blood sample project attached to breast cancer.
Laboratory of Human Genetics, Faculty of Medicine UAEM, Maternal Perinatal Hospital Pretelini Monica, Deputy Secretary of Health, Breast Cancer, CICMED Molecular Biology Laboratory, Department of Pathology (HMPMP).

Technical skills and methodological: Blood sampling, Information management, patient reception and treatment, Transport and storage of biopsy samples, Cell culture and in suspension by explants.

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