16th International Online Mini-Symposium of The Protein Society of Thailand

17-18 November 2021

09:00– 16:30
via CISCO Webex

ABSTRACTS

pstsymposium2021@gmail.com
https://science.mahidol.ac.th/pst2021
Mahidol Science
The Protein Society of Thailand

ABSTRACTS AND PROCEEDINGS

16th International Online Mini-Symposium of the Protein Society of Thailand

November 17-18, 2021

Hosted by

Faculty of Science, Mahidol University

Via

Zoom Meeting

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RATIONALE AND REMARK

Since its foundation in 2006, Protein Society of Thailand (PST) has arranged annual symposia for protein researchers and students to exchange their knowledge, experience, and expertise. This year, due to the current COVID-19 pandemic situation, Faculty of Science, Mahidol University collaborates with the Protein Society of Thailand to organize the 16th annual symposium as a free virtual conference titled "16th International Online Mini-Symposium of the Protein Society of Thailand." This symposium demonstrates that advanced research can still be carried on albeit difficulties the pandemic has brought about to all researchers. Most of the invited speakers are rising young scientists conducting frontier research on protein and/or related fields. Participants also have opportunities to present and exchange their research through poster and/or oral presentation. Option to publish a proceeding is also available. The manuscripts of which are as critically reviewed with the same quality standard as any of the preceding PST conferences in previous years.

On behalf of the Organizing Committee, I would also like to thank companies that provide support for this symposium. As always, this kind interaction between academia and industry has been an important hallmark of the PST symposium.

Lastly, I hope that all participants can enjoy and benefit from scientific program and interactions through this virtual conference.

Associate Professor Kittisak Yokthongwattana, Ph.D.
Chair of the Organizing Committee
16th International Online Mini-Symposium of the Protein Society of Thailand

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SCIENTIFIC PROGRAM

16th International Online Mini-Symposium of the Protein Society of Thailand

Wednesday November 17, 2021

09:00 – 09:15	Opening Session
09:15 – 09:45	**Invited Lecture 1 – Dr. Erik Procko** Engineered decoy receptors as potent neutralizers of SARS-CoV-2 variants and therapeutic candidates for the treatment of COVID
09:45 – 10:15	**Invited Lecture 2 – Dr. Kittikhun Wangkanont** Inhibitor discovery and a novel inhibitor binding assay for SARS-CoV-2 main protease
10:15 – 10:45	**Invited Lecture 3 – Dr. Bunyarit Meksiriporn** An engineered survival selection strategy for synthetic binding proteins against difficult-to-drug targets
10:45 – 11:00	Break
11:00 – 11:30	**Invited Lecture 4 – Dr. Sakonwan Kuhaudomlarp** Development of inhibitors targeting pathogenic sugar binding proteins
11:30 – 12:00	**Bruker Academic Talk – Dr. Sri Ramarathinam** Immunopeptidomic analysis of SARS-CoV2 infected lung epithelial cells reveals new targets for antiviral immunity
12:00 – 13:00	Lunch Break
13:00 – 14:00	**PST Annual Meeting / Poster Session**
14:00 – 15:00	**Poster Session** (continued)
15:00 – 15:30	**Invited Lecture 5 – Dr. Patompon Wongtrakoongate** Science and translation toward prevention and treatment of COVID-19
15:30 – 16:00	**Invited Lecture 6 – Dr. Somchai Chutipongtanate** Breast Milk, BigMAC and Crab Cracker: My research on therapeutic peptides

Thursday November 18, 2021

09:00 – 09:30	**Invited Lecture 7 – Dr. Waranyoo Phoolcharoen** Towards clinical trial phase I of plant-based COVID-19 vaccine in Thailand
09:30 – 10:00	**Invited Lecture 8 – Dr. Vimvara Vacharathit** Antibody and cytokine responses in COVID-19 patients and vaccines
10:00 – 10:30	**Invited Lecture 9 – Dr. Waradon Sungnak** Cellular immune response to COVID-19 deciphered by single-cell multi-omics
10:30 – 10:50	Break
10:50 – 11:10	**Oral Presentation 1 – Ms. Wichuda Phothichaisri** Host-derived cell-wall-binding domain of phage endolysin CD16/50L anchors to the surface polysaccharide of *Clostridioides difficile* to preserve neighboring host cells and ensure progeny expansion
11:10 – 11:30	**Oral Presentation 2 – Ms. Kankamol Kerdkumthong** Proteomic analysis of 5-Fluorouracil resistant cholangiocarcinoma cell line
13:30 – 13:00	Lunch Break
13:00 – 13:30	**Waters Academic Talk – Dr. Dhaval Patel** How LC-MS can support our fight against Covid-19
13:30 – 15:00	**Poster Session** (continued)
15:00 – 15:15	Break
15:15	**Closing Session**

Invited Lectures

Company Academic Talks

Oral Presentations

IL-01

Engineered decoy receptors as potent neutralizers of SARS-CoV-2 variants and therapeutic candidates for the treatment of COVID

Erik Procko

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ABSTRACT

Soluble receptors can act as decoys to neutralize virus infections with reduced risk for the emergence of resistance. However, in the case of SARS-CoV-2, the ACE2 receptor lacks tight affinity and potency. Using deep mutagenesis, decoys are engineered for SARS-CoV-2 that resolve these challenges and are demonstrated to be safe and effective in animal infection models. The decoys broadly bind virus variants with tight affinity and limited potential for escape.

IL-02

Inhibitor discovery and a novel inhibitor binding assay for SARS-CoV-2 main protease

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ABSTRACT

The main protease of SARS-CoV-2 is responsible for viral polyprotein cleavage. This process is crucial for viral multiplication. Thus, the main protease is a promising target for antiviral drug development. Traditional enzyme activity assays for inhibitor identification rely on peptide-based substrates. However, the COVID-19 pandemic has limit or delay access to peptide synthesis services, especially for researchers in developing countries. We explored the application of 8-anilinonaphthalene-1-sulfonate (ANS) as a fluorescent probe for inhibitor identification. Fluorescence enhancement upon binding of ANS to the main protease was observed. This interaction was competitive with a peptide substrate, indicating that ANS bound within the active site. The utility of ANS-based competitive binding assay to identify main protease inhibitors was demonstrated with the flavonoid natural products baicalein and rutin. The molecular details of ANS and rutin interaction with the main protease was investigated with molecular modeling. Our results suggested that ANS could be utilized in a competitive binding assay to facilitate the identification of novel SARS-CoV-2 antiviral agents.

IL-03

An engineered survival-selection strategy for synthetic binding proteins against difficult-to-drug targets

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ABSTRACT

Protein phosphorylation plays an important role in the regulation of protein function and many cellular processes. Aberrant phosphorylation has been shown to be a cause of cell death as well as maligination. As such, there is an urgent need for affinity reagents that target phospho-modified sites on individual proteins. Currently, generation of phospho-specific antibodies relies primarily on hybridoma technology which requires phospho-epitope mapping through mass spectrometry, selection of the phospho-epitope to be targeted, and synthesis of a short phosphopeptide to be injected. As an alternative to immunization, protein display technologies (*e.g.*, phage, yeast, and ribosome display) have been employed as a viable approach to specifically select for binders against phospho-modified sites on individual targets. Though up-front phospho-amino acid identification is eliminated in protein display technologies, purification of kinases is still required. Another challenge is that the resulting antibody fragments require intradomain disulfide bonds for conformational stability, thus precluding their use as "intrabodies" in the reducing intracellular environment where most phosphoproteins of interest naturally reside. This bottleneck can be overcome by using alternative non-antibody binding scaffolds for molecular recognition such as designed ankyrin repeat proteins (DARPins), which contain no disulfide bonds and can be expressed in soluble form with high yields in the cytoplasm of living cells. Here, we describe a genetic selection strategy for routine laboratory isolation of phospho-specific designed ankyrin repeat proteins (DARPins) by linking in vivo affinity capture of a phosphorylated target protein with antibiotic resistance of *Escherichia coli* cells. The assay is validated using an existing panel of DARPins that selectively bind the nonphosphorylated (inactive) form of extracellular signal-regulated kinase 2 (ERK2) or its doubly phosphorylated (active) form (pERK2). We then use the selection to affinity-mature a phospho-specific DARPin without compromising its selectivity for pERK2 over ERK2 and to reprogram the substrate specificity of the same DARPin towards non-cognate ERK2. Collectively, these results establish our genetic selection as a useful and potentially generalizable protein engineering tool for studying phospho-specific binding proteins and customizing their affinity and selectivity.

IL-04

New glycomimetics inhibitors targeting sugar-binding proteins from pathogenic bacteria

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ABSTRACT

Pathogenic bacteria utilise glycan epitopes on host tissue for specific recognition and host infection, the processes which are mediated by the interaction between host glycan epitopes and the pathogenic sugar-binding protein called lectins. Opportunistic pathogens such as *Pseudomonas aeruginosa* produces multivalent sugar-binding proteins, LecA and LecB, which plays crucial roles in host infection and biofilm formation. Through rational design and drug screening approaches, our research works aimed at developing new glycomimetics inhibitors targeting these proteins to interfere with host glycan binding process. In drug screening approach, we performed virtual drug screen against chemical libraries to identify hit molecules that were subsequently validated by several orthogonal biophysical assays (surface plasmon resonance, thermal shift assays, fluorescent polarisation, and nuclear magnetic resonance). X-ray crystallographic study was used to determine the binding mode of the hit molecules to LecA, providing the first evidence of non-carbohydrate glycomimicry for lectins from pathogens. The identified non-carbohydrate inhibitors can be used as a starting point for the development of a new class of LecA inhbitors.

Bruker Academic Talk

Immunopeptidomic analysis of SARS-CoV2 infected lung epithelial cells reveals new targets for antiviral immunity

Sri Ramarathinam

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ABSTRACT

Peptides presented by Human Leukocyte antigen (HLA) class I and II molecules form an important target of the adaptive immune response against viruses, bacteria and tumours. Identifying HLA-bound peptides is therefore crucial to understand the specificity of T cell responses in cancer and infectious disease. This mode of antigen presentation facilitates broad immunosurveillance by T-lymphocytes (T-cells) which can recognize foreign or abnormal peptides associated with these HLA molecules and elicit an appropriate immune response to eradicate the infected or malignant cells. It is therefore vital to identify these peptides to enable better design of vaccines and immunotherapies.

Mass spectrometry is emerging as a gold-standard to identify the peptide cargo of HLA molecules and the study of these peptides is termed immunopeptidomics. We applied this approach to understand the antigenic landscape of SARS-CoV2 infected Calu3 lung epithelial cells. Virus infected and uninfected cells were lysed and the HLA complexes immunoprecipitated using antibodies specific for HLA class I and II molecules. The peptide cargo was then separated and analysed using a Bruker timsTOF PRO coupled with a NanoElute nano-UHPLC. Leveraging the high sensitivity and trapped ion mobility features of this instrument aided the identification of over 9000 Class I and 10000 class II ligands, that included over 100 SARS-CoV2 derived peptides. We show that peptides spanning the entire viral proteome contribute to the SARS-CoV2 immunopeptidome, revealing new opportunities for rational vaccine design that extend beyond the currently targeted viral Spike protein.

IL-05

Science and translation toward prevention and treatment of COVID-19

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ABSTRACT

In this talk, I will discuss three technologies in prevention and treatment of COVID-19 which have been developed under our COVID-19 research group. First, we have identified two small molecule derivatives of a plant-derived compound which possess a strong anti-SARS-CoV-2 activity. Preliminary data suggest that the two compounds exert their effect via both viral entry and egress pathways. Second, we have recently shown a neutralizing activity of sera collected from mice receiving a prime-boost regimen of HexaPro spike subunit vaccine; this conformation of the SARS-CoV-2 spike glycoprotein is employed by NDV-HXP-S vaccine developed by Mahidol-GPO. Third, we isolated spleenocytes from the mice and used them to create hybridomas. Monoclonal antibody derived from one of these clones possesses a strong neutralizing activity against live viruses. cDNA isolated from this hybridoma clone will be utilized to create a humanized therapeutic monoclonal antibody. Lastly, I will discuss our ongoing work on a novel class of mRNA vaccine. We have demonstrated that this novel class of mRNA vaccine is more potent than a conventional linear mRNA counterpart. Moreover, the mRNA technology will be applied not only for other infectious diseases, but also for genetic diseases, regenerative medicine, and cancers.

IL6

Breast milk, BigMAC and Crab Cracker: My Research on Therapeutic Peptides

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ABSTRACT

Peptides have gained much attention during the last few years as new therapeutic agents, particularly for cancers. While the exact mechanisms and cancer selectivity have yet to be elucidated, anti-cancer peptides may have oncolytic activities depending on their intrinsic characteristics. Some of these anti-cancer properties are predictable from amino acid sequences. Here, our group has explored the advent of Big data and Machine learning to facilitate the discovery of new Anti-Cancer peptides (Big-MAC). We applied this approach to breast milk peptidomics and recently reported HMP-S7 as a novel anti-leukemic peptide against leukemic cells *in vitro* and patient-derived cells *ex vivo*. Further investigations using a more extensive peptide library and a new algorithm for anti-cancer peptide mutagenesis may be enable the right way to crack the crab by therapeutic peptides in the future.

This research was supported by the New Discovery and Frontier Research Grant of Mahidol University, Thailand (NDFR19/2563).

IL-07

Towards Clinical Trial Phase I of Plant-based COVID-19 Vaccine in Thailand

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ABSTRACT

SARS-CoV-2 causes the COVID-19 outbreak since January 2020 and the virus spreads rapidly worldwide. Within one year of its outbreak, COVID-19 vaccines were developed and approved under emergency application. Several countries have begun mass administration of COVID vaccines from early 2021. However, the accessibility of the vaccines in most countries have been questioned as the vaccines are reserved by wealthy nations. Hence the people in low-income and middle-income countries are still waiting to get a vaccine shot due to the unequal distribution of COVID-19 vaccines around the globe. In view of the vaccine security, plant-based COVID-19 vaccine production has been initiated in Thailand as plants offer many advantages, including high scalability, rapid production time, and low infrastructural cost. Plant-produced COVID-19 vaccine was developed by producing SARS-CoV-2 RBD-Fc fusion protein in *Nicotiana benthamiana* (Baiya SARS-CoV-2 Vax 1). SARS-CoV-2 RBD-Fc showed specific binding to ACE2 protein. The immunogenic potential of Baiya SARS-CoV-2 Vax 1 was demonstrated in mice. The results showed that a single dose of Baiya SARS-CoV-2 Vax 1adjuvanted with alum could induce immune response in mice, however the titer of RBD-specific antibody and neutralizing antibody was increased after the second dose. Also of note, the *in vitro* neutralization of live SARS-CoV-2 virus with the sera collected from Baiya SARS-CoV-2 Vax 1 immunized mice and monkeys was evaluated. Further, the results from SARS-CoV-2 challenge study suggest that Baiya SARS-CoV-2 Vax1 reduces the symptoms and mortality associated with SARS-CoV-2 infection in K18hACE2 mice. The toxicity study also showed that Baiya SARS-CoV-2 Vax1 was well tolerated in Wistar rats with no unanticipated findings or toxicity observed up to 100 µg dose. Collectively, the results from mice, rats and monkeys have shown that Baiya SARS-CoV-2 Vax1 induced potent neutralizing antibody response against SARS-CoV-2, including the variants of concern.

IL-08

Antibody and cytokine responses in COVID-19 patients and vaccines

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ABSTRACT

The ongoing COVID-19 pandemic necessitates continued monitoring of both naturally acquired and vaccine-induced immunity against SARS-CoV-2 and its variants of concern (VOCs). A longitudinal study of neutralizing antibody (NAb) titers in patients hospitalized in early 2020 with mild symptoms, pneumonia, or severe pneumonia revealed that peak NAb titers correlated with disease severity and that NAb titers declined over the course of 1 year regardless of severity. Serum-derived inflammatory cytokine profiling in all severity groups unveiled key cytokines linked to severe pneumonia, and cytokine networks were found to be distinct between severity groups. Furthermore, assessment of NAb titers in a separate cohort of healthcare workers who had received 2 doses of CoronaVac revealed that the regimen conferred relatively low levels of NAb-mediated protection against SARS-CoV-2 VOCs compared to WT, and that vaccine-induced NAb potency was lower than that derived from natural infection. Our results highlight the need for constant vigilance and appropriate vaccine monitoring, especially against the highly transmissible delta strain, which was found to be most refractory to neutralization.

IL-09

Cellular immune response to COVID-19 deciphered by single-cell multi-omics

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ABSTRACT

Single-cell gene and multi-omic profilings have provided insights into the biology of COVID-19. We surveyed expression of viral entry-associated genes in single-cell RNA-sequencing data from multiple tissues from healthy human donors and co-detected these transcripts in specific respiratory, corneal, and intestinal epithelial cells, potentially explaining the high efficiency of SARS-CoV-2 transmission. Subsequently, we performed single-cell transcriptome, surface proteome, and T and B lymphocyte antigen receptor analyses of over 780,000 peripheral blood mononuclear cells from a cross-sectional cohort of 130 patients with varying severities of COVID-19. We identified the expansion of nonclassical monocytes expressing complement transcripts (*CD16*$^{+}$*C1QA/B/C*$^{+}$) that sequester platelets and were predicted to replenish the alveolar macrophage pool in COVID-19. Early, uncommitted CD34^{+} hematopoietic stem/progenitor cells were primed toward megakaryopoiesis, accompanied by expanded megakaryocyte-committed progenitors and increased platelet activation. Clonally expanded CD8^{+} T cells and an increased ratio of CD8^{+} effector T cells to effector memory T cells characterized severe disease while circulating follicular helper T cells accompanied mild disease. We observed a relative loss of IgA2 in symptomatic disease despite an overall expansion of plasmablasts and plasma cells. Our study highlights the coordinated immune response that contributes to COVID-19 pathogenesis and reveals discrete cellular components that can be targeted for therapy.

Waters Academic Talk

How LC-MS can support our fight against Covid-19

Dhaval Patel

Waters Pacific Pte Ltd, Singapore

ABSTRACT

Research organizations have risen to the challenge of fighting the covid-19 pandemic across the globe. Key areas of research related to covid-19 include improving fundamental understanding of COVID-19 infection; characterizing the virus and how it causes infection; development of rapid and accurate assays for the diagnosis; identifying and evaluating potential treatments for COVID-19 infection and development of safe and effective vaccines.

The presentation will provide high-level context to explain the role of LC-MS based approaches for covid-19 research related applications.

OR-01

Host-derived cell-wall-binding domain of phage endolysin CD16/50L anchors to the surface polysaccharide of *Clostridioides difficile* to preserve neighboring host cells and ensure progeny expansion

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Abstract

Endolysin is a phage-encoded cell-wall hydrolase which degrades the peptidoglycan layer of the bacterial cell wall. The enzyme is often expressed at the late stage of the phage lytic cycle and is required for progeny escape. Endolysin of bacteriophage that infects Gram-positive bacteria often comprises two domains: a peptidoglycan hydrolase and a cell-wall binding domain (CBD). Although the catalytic domain of endolysin is relatively well-studied, the precise role of CBD is largely unknown and remains controversial. For example, while certain endolysins absolutely require CBD for their lytic activity, others do not need it at all, raising the question of its necessity. Here, we focus on the function of CBD of endolysin encoded in the genome of recently isolated *Clostridioides difficile* phages. We found that CBD of this endolysin is not required for the lytic activity, which is strongly prevented by the surface layer of *C. difficile*. Intriguingly, hidden Markov model (HMM) analysis suggested that the endolysin CBD is likely derived from the cell-wall-binding-2 domain of host's cell-wall proteins but possesses a higher binding affinity to polysaccharide components of bacterial cell wall. Moreover, the CBD is able to form a homodimer, formation of which is necessary for interaction with the peptidoglycan layer. Importantly, endolysin diffusion and sequential cytolytic assays showed that CBD of endolysin is required for anchoring to cell-wall remnants, suggesting its physiological roles in controlling diffusion of the enzyme, preserving neighboring host cells, and thereby enabling phage progeny to initiate new rounds of infection. Taken together, this study provides an insight into regulation of endolysin through CBD and can potentially be applied for endolysin treatment against *C. difficile* infection.

Acknowledgement
This research was supported by Science Achievement Scholarship of Thailand (SAST).

OR-02

Proteomic analysis of 5-Fluorouracil resistant cholangiocarcinoma cell line

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ABSTRACT

Resistance to commonly used chemotherapeutic drugs is a major problem of cholangiocarcinoma (CCA) treatment, therefore more effective treatment strategy is urgently needed. Drug resistant cell lines have been used for elucidating drug resistance mechanism in various cancers leading to the discovery of novel treatments. Here in, we have established 5-Fluorouracil resistant (FR) CCA cell line by culturing the parental CCA cell line (KKU-213A) in the stepwise increased concentration of 5-Fluorouracil (5-FU). The established cell line was designated as KKU-213A-FR, confirmed for drug resistance, and use for further analyses. The results showed that, under low serum condition, KKU-213A-FR had slower growth rate than its parental cell line. Conversely, cell migration and invasion ability were increased significantly. Moreover, the proteomic analysis using LC/MS/MS identified the total of 5,680 proteins. In which, 431 were identified only in parental cells, 1,105 were found only in FR cells, whereas 4,144 were expressed in both cell lines. Heatmap analysis showed the 25 most up- and down-regulated proteins in KKU-213A-FR. The up-regulated proteins were further analyzed. Gene ontology revealed that most of these proteins were classified in cellular process group. The protein-protein interaction analysis showed 3 clustering groups that involve in N-linked glycosylation, ubiquitin-dependent protein catabolic process, and organic substance catabolic process. In conclusion, the KKU-213A-FR exhibits aggressive phenotypes and a unique proteomic profile as compared to KKU-213A. More in-depth study on the differentially expressed proteins may lead to better understanding of drug resistance mechanism and more effective treatment for CCA.

Poster Abstracts

PP001

In silico prediction and experimental validation of a novel anti-cancer peptide

Tassanee Lertsuthirat[1#], Pasinee On-yam[2#] Sermsiri Chitphuk[1], Wasana Stitchantrakul[1], Suradej Hongeng[3], Wararat Chiangjong[3*], Somchai Chutipongtanate[3*]

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ABSTRACT

INTRODUCTION: Cancer is the leading cause of death worldwide. To improve patient outcomes, a more effective treatment agent is required. Our previous work on discovering human milk peptides that selectively kill leukemia cells has shown the approach of using mass spectrometry to identify the candidate anticancer peptides. It is limited by the small peptide library. Thus, we propose to expand the peptide library by using *in silico* generation of peptides from the protein of interest.

MATERIALS AND METHODS: We *in silico* produced peptides from a protein with anticancer potential, alpha-lactalbumin. On-line tools were used to predict anticancer properties of the filtered peptides. Lastly, we chose four candidates for *in vitro* testing on cancer cells.

RESULTS AND DISCUSSION: We *in silico* obtained four candidates (A1-A4) peptides with the highest anticancer scores for further *in vitro* testing on five cancer cell lines, SH-SY5Y, MDA-MB-231, A549, HT29, and K562. A2 was able to solubilize in media, whereas three peptides (A1, A3, and A4) precipitated. Preliminary screening revealed A2 demonstrated the most anticancer activity. To avoid the interference of peptide precipitants on cell viability, we therefore solubilized peptide in DMSO. A1, A3, and A4 exhibited dose-dependent cytotoxicity toward A549, but none of the three exhibited significant cytotoxicity toward the remaining cancer cells.

CONCLUSION: This approach enables us to rapidly identify potential anticancer candidates prior to conducting *in vitro* experiments, while also expanding the pool of bioactive peptides that may be missed by mass spectrometry analysis.

REFERENCE: Chiangjong, W., et al., HMP-S7 Is a Novel Anti-Leukemic Peptide Discovered from Human Milk. Biomedicines, 2021. 9(8): p. 981.

PP002

Identification of protein A, B, C, D and E as biomarkers to distinguish between hepatocellular carcinoma and cholangiocarcinoma in 3D culture using Label-free quantitative proteomics

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ABSTRACT

Precise monitoring and diagnosis of cancer patients are the key to cancer therapeutic. Therein, to find the new specific biomarker is the challenge for cancer prevention and treatment. Discovery of new protein markers employ high throughput of sample characterization by mass spectrometry. Here, 3D cell culture was used as a model to mimic tumor environment. Cells were digested and identified by label-free mass spectrometry to differentiate candidate cancer biomarkers between hepatocellular carcinoma and cholangiocarcinoma, which located at nearby area and difficult to diagnose. Mass spectrometry results were then analyzed by Progenesis QI software. More 500 proteins were changed between two types of cancer. All changed proteins involved in protein binding, catalytic activity, structural molecule activity, transport regulator activity and transporter activity. Among these proteins, we found that 4 proteins named protein A, B, C and D were highly expressed in cholangiocarcinoma cells compared to hepatocellular carcinoma. Furthermore, we also found protein E was highly express in hepatocarcinoma compared to cholangiocarcinoma. All candidate proteins were validated by immunoblotting. All of these proteins were analyzed by String and Cytoscape software. However, tissue samples from patients are needed for further study. Taken together, our results provide 5 candidate biomarkers to distinguish the difference between hepatocellular carcinoma and cholangiocarcinoma.

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PP004

Design of enzyme immobilization system for chitin bioconversion

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ABSTRACT

Chitooligosaccharides (COS) produced by the enzymatic hydrolysis of chitin are of significant interest; their high value indicates that they have interesting bioactivities such as anticancer, antifungal, and anti-inflammatory properties, making them a viable pharmaceutical product. Utilizing of immobilized enzyme for COS production is interesting, as long as the enzyme is stable enough for industrial application. In this study, chitinase A from the marine bacterium *Vibrio harveyi* (*Vh*ChiA) was fermented and purified by a Ni-NTA column. Chitosan coated magnetic nanoparticles (CS@MNPs) were synthesized by in situ co-precipitation method and were then functionalized with hemin through amidation reaction. *Vh*ChiA was immobilized on to three different types of magnetic nanoparticles including uncoated MNPs, CS@MNPs, and Hemin@CS@MNPs. Transmission electron microscopy (TEM), Scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and thermal gravimetric analysis (TGA) were used to illustrate the MNPs and immobilized *Vh*ChiA. Among of three types of magnetic nanoparticles, CS@MNPs provided the highest immobilization yield around 95%, followed by Hemin@CS@MNPs, and uncoated MNPs which gave 87% and 29%, respectively.

PP005

Virtual screening of novel *M. tuberculosis* PknA/PknB dual inhibitors as anti-tuberculosis agents

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ABSTRACT

In this research, we applied virtual screening and pharmacokinetic predictions to identify novel PknA/PknB dual inhibitors for tuberculosis agents from kinase inhibitor database. The obtained results demonstrated that four compounds were identified as new *M. tuberculosis* PknA/PknB dual inhibitors which showed strong biding affinity with ATP binding site. These compounds shared hydrogen bond interaction at the hinge residue of Val98 and Val95 backbone of PknA and PknB, respectively. In addition, sigma-pi andhydrophobic interactions of hit compounds with amino acid residues enhanced the binding affinity in the ATP binding site. Based on the pharmacokinetic prediction, the Caseum FU was ranging from 0.20 to 18.97 % which indicated that compounds will inhibit mycobacterialwith low MIC value. The low maximum tolerated dose and oral rat acute toxicity suggested that hits compounds should be low toxicity. Therefore, this work aided to identify novel PknA/PknB dual inhibitors as potent anti-tuberculosis agents.

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PP006

Elucidating the binding interaction of pyrimido[4,5-b]indol-8-amine derivatives as potential GyrB inhibitors against *M. tuberculosis* using molecular docking calculations

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ABSTRACT

Tuberculosis (TB) caused by *M. tuberculosis* remains as a major world health problem due to drug resistance of *M. tuberculosis*. DNA gyrase subunit B (GyrB) has been studied. GyrB is a significant target for *M. tuberculosis* because it is required for DNA replication and transcription. The pyrimido[4,5-b]indol-8-amine derivatives have been reported as GyrB ATPase inhibitors against tuberculosis. In this work, to understand the binging mode, binding interaction and binding energy of compounds, molecular docking calculations were carried out against GyrB. The crucial interactions showed H-bond interactions between pyrimido[4,5-b]indol-8-amine derivatives with Asn52 and Arg141 residues. The hydrophobic interactions between compounds with Val49, Ile84, Val99, Val123, Val125, Ile171 residues were obtained. In addition, the binding affinity increase with pi-cation between pyrimido[4,5-b]indol-8-amine derivatives and Arg82 residue of GyrB pocket. Based on the results, molecular docking calculations of the pyrimido[4,5-b]indol-8-amine derivatives showed crucial interactions between ligands and binding pocket. It may be said that the antitubercular property of the molecule could be via the inhibition of ATPase domain of GyrB enzyme. Therefore, the docking studies of pyrimido[4,5-b]indol-8-amine derivatives are important results for further rational design of novel GyrB inhibitor to combat drug resistant tuberculosis.

This research was supported by the Health Systems Research Institute (HSRI.60.083), Ubon Ratchathani University (DR2564SC01200) and the Excellence for Innovation in Chemistry (PERCH-CIC). Thailand Graduate Institute of Science and Technology (TGIST) Ph.D. Program (SCA-CO-2561-6946TH) to P. Thongdee is appreciated. Faculty of Science, Ubon Ratchathani University, Faculty of Science, Kasetsart University, Faculty of Science, Nakhon Phanom University, School of Chemistry, University of Bristol, and Thailand: National Electronics and Computer Technology (NECTEC) are gratefully acknowledged for supporting this research. We thank EPSRC for funding via BristolBridge (grant number EP/M027546/1) and CCP-BioSim (grant number EP/M022609/1).

PP007

Discovery of novel and potential InhA inhibitors based on virtual screening approaches

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ABSTRACT

The virtual screening from Specs database was carried out to identify novel 2-*trans* enoyl-acyl carrier protein reductase (InhA) inhibitors as anti-tuberculosis agents. 6 compounds were started from similarity search of cannabigerol (CBG) as potential InhA inhibitor with IC_{50} value of 5.2 μM. 5 compounds passed filtering cutoff with Lipinski's rule of five employed to investigate the binding energy, binding mode, and binding interactions via molecular docking calculations. 3 hit compounds were obtained from the predicted biological activity by way2drug program and anti-bacterial prediction focused on the inhibition of *M. tuberculosis* H37Rv and H37Ra strains. Therefore, the virtual screening provides useful information for rational design new and more potent InhA inhibitors as anti-tuberculosis agents.

This work was supported Thailand Graduate Institute of Science and Technology (TGIST) (SCA-CO-2563-12135-TH) to N. Phusi and the Excellence for Innovation in Chemistry (PERCH-CIC) are appreciated. Faculty of Science, Ubon Ratchathani University, Kasetsart University, Nakhon Phanom University, School of Chemistry, University of Bristol, and Thailand: National Electronics and Computer Technology (NECTEC) are gratefully acknowledged for supporting this research.

PP008

CRISPR-based Detection for Identifying Snake Species from Snakebite Envenoming

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ABSTRACT

Snakebite envenoming is a neglected tropical disease which causes broad symptoms ranging from mild to high severity, affecting healthcare management and economy of tropical developing countries including Thailand. Multiple venomous snake species are distributed throughout different regions in Thailand, and it is not trivial to identify snake species upon getting bitten or threated based on old-fashioned means, rendering timely administration of the antivenom difficult or impossible. Here, we propose the development of a point-of-care genotyping tool based on CRISPR for rapid and accurate identification of snake species from bite wounds. DNA regions within the mitochondrial *cytochrome b* (*cytb*) gene of eight venomous snake species endemic to Thailand—*O. hannah*, *N. kaouthia*, *C. rhodostromata*, *D. russelii*, *D. siamensis*, *T. albolabris*, *B. fasciatus*, and *B. candidus*—were chosen as targets for CRISPR-Cas13a-mediated detection, which utilizes recombinase-polymerase amplicification (RPA) and *Leptotrichia wadei* (Lwa) Cas13a for maximal detection sensitivity and specificity. We successfully prepared all biomolecular components necessary for the RPA/LwaCas13-based detection of the *cytb* gene from different snake species, optimized their detection conditions, validated their specificity, and assessed their analytical sensitivity upon detection of surrogate DNA substrates. Detection modules with sufficiently high sensitivity will be used to detect the *cytb* gene from biological fluids obtained from snakes in the near future.

PP010

Global analysis of protein expression of A549 cells after prolonged nicotine exposure by using label-free quantification

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ABSTRACT

Lung cancer is the leading cause of cancer death worldwide, and cigarette smoke is considered as the most important risk factor for development of lung cancer. Nicotine, an addictive component in cigarettes, is generally considered as non-carcinogenic. However, growing evidence indicates that prolonged nicotine exposure is a potential factor associated with tumorigenesis. Therefore, there is a need to gain insight into the molecular events during prolonged exposure to nicotine. Here, the effect of prolonged nicotine exposure on A549 lung adenocarcinoma cells was investigated, using label-free quantitative proteomic analysis. Selection of invasive subpopulation from A549 cell line was performed to reveal the differential expression and functional annotation of proteins in relation to prolonged nicotine exposure, using Boyden chamber assays in combination with the proteomics approach. One hundred proteins from the NicoA549-L5 subline were identified showing significant change in expression compared to those from A549-L5 subline and their A549 parental cell line. Western blotting was then employed to validate the candidate proteins. The results indicated that prolonged exposure of nicotine promoted invasion on A549 cells. Interestingly, legumain, heat shock protein HSP 90-alpha, heat shock related 70 kDa protein 2, protein disulfide isomerase A3 and profilin-1 were found to show higher expression in A549 cells after prolonged exposure to nicotine. Our findings suggested that these aberrant proteins might serve as novel cancer biomarkers for cigarette smokers.

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PP011

Investigation of malathion sensitivity in *Saccharomyces cerevisiae* lacking the mitophagy receptor Atg32p

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ABSTRACT

Organophosphate (OP) insecticides are widely used in agriculture for controlling insects that can damage crops. Malathion is among the most commonly used of this class. Misapplication and extensive use of insecticides has contributed to the development of OP resistance in insect populations as well as decreasing efficacy of insecticides resulting in crop loss and the spread of insect-borne diseases. In addition, increased insecticide use can lead to environmental contamination and human exposure. However, the limited genetic tools available for insect systems prevents comprehensive screening for mutations or deletions of genes that promote insecticide sensitivity directly. Simple model systems such as the baker's yeast *Saccharomyces cerevisiae* can be utilized to aid in the identification of pathways that lead to insecticide sensitivity. A collection of yeast deletion strains was screened for malathion sensitivity under conditions requiring respiratory growth. This screen identified *ATG32* as being required for malathion tolerance. However, the role of this protein in autophagic processes under other stress conditions has not been previously examined. Using the dual fluorescence Rosella system increased levels of autophagy were observed WT and yeast lacking Atg32p following malathion exposure. In contrast, increased levels of mitophagy were not apparent due to malathion treatment. It appears that malathion sensitivity in yeast lacking Atg32p is not due to disruption in autophagy. Overall, these findings suggest that loss of Atg32p can promote malathion sensitivity. Further characterization of pathways affected in yeast lacking Atg32p may identify molecular targets with utility in the development of agents to sensitize cells to malathion.

Keywords: Organophosphate, malathion, *Saccharomyces cerevisiae, ATG32*, Rosella, autophagy

PP012

Elucidating the binding mode and binding interactions of 1,2,4-triazole-5-thione derivatives as InhA inhibitor using molecular docking calculations

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ABSTRACT

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* remains a major worldwide public health problem. The enoyl-acyl carrier protein reductase, InhA has been focused on numerous drug discovery efforts as this is the target of the first line prodrug isoniazid. However, resistance to this drug has been becoming more common. Direct InhA inhibitors are remain effective against InhA variants with mutations associated with isoniazid resistance. 1,2,4-triazole-5-thione derivatives were reported as antimycobacterial agents against *Mycobacterium tuberculosis*. 1,2,4-triazole-5-thione derivative was identified as anti-mycobacterial direct InhA inhibitor. In this study, binding mode and binding interactions of 1,2,4-triazole-5-thione derivatives in InhA binding site were evaluated using molecular docking calculations. The results indicating the crucial interactions of 1,2,4-triazole-5-thione derivatives in InhA binding site were found. 1,2,4-triazole-5-thione derivatives formed pi-pi interaction with Phe149. Hydrogen bond interaction was found with Met199. Moreover, hydrophobic interactions were found with Met161, Pro156, Ala198, Val203, Met193, Ala157 Met155, Leu218. Finally, the obtained results from these studies are fruitful to further design the promising InhA inhibitors to overcome tuberculosis drug resistance.

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PP013

Identification of *Pseudomonas pseudomallei* FabI1 inhibitors of antimicrobial agents against melioidosis using virtual screening approaches

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ABSTRACT

Melioidosis is a complex disease because of its rapid progression and tendency to generate latent infections. The etiologic agent of melioidosis is the gram-negative organism *Burkholderia pseudomallei*. To identify new and potential melioidosis inhibitors, Zinc database was applied to screen novel melioidosis inhibitor. Then, drug-likeness properties and antibacterial predictions were elucidated. Molecular docking calculations using Glide program were then performed. Based on the obtained results, top three compounds which showed docking score closed to x-ray ligand including **ZINC93953430, ZINC94081106** and **ZINC94708668** were selected. The crucial interactions are hydrogen bond interactions with Gly93 residue and NAD^+ cofactor and hydrophobic interactions with Phe94, Ile100, Ile153, Pro154, Met159, Pro191, Ile192, Ala196, Ala197, Ile200, Phe203 and Ile206 residues were found as crucial interactions. The integrated results provide fruitful information for discovery of melioidosis inhibitor with highly and more potent.

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PP014

Discovery of novel main protease inhibitors of SARS-CoV-2 using virtual screening and pharmacokinetic predictions

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ABSTRACT

An infectious disease, COVID-19 disease caused by SARS-CoV-2 virus is the major health concerned. The main protease enzyme has been validated as a drug development target to stop SARS-CoV-2. Herein, we attempted to identified new promising main protease inhibitors from Specs commercial database to prepose as novel main protease inhibitors of SAES-CoV-2 Based on virtual screening and pharmacokinetic prediction, three compounds, **AA-504/07472048**, **AK-968/40046672** and **AH-034/04906059** with good binding affinity and pharmacokinetic properties were obtained. Hydrogen bond interactions with Asn142 residue, pi-sigma interactions with Met165 residue and hydrophobic interactions with Met165, Gly143 and Leu27 residues in the SARS-CoV-2 main protease binding site were found as crucial interactions. Based on pharmacokinetic properties predictions, these compounds were suitable to propose for biological assay evaluation and develop as anti-COVID-19 agents.

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PP015

Investigating crucial interactions of the 2-(benzylideneamino)-*N*'-(7-chloroquinolin-4-*yl*)benzohydrazide derivatives and GyrB as potent GyrB inhibitors through molecular docking calculations

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ABSTRACT

Multi-drug resistant tuberculosis is considered as major bottleneck in the treatment and cure of tuberculosis. So, developing of new tuberculosis drugs to overcome drug resistant have been carried out to develop effective anti-TB drugs. *Mycobacterium tuberculosis* DNA gyrase is a tetrameric A_2B_2 protein. The A subunit (GyrA) carries the breakage-reunion active site, whereas the B subunit (GyrB) promotes ATP hydrolysis. GyrB of DNA gyrase is an attractive target for developing inhibitors against drug resistant tuberculosis. The 2-(benzylideneamino)-*N*′-(7-chloroquinolin-4-*yl*)benzohydrazide derivatives were reported as antimycobacterial activity against *Mycobacterium tuberculosis.* In the present work, the binding mode, binding interactions and binding energy between the 2-(benzylideneamino)-*N*′-(7-chloroquinolin-4-*yl*)benzohydrazide derivatives and GyrB were performed using molecular docking calculations. The crucial interactions showed hydrogen bond interactions via mediated interactions the carbonyl substituent on benzohydrazide with Asp79 residue and nitrogen atom of *N*-benzylidene with Asn52 residue. The 2-(benzylideneamino)-*N*'-(7-chloroquinolin-4-*yl*)benzohydrazide derivatives formed hydrophobic interactions with Ile84, Prp85, Val99, Val123 and Val125 residues. Therefore, our results obtained from this study aid to better understand the crucial binding characteristic of the 2-(benzylideneamino)-*N*′-(7-chloroquinolin-4-*yl*)benzohydrazide derivatives with GyrB are beneficial for rational design of novel GyrB inhibitors as potential anti-tuberculosis agents.

This research was supported by the Health Systems Research Institute (HSRI.60.083), Ubon Ratchathani University (DR2564SC01200) and the Excellence for Innovation in Chemistry (PERCH-CIC). Royal Golden Jubilee (RGJ) Ph.D. Program (PHD/0132/2559) to B. Kamsri are appreciated. Faculty of Science, Ubon Ratchathani University, Faculty of Science, Kasetsart University, Faculty of Science, Nakhon Phanom University, School of Chemistry, University of Bristol, and Thailand: National Electronics and Computer Technology (NECTEC) are gratefully acknowledged for supporting this research. We thank EPSRC for funding via BristolBridge (grant number EP/M027546/1) and CCP-BioSim (grant number EP/M022609/1).

PP016

Investigation of the binding mode and binding interactions of ML300-derivatives as potential anti-SARS-CoV-2 agents through molecular docking calculations

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ABSTRACT

Coronaviruses 19 (COVID-19) are a group of enveloped positive-strand RNA pathogenic viruses that can cause a variety of acute and chronic illnesses, including central nervous system disorders, the common cold, lower respiratory tract infections, and diarrhea. COVID-19 is a serious health risk caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The main protease (M^{pro}) is an enzyme of SARS-CoV-2. The M^{pro} protein of the virus has also been investigated as a development target for inhibiting SARS-CoV-2 virus. Noncovalent inhibitors, ML300 derivatives have been developed against SARS-CoV-2. Herein, we attempted to elucidate ML300-derivatives as novel M^{pro} inhibitors of SARS-CoV-2 based on molecular docking calculations. Hydrogen bond interactions between OH group of all selected compounds with Asn142 and Glu166 residues, pi-sigma interactions between benzene ring with Gln189 residue, and hydrophobic interactions with Met49, Met165 and Pro168 residues in the SARS-CoV-2 M^{pro} binding site were found as crucial interactions. Therefore, the obtained docking results of selected ML300-derivatives are beneficially informative for further rational design of new and potential inhibitors to combat COVID-19.

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PP017

Insight into binding mode and crucial interaction of aminopyrimidine derivatives as potential PknB inhibitors using molecular docking calculations

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ABSTRACT

Tuberculosis caused by *mycobacterium tuberculosis* is major global public health concern. Protein serine/threonine kinase B or PknB is an attractive drug development target because of its central importance in several critical signaling cascades. Here, molecular docking calculations were applied to investigate the binding mode and crucial interactions of aminopyrimidine derivatives as anti-tuberculosis agents. The obtained result indicated that crucial interactions are including, hydrogen bond interaction with Val95 residue, pi-sigma interactions with Met145 and Met155 residues. Hydrophobic interactions with Leu17, Phe19, Ser23, Val25, Ala38, Val72, Met92 and Ala142. Based on the obtained results, it could be fruitful guideline for rational design of novel PknB inhibitor as anti-tuberculosis agents.

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PP018

Insight into binding mode and crucial interaction of tetrahydropyran derivatives as potential InhA inhibitors using molecular docking calculations

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ABSTRACT

Tuberculosis (TB) remains a major global health problem, caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). Enoyl-ACP reductase or InhA is an attractive target responsible for mycolic acid synthesis by preventing the fatty acid biosynthesis pathway. Tetrahydropyran derivatives were selected to study because it is a classical substructure for glycomimetics inhibition of proteins and exhibits good InhA inhibitory potency withmoderate antimycobacterial activity. In this work, molecular docking calculations were applied to investigate binding mode and crucial interactions of tetrahydropyran derivatives using Glide program. The obtained results indicated that hydrogen bond interaction between oxygen atom of tetrahydro-2H-pyran ring with Gln100 residue, pi-sigma interactions with between aromatic ring of ligand with Tyr 158 residue and hydrophobic interactions with Phe97, Met103, Ala201, Leu207 and Ile215 residues were found as crucial interaction in InhA binding pocket. These results provide beneficial guideline to rational design new and effective inhibitor against *M. tuberculosis.*

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PP019

Identification of novel JAK2 inhibitors as erythropoiesis stimulant agents for thalassemia therapy

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ABSTRACT

Janus kinase 2 (JAK2) is an enzyme responsible for regulating erythropoiesis. associated with the ineffective erythropoiesis process. This is currently a key target for developing inhibitors in the new pathway therapeutic option for thalassemia. Herein, we applied the computer aided molecular design to identify novel JAK2 inhibitors for erythropoiesis stimulating agent (ESA) from Specs database. Based on multistage virtual screening process, seventy-six compounds were predicted to be active against both JAK2 and ESA. Based on pharmacokinetic properties prediction, two promising compounds, **AN-979/41713534** and **AN-648/15101115** were obtained. In addition, the binding mode and binding interactions in the ATP binding site of JAK2 were investigated. The obtained results revealed that the hydrogen bond interactions with Leu932 backbone in the ATP binding site of JAK2 are key interaction for binding of new finding compounds. The Caco2 permeability of these two compounds was high. The BBB and CNS permeability values suggested that the selected compounds were poorly distributed to the brain and unable to penetrate the CNS. Therefore, the finding compounds were proposed as novel and potential JAK2 inhibitors as ESA for thalassemia therapy.

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PP020

In-house production of brain-derived neurotrophic factor (BDNF) for differentiation of SH-SY5Y cells into matured neurons

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ABSTRACT

The SH-SY5Y neuroblastoma cell line is extensively used in neuroscience research. This cell expresses tyrosine hydroxylase and dopamine-β-hydroxylase, as well as the dopamine transporter. These proteins are important markers in the dopamine synthesis pathway. Although the cells possess the dopaminergic neuron characteristics, they can be differentiated into a more functionally mature neuronal phenotype. The differentiated cells become flat with neurites that produce longer projections connecting to neighboring cells. These neurite outgrowths display a morphological similarity to mature neurons in the human brain. A number of protocols have been used to differentiate SH-SY5Y cells to a terminally mature neuron-like phenotype. Different protocols induce different neuronal phenotypes with different biochemical properties. In our laboratory, the sequential differentiation of cells is routinely performed with retinoic acid (RA) and then brain-derived neurotrophic factor (BDNF).

For our research interest on Parkinson's disease (PD), the differentiated cells are a very good model. Once cells are differentiated, BDNF must be maintained in all media. As the commercially available BDNF can be a continuous expense we have produced a more economical alternative by cloning, expressing and purifying a recombinant BDNF protein in our laboratory and then comparing it to commercially available protein.

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PP021

Sensor for direct detection of *Vibrio cholerae* in frozen seafood

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ABSTRACT

This work was developed a new sensor for Vibrio cholerae based on molecularly imprinting technique coupled with quartz crystal microbalance (QCM). A film of molecularly imprinted polymers (MIPs) coated on quartz was carried out for direct detection. Firstly, V. cholerae O1 was used as a template for imprinting on polyacrylamide (PAA) and washed with 10% acetic acid in 0.1% SDS solution. The characterization of MIP's *V. cholerae O1* was studied by using SEM. Under the optimum of developed method, a good linearity was obtained in the concentration of 1.0 x 103 to 1.0 x 108 CFU/mL with the limit detection of 1.5 x 10^2 CFU/mL. The developed method provided good reproducibility (%RSD < 7) and high selectivity, can be applied in seafood. The method accuracy was evaluated using recovery measurements in standard spiked samples and good recoveries of 84.0–114.3% with relative standard deviations of less than 10% have been achieved

PP022

Investigation of the mechanism of anoikis resistance involving protein X in thyroid carcinoma cells

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ABSTRACT

Anoikis resistance is an important characteristic of metastatic tumor cells, thus impairing anoikis resistance can be a promising strategy for the therapy of metastatic cancers. Previously, we have identified protein X to be important in anoikis resistance of thyroid cancer cells. Remarkably, protein X does not trigger anoikis resistance via epithelial mesenchymal transition (EMT) or integrin switch. In other words, there should be an alternative mechanism of anoikis resistance in thyroid cancer cells involving protein X, which may be through receptors A and/or B. In this study, we investigated the underlying molecular mechanism involving protein X using follicular-type (FTC133) and the highly metastatic anaplastic-type (ARO) thyroid carcinoma cells. siRNA knockdown of protein X was used to study the mechanism of anoikis resistance. Anoikis resistance of these cell lines was determined using flow cytometry and the expression of receptors A and B was determined using Western blot and flow cytometry. The percentage of dead floating cells or anoikis increased in protein X-knockdown cells of both cell lines when compared to that of si-Control cells. The expression of receptors A and B was significantly increased in knockdown ARO cells but not in knockdown FTC133 cells. In summary, protein X induces anoikis resistance in highly metastatic anaplastic-type thyroid cancer cells via the reduction in the expression of receptors A and B, consequently promoting thyroid cancer metastasis. Further studies to identify protein X inhibitor(s) will help to treat thyroid cancer metastasis, therefore reducing the mortality of thyroid cancer patients.

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PP023

Vanillin induces apoptosis in floating colorectal cancer cells under a metastasis-associated condition

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ABSTRACT

Drug resistance is considered as a major cause of treatment failure in 90% of cancer patients with metastatic stage. Metastatic cancer can be studied in vitro by means of non-adherent cell culture. Our previous study suggested that under non-adherent culture conditions, SW480 human colorectal cancer cells naturally developed drug resistance as a result of slow growth rate and cell cycle progression delay. Therefore, finding new compounds that are safe and effectively kill cancer cells, especially metastasizing cancer cells, is an important research topic nowadays. Vanillin is indicated as safe and used as flavoring agent in a variety of food products. Moreover, vanillin has been reported for its potent antioxidant, anti-inflammatory, and anticancer activities. In the present study, we examined the anticancer effect of vanillin on SW480 cells. The cells were cultivated as attached cells by standard monolayer culture to mimic primary cancer, or cultivated under non-adherent culture condition to obtain floating cells which represented metastasizing cancer cells when floating in blood or lymphatic circulation. Treatment of SW480 cells with vanillin led to a dose-dependent decrease in survival rate of both attached and floating cells. In addition, apoptosis analysis showed that vanillin also increased apoptotic rate in both attached and floating cells, in a dose-dependent manner. Interestingly, cytotoxicity of vanillin was stronger in floating cancer cells compared with attached cells. In conclusion, SW480 floating cells under non-adherent conditions were more susceptible to cytotoxicity of vanillin. The mechanism of action of vanillin is under investigation. Our results suggest that vanillin might be an effective anticancer agent for metastatic colorectal cancer treatment.

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PP024

Short-term exposure to a chemotherapeutic drug oxaliplatin increases metastatic potential of well-differentiated hepatocellular carcinoma cells

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ABSTRACT

Hepatocellular carcinoma (HCC) ranks on top of the high mortality cancer types. Chemotherapeutic agents are utilized as common way to cure cancer patients. Nevertheless, numerous evidences demonstrate a new adverse effect of chemotherapeutic drugs contributing to treatment failure by acceleration of invasion and metastasis. Here, we investigated the influence of a short-term treatment (24 h) of chemotherapeutic drug oxaliplatin on invasion process, a critical step of metastasis, in a HCC cell line, HepG2. First of all, cytotoxicity of oxaliplatin on HepG2 cells was determined by MTT assay. A selected sub-lethal concentration (1 μM) was used to explore impact of the drug on cancer cell invasion, using Transwell assay. Oxaliplatin treatment significantly increased invasion rate of HepG2 cells, compared with untreated group. Invasion process involves cell migration and extracellular matrix (ECM)-remodeling enzymes. Oxaliplatin treatment also elevated migration rate of HepG2 cells, determined by Transwell assay. Matrix metalloproteinase-2 (MMP-2) is an ECM-remodeling enzyme that plays a role in cancer cell invasion of several cancer types. Gelatin zymography demonstrated the significant increase in level and activity of MMP-2 secreted by HepG2 cells after oxaliplatin treatment, compared with untreated group. In conclusion, the invasion-promoting effect of oxaliplatin could be observed in HepG2 cells after short-term exposure to the drug, and the underlying mechanism of this effect is the enhancement of cell migration capacity and MMP-2 production induced by oxaliplatin. This study might lead to improve the chemotherapeutic strategies and treatment outcome.

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PP0025

Molecular docking study of benzosuberone-thiazole derivatives for rational design of novel GyrB inhibitors as anti-tuberculosis agents

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ABSTRACT

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB). DNA Gyrase is attractive target for antibacterial which is an essential enzyme that has roles in the fundamental biological processes of replication, transcription and recombination. Mutations of DNA gyrase affected to fluoroquinolone drug resistance are the main problem of treating tuberculosis. Therefore, discover of new potential DNA gyrase inhibitors are urgent to overcome this drug resistant problem. Benzosuberone-thiazole moieties were reported for their antibacterial activity against *Mycobacterium tuberculosis* and ATPase activity. In this work, we are objective to evaluate binding mode, binding interaction and binding energy using molecular docking calculations. The result revealed the hydrogen bond interactions of compound 22 and 27 with Arg82 residue in the GyrB pocket. Another hydrogen bond interaction was found to bind between the sulfur atom of thiazole moiety of compound 22 with Arg141 residue of the GyrB active site. The hydrophobic interaction between compound with Val49, Ile84, Pro85, Val99, Val123, Val125 and Ile171 residues were found. Glide docking score was -4.583 for compound 22 and -3.374 for compound 27. In addition, compound 22 has two hydrogen bond interactions to correlate with low Glide docking score. Therefore, the results obtained from this work provide better understanding crucial interactions of benzosuberone-thiazole derivatives with GyrB pocket for rational design new and more potent ATPase inhibitors as novel anti-tuberculosis agents.

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PP026

Polymer-lipid hybrid nanoparticles improve drug-resistant lung cancer treatment

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ABSTRACT

The purpose of this study was to develop polymer-lipid hybrid nanoparticles for delivery of anticancer drug, paclitaxel (PTX) to overcome multidrug resistance (MDR) of human lung cancer cells. The PTX-loaded polymer-lipid hybrid nanoparticles were prepared through modified nanoprecipitation. Nanoparticles are composed of a biodegradable poly(D,L-lactide-co-glycolide) (PLGA) as polymeric core encapsulating PTX, wrapped with a mixture of lecithin and PEGylated phospholipids as lipid shell. The resulting nanoparticles had an average particle size of 103.0 ± 1.6 nm, the zeta potential value of -52.9 mV with monodisperse distributions. Nanoparticles were further evaluated for the *in vitro* cytotoxicity against two MDR models with different resistant mechanisms originated from the same cell line A549. An MDR cell line, A549RT-eto with overexpression of drug transporter P-glycoprotein, was used as a drug-selected MDR cells model. A model of metastasis-associated MDR cells (A549 floating cells) was obtained by culturing A549 cells under a metastasis-mimic condition to induce acquisition of MDR phenotype without experience of drug exposure. Comparisons were made with A549 cells. Nanoparticles presented superior cytotoxicity by increases PTX potency as indicated by the decrease in IC_{50} values compared to non-encapsulated PTX. The IC_{50} values were decreased by 49-fold and 10-fold in A549 and A549RT-eto cells treated with nanoparticles, respectively. The IC_{50} values of A549 attached and A549 floating cells treated with nanoparticles were decreased by 43-fold and 256-fold, respectively. These findings indicated that the PTX-loaded polymer-lipid hybrid nanoparticles provide a promising delivery system for treatment of drug resistant lung cancer.

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PP027

Characterization and quantitative proteomics of mammospheres-forming cells of triple negative breast cancer

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ABSTRACT

Breast cancer is a leading cause of death in women worldwide. Although several therapeutic approaches are combined to successfully cure this disease, some cancer cells can escape and are recurrent, especially in triple negative breast cancer (TNBC). Several lines of evidence suggest that cancer cells which possess stemness-related characters are contributed to drug resistance thereby leading to cancer relapse. Thus, this study aims to establish breast cancer cell – derived mammospheres to investigate its stemness properties and identify key protein changes using a label-free proteomics approach. In this study, mammospheres formation was assay using a TNBC cell line, MDA-MB231. MDA-mammospheres showed upregulated stemness-related genes. Moreover, these mammospheres demonstrated a significant resistance to paclitaxel, a first-line chemotherapy medication for patients with TNBC, compared to that of their parental cells, MDA-MB231. Therefore, protein identification and quantification were performed using a nano-LC coupled with Orbitrap mass spectrometer (LC-MS/MS) and label-free quantitative analysis (Progenesis QI), respectively. Then, almost 200 proteins were identified and compared. With a cut-off value of > 1.5 fold change, there were 37 and 10 proteins that up-regulated and down-regulated in MDA-mammosphers, respectively. Furthermore, the levels of some selected proteins were confirmed by westernblotting. Lastly, all altered proteins were subjected to protein-protein interaction network analysis by STRING database. Several altered proteins expressed in mammospheres were categorized into many biological processes including regulation of cell death, cell activation involved in immune responses, and cytoskeletal reorganization. Collectively, these proteins and predicted molecular pathways enlighten potential markers and therapeutic targets within stemness-possessed breast cancer mammospheres.

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PP0028

Host-derived cell-wall-binding domain of phage endolysin CD16/50L anchors to the surface polysaccharide of *Clostridioides difficile* to preserve neighboring host cells and ensure progeny expansion

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ABSTRACT

Endolysin is a phage-encoded cell-wall hydrolase which degrades the peptidoglycan layer of the bacterial cell wall. The enzyme is often expressed at the late stage of the phage lytic cycle and is required for progeny escape. Endolysin of bacteriophage that infects Gram-positive bacteria often comprises two domains: a peptidoglycan hydrolase and a cell-wall binding domain (CBD). Although the catalytic domain of endolysin is relatively well-studied, the precise role of CBD is largely unknown and remains controversial. For example, while certain endolysins absolutely require CBD for their lytic activity, others do not need it at all, raising the question of its necessity. Here, we focus on the function of CBD of endolysin encoded in the genome of recently isolated *Clostridioides difficile* phages. We found that CBD of this endolysin is not required for the lytic activity, which is strongly prevented by the surface layer of *C. difficile*. Intriguingly, hidden Markov model (HMM) analysis suggested that the endolysin CBD is likely derived from the cell-wall-binding-2 domain of host's cell-wall proteins but possesses a higher binding affinity to polysaccharide components of bacterial cell wall. Moreover, the CBD is able to form a homodimer, formation of which is necessary for interaction with the peptidoglycan layer. Importantly, endolysin diffusion and sequential cytolytic assays showed that CBD of endolysin is required for anchoring to cell-wall remnants, suggesting its physiological roles in controlling diffusion of the enzyme, preserving neighboring host cells, and thereby enabling phage progeny to initiate new rounds of infection. Taken together, this study provides an insight into regulation of endolysin through CBD and can potentially be applied for endolysin treatment against *C. difficile* infection.

Acknowledgement
This research was supported by Science Achievement Scholarship of Thailand (SAST).

PP029

Understanding the binding mode and binding interactions of novel main protease pyridyl ester derivatives with SARS-CoV-2 using molecular docking calculations

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ABSTRACT

Currently, the infectious disease, COVID-19 disease caused by SARS-CoV-2 virus is spreading over a large number. Resulting in major health problems main protease enzymes (M^{pro})have been investigated as a prime drug development target to inhibit SARS-CoV-2 virus. In this study, pyridyl ester derivatives were selected to investigate the binding mode and crucial interactions in M^{pro} binding site due to the inhibitors are equipped with the unique azanitrile warhead exhibited concomitant inhibition of M^{pro} and cathepsin L, a protease relevant for viral cell entry. Glide program was appled to understand the binding mode and binding interactions of pyridyl ester derivatives. The obtained results showed that hydrogen bond interactions were found between carbonyl group of the ligands with Glu166and Cys145 residues. Moreover, hydrophobic interactions with Phe140, Leu141, Met49 and Met165, residues in the SARS-CoV-2 main protease binding site were found as crucial interactions. These results provide fruitful information for further design of pyridyl ester derivatives as SARS-CoV-2 main protease agent.

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PP030

The Major Peanut (*Arachis hypogaea*) Allergens Potentially Contain Antibacterial and Antiviral Peptides: An *In Silico* Study

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ABSTRACT

One of the problems in biomedical science is the emergence of antibiotic resistance among pathogenic microorganisms. This led to the exploration of potential alternative agents to combat this global concern. Bioactive peptides became interesting molecules to study against microorganisms due to its unique microbicidal mechanisms. In this study, the peanut (*Arachis hypogaea*) major allergens (Ara h 1 or A1, Ara h 2 or A2, and Ara h 3 or A3) were enzymatically hydrolyzed *in silico* to generate potential bioactive peptides, which were then screened for their antibacterial and antiviral probabilities using various computational tools. The determined probabilities were correlated with their hydropathicity index (GRAVY) using Spearman's correlation. Sequentially, the residue-level interactions between the peptide and chosen proteins were visualized through molecular docking. Screening showed that the percentages of potential antibacterial and antiviral peptides using iAMPpred were higher than those screened using MLAMP and Meta-iAVP. Analysis revealed that positive monotonic correlations predominated between GRAVY values and bioactive probabilities obtained. Negligible correlations were obtained for antibacterial probabilities of the peptides derived from A1 and A3 using iAMPpred and antiviral probabilities of peptides from A1 and A3 using MetaiAVP and iAMPpred respectively. The antibacterial ^{393}NAHTIVVA^{400} peptide (prob=0.92, GRAVY=1.138) and antiviral ^{64}QCAGVA^{69} peptide (prob=1.00, GRAVY=1.067), both derived from A3, showed docking-based hydrophobic interactions with the *S. aureus* membrane enzyme and dengue virus-1 envelope protein respectively. These hydrophobic interactions were consistent with the correlated hydrophobicity of the bioactive peptides. Further work should focus on determining the *in vitro* antibacterial and antiviral activities of these peptides.

PP0031

Analysis of NaOCl-sensing Transcriptional Repressor NieR in *Agrobacterium tumefaciens* C58

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ABSTRACT

Hypochlorous acid (HOCl) is naturally produced by neutrophils in the innate immune system and is commonly used as the active ingredient of household bleach (sodium hypochlorite) for disinfection of various microorganisms. *Agrobacterium tumefaciens* C58, a plant pathogen causing crown gall disease, was used as a model to study the hypochlorite stress response in bacteria. In this study, a NaOCl-sensing transcriptional regulator (NieR) and its target genes were identified. *A. tumefaciens* NieR belonging to TetR family shares a high amino acid identity with the *Escherichia coli* NemR. The C106 and K175 of *E. coli* NemR protein were reported as NaOCl responding residues, whereas C104 and R166 of *A. tumefaciens* NieR were particularly responsible for sensing NaOCl. NieR was autoregulated and repressed the NaOCl-inducible efflux operon (*nieAB-sdh)*. The results from DNase I footprinting assays revealed the NieR-binding motifs (imperfect inverted repeats), 5′-TAGATTTAGGATGCAATCTA-3′ (boxA) and 5′-TAGATTTCACTTGACATCTA-3′ (boxR) locating in an intergenic region of the divergent *nieA* and *nieR* genes. Electrophoretic mobility shift assays demonstrated that NieR specifically bound to these boxes and NaOCl prevented the NieR-DNA interaction. This implied that NieR was sensitive to NaOCl oxidation. The promoter-*lacZ* fusions and mutagenesis of either boxA or boxR further confirmed a crucial role of these boxes for NieR repression. In addition, the *nieR* mutant exhibited a small colony phenotype and hypersensitivity to NaOCl and antibiotics, including ciprofloxacin, nalidixic acid, novobiocin, and tetracycline. In summary, we report a novel NaOCl-inducible transcriptional repressor NieR that controls an expression of genes encoding efflux pump components in *A. tumefaciens*. Understanding of NaOCl-sensing mechanisms in bacteria may be useful for developing new treatments to combat infectious diseases.

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PP032

Identification and functional characterization of *gaa* gene mutations in Thai patients with infantile-onset pompe disease

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ABSTRACT

Pompe disease is a lysosomal storage disorder caused by the deficiency of acid alpha-glucosidase (EC. 3.2.1.20) due to mutations in human *GAA* gene. Twelve patients with infantile-onset Pompe disease (IOPD) including 10 Thai, one Burmese, and one Vietnamese were enrolled. To examine the molecular characteristics of Pompe patients, *GAA* gene was analyzed by PCR amplification and direct Sanger-sequencing of 20 exons coding region. The novel mutations were transiently transfected in COS-7 cells for functional verification. The severity of the mutation was rated by study of the GAA enzyme activity detected in transfected cells and culture media, as well as the quantity and quality of the proper sized GAA protein demonstrated by western blot analysis. All patients had hypertrophic cardiomyopathy, generalized muscle weakness, and undetectable or <1% of GAA normal activity. Seventeen pathogenic mutations including four novel variants: c.876C>G (p.Tyr292X), c.1226insG (p.Asp409GlyfsX95), c.1538G>A (p.Asp513Gly), c.1895T>G (p.Leu632Arg), and a previously reported rare allele of unknown significance: c.781G>A (p.Ala261Thr) were identified. The rating system ranked p.Tyr292X, p. Asp513Gly and p. Leu632Arg as class "B" and p. Ala261Thr as class "D" or "E". The present study provides useful information on the mutations of *GAA* gene in the underrepresented population of Asia which are more diverse than previously described and showing the hotspots in exons 14 and 5, accounting for 62% of mutant alleles. Almost all mutations identified are in class A/B. These data can benefit rapid molecular diagnosis of IOPD and severity rating of the mutations can serve as a partial substitute for cross reactive immunological material (CRIM) study.

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PP033

Gymnanthemum extensum extracts exerted dose-dependent cytostatic and cytotoxic effects on A549 human lung carcinoma cells

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ABSTRACT

Lung cancer remains one of the primary cancer-related causes of death in both men and women worldwide due to drug resistance and disease recurrence. The limited efficiency of current conventional chemotherapies necessitates the search for new effective anticancer agents. The present study demonstrated anti-proliferative effect and apoptosis-inducing activity of three sesquiterpene lactones isolated from *Gymnanthemum extensum*, including vernodalin (VDa), vernolepin (VLe) and vernolide (VLi), on A549 human lung cancer cells. Treatment with sub-cytotoxic doses (cell viability remaining > 75%) of VDa, VLe and VLi, arrested progression of A549 cell cycle in the G0/G1 phase, while cytotoxic doses of the three compounds induced G2/M phase arrest and apoptosis. Mechanistic studies revealed that VDa, VLe and VLi may exert their anti-tumor activity through the JAK2/STAT3 pathway. Molecular docking study confirmed that VDa, VLe and VLi formed hydrogen bonding interactions with the FERM domain of JAK2 protein. The overall finding of the present study highlights the potential therapeutic value of VDa, VLe and VLi to be further developed as anticancer agents for treatment of lung cancer.

This research was supported by the Thailand Science Research and Innovation, Chulabhorn Research Institute (grant no. 302/2205).

PP034

Proteomic profiling of skin fibroblasts from patients with Parkinson's disease carrying heterozygous variants of glucocerebrosidase and parkin genes

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ABSTRACT:

Parkinson's diseases (PD) is a neurodegenerative disorder affecting movement and balance which mostly found in aged population. While the exact cause of PD is generally unknown, the development and progression of the disease is believed to be associated with environmental and genetic factors. The variants of glucocerebrosidase (*GBA*), a lysosomal enzyme responsible for Gaucher's disease, and parkin (*PARK2*), a "parkin" protein which is a E3 ubiquitin ligase, are important risk factors for developing PD. However, the mechanisms how these mutations cause PD remain largely unknown. In this study, we aim to investigate the protein expression patterns of skin fibroblasts from PD patients carrying heterozygous variants of *GBA* and *PARK2* genes in comparison to those of healthy controls. Proteins extracted from these fibroblasts were analyzed by a liquid chromatography coupled with tandem mass spectrometer (LC-MS/MS). Protein identification and quantification were performed using a label-free quantitative proteomics by Progenesis QI. A number of proteins was identified and compared among all sample groups. Using label-free quantitative proteomic analysis, several proteins with changed in their expressions in *GBA* and *PARK2* groups were compared to those in the healthy controls and some were confirmed by western blot analysis. In addition to those alterations, analysis of protein-protein interactions (PPI) by STRING analysis revealed potential unique molecular features of skin fibroblasts from PD patients carrying heterozygous variants of *GBA* and *PARK2*. This study may provide additional information about protein network regulation and contribution of *GBA* and *PARK2* variants related to PD.

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PP035

Different mechanisms of drug resistance in A549 human lung cancer cells under drug pressure or metastasis-associated conditions

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ABSTRACT

Failure of chemotherapy is due to the drug resistance emerging and the spread of cancer in the body (metastasis). Drug resistance can result from selection of drug-resistant cell populations under drug pressure. Alternatively, during metastasis progression, cellular adaptation to microenvironmental changes can lead to drug-resistant phenotype without prior drug exposure. Both resistances can be co-emerged in the same patient. Here, we explored the differences between two drug resistances originated from the same cell line A549 human lung cancer. A drug-selected cell line A549RT-eto was established from A549 by Etoposide selection (drug pressure condition). In metastasis-associated resistance model, A549 cells were cultured under non-adherent conditions without drug (metastasis-associated condition) to obtain A549 floating cells, which mimic adaptation of metastasizing cancer cells in lymphatic vessels. Comparing to A549 parental attached cells, the Etoposide and Paclitaxel resistances in A549RT-eto cells were 17.4-fold and 1.8-fold, respectively, while the resistances in A549 floating cells were 11.6-fold and 57.8-fold, respectively. Immunoblot detection showed an increased expression of a drug transporter ABCB1 (MDR1/P-gp) by 1.9-fold in A549RT-eto cells compared to A549 parental attached cells, while these change was not found in A549 floating cells. qRT-PCR analysis revealed that A549 floating cells up-regulated expression of a Paclitaxel-resistant β-tubulin isotype βIVa by 2.0-fold compared to A549 parental attached cells, while expression level of the gene in A549RT-eto cells was 0.3-fold. In conclusion, drug-selected and metastasis-associated resistances developed from the same cell line exhibited different drug-resistant profiles and its underlying mechanisms, and this might occur with cancer patients.

This research was supported by the Thailand Science Research and Innovation, the Chulabhorn Research Institute (grant no. 302/2128), and the Center of Excellence on Environmental Health and Toxicology (EHT).

PP037

Plasma proteomic profiling identifies novel biomarkers for cholangiocarcinoma

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ABSTRACT

Cholangiocarcinoma (CCA) is a malignant tumor derived from bile duct epithelium which occurs with relatively high incidence in Thailand. CCA is also highly lethal because most are locally advanced at patient presentation where therapies have limited benefit. Currently available tumor markers, e.g., CA19-9 and CEA, are not specific to CCA, thereby novel CCA biomarker is an unmet need. We explored the feasibility of a translational proteomic approach on CCA biomarkers discovery. The possible candidates were obtained from our previous work, together with the label-free quantitation technique which employed on 27 plasma specimens (9 CCA, 9 disease controls and 9 normal individuals). After immunoblot verification, four interesting proteins (A, B, C and D) were selected and translated into clinically compatible ELISA immunoassay to examine the diagnostic performances in a larger cohort (n=63; 26 CCA vs. 37 non-CCA including 17 disease controls and 20 healthy controls). Receiver Operating Characteristics showed that protein A had higher performance with the area under the curve (AUC) of 0.835 (80.8% sensitivity, 83.8% specificity) as compared to other proteins. Among the combined indexed models, a combination of protein A, protein B and protein D enhanced the diagnostic performance with the AUC of 0.849 (76.9% sensitivity, 89.2% specificity). Our results supported that the combination of three proteins (A, B and D) hold good promise as a potential multiplexing biomarker of CCA, in which the real performance should be further validated in an independent cohort or multicenter study. Translational proteomic approach is feasible for CCA biomarker investigation.

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PP038

7-Methoxyheptaphylline Sensitizes TRAIL-induced Colorectal Adenocarcinoma Cells Death through Up-Regulation of DR5 Expression by Activation of JNK

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ABSTRACT

TRAIL (TNF-related apoptosis-inducing ligand) is a cytokine that can selectively induce apoptosis in cancer cells without damaging normal cells. Regarding current research findings, a number of cancer cells are resistant to TRAIL-induced apoptosis. This study was designed to investigate the molecular mechanisms of the antitumor activity of heptaphylline and 7-methoxyheptaphylline from *Clausena harmandiana* on TRAIL-treated HT29 colorectal adenocarcinoma cells. The quantification of cell viability was performed using a cell proliferation assay (MTT assay) and cell morphology was investigated by phase contrast microscopy. The molecular mechanisms were studied by Western blotting, RT-PCR and real-time RT-PCR. The results showed that 7-methoxyheptaphylline induced cancer cell death in a concentration dependent manner without cytotoxic effect on normal colon FHC cells, while hepataphylline showed cytotoxicity to normal cells. In combination with TRAIL, heptaphylline had no significant effects on TRAL-induced HT-29 cell death, while the cleavage of caspase-3 and PARP-1 was increased when combining 7-methoxyheptaphylline with TRAIL. The investigation of mechanism found that 7-methoxyheptaphylline increased TRAIL receptor, death receptor 5 (DR5) mRNA and protein through the JNK pathway. The findings suggested that 7-methoxyheptaphylline of *Clausena harmandiana* sensitized TRAIL-induced HT29 cell death by increasing expression of DR5 via the JNK pathway.

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PP039

Anticancer effect of protocatechuic aldehyde

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ABSTRACT

Protocatechuic aldehyde (3,4-dihydroxybenzaldehyde) is a water-soluble compound found in the root of *Salvia miltiorrhiza* which is a herbal plant used in traditional Chinese medicine to treat cardiovascular and liver diseases. This study have evaluated anticancer effect of protocatechuic aldehyde *in vitro* and elucidated its mechanism of action. WST-1 assay was used to determine growth inhibitory effect of protocatechuic aldehyde and its structural-related compounds including protocatechuic acid, vanillin, and vanillic acid on 4T1 mouse mammary adenocarcinoma cell line. After 48 h treatment, IC_{50} value of protocatechuic aldehyde was significantly lower than IC_{50} of vanillin (~187 fold). Caspase-3 activation and PARP cleavage were observed in 4T1 cells when treated with protocatechuic aldehyde or vanillin, indicating induction of apoptotic cell death. Effect of protocatechuic aldehyde and vanillin on MAPKs (ERK, p38, JNK) and PI3K/Akt signaling pathways were determined by western blot analysis. Both protocatechuic aldehyde and vanillin could decrease basal level of Akt phosphorylation, but had no effects on p38 phosphorylation. Protocatechuic aldehyde inhibited Erk phosphorylation and activated JNK phosphorylation, whereas vanillin did not have such effects. These might be an explanation for the large fold difference between IC_{50} values of the two compounds in 4T1 cells.

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PP040

Proteomic analysis of anti-cancer effects by 5'-deoxy-5'-methylthioadenosine treatment in cholangiocarcinoma cell line

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ABSTRACT

The treatment of advanced cholangiocarcinoma (CCA) is mostly ineffective due to the intrinsic and acquired chemotherapeutic drug resistance phenotypes. Clearly, a novel and effective therapies are urgently needed. 5'-deoxy-5'-methylthioadenosine (MTA) is a natural-derived bioactive compound that has been shown to possess anti-cancer activity in various human cancers. In the present study we demonstrated the MTA exhibited a potent cytotoxic effect against a CCA cell line, KKU-213A in a dose dependent manner. In addition, it strongly inhibited cell migration and invasion of CCA cells. Proteomic analysis using LC/MS/MS identified the total of 5,297 proteins. In which, 507 were identified only in untreated cells, 710 were found only in MTA treated cells, whereas 4,080 were expressed in both. The 25 most up-regulated and down-regulated proteins in MTA-treated KKU-213A cell line were displayed using heatmap analysis. The results showed that MTA significantly suppressed numbers of oncoproteins such as RYR1, RCOR2, KLC1, GRAMD1C, and HLA-DQB1. Protein-protein interaction analysis of 25 most down-regulated proteins predicted 3 major clusters namely calcium ion transmembrane transport, antigen processing and presentation of exogenous peptide, and microtubule motor activity. These clusters were reported to involved in tumor progression and they might be the main target of MTA. The detailed study of molecular mechanisms underlying MTA effect on CCA cells both *in vitro* and *in vivo* should be further explored with the anticipation that this promising natural bioactive compound might be an alternative treatment for CCA in the future.

PP041

Expression and characterization of recombinant alkaline protease from *Aspergillus sojae* in yeast *Pichia pastoris*

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ABSTRACT

Alkaline protease (AP) is a group of enzymes that breaks peptide bonds within polypeptide chain under alkaline condition. It has been extensively used in many industries in particularly food and detergent industries. AP can be obtained from wide variety of alkalophilic microorganisms including bacteria and fungi. Yeast expression system has been utilized for production of recombinant protein in large scales. In this study, we produced a recombinant alkaline protease (rAP) via cloning AP gene from *Aspergillus sojae* (*A. sojae*) into pPIC9K expression vector and introduce into the yeast *Pichia pastoris* KM71. The rAP was successfully expressed and secreted to culture medium. The resultant rAP was composed of 389 amino acids and had molecular weight of approximately 35 kDa. Two positive clones, rAP-3 and rAP-4, were further analyzed and showed positive on skim milk agar plate. Folin-phenol method using casein as a substrate revealed that the enzyme activity of rAP-3 and rAP-4 were 2.966 and 3.923 U respectively. The rAPs had higher enzyme activity as compared with the commercial alkaline proteases from *Bacillus Subtilis* (*B. subtilis*) whose enzyme activity was 2.026 U. SDS-PAGE analysis showed that rAP-3 and rAP-4 completely digested protein casein and protein from cell line lysate. This result suggests that rAP might have broad range of substrate specificity. In summary, we successfully expressed AP gene from *A. sojae* in *P. pastoris* in the form of secreted enzymes in high quantity and satisfactory activity. This established platform could be used in various applications in the future.

PP042

Proteomic analysis of 5-Fluorouracil resistant cholangiocarcinoma cell line

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ABSTRACT

Resistance to commonly used chemotherapeutic drugs is a major problem of cholangiocarcinoma (CCA) treatment, therefore more effective treatment strategy is urgently needed. Drug resistant cell lines have been used for elucidating drug resistance mechanism in various cancers leading to the discovery of novel treatments. Here in, we have established 5-Fluorouracil resistant (FR) CCA cell line by culturing the parental CCA cell line (KKU-213A) in the stepwise increased concentration of 5-Fluorouracil (5-FU). The established cell line was designated as KKU-213A-FR, confirmed for drug resistance, and use for further analyses. The results showed that, under low serum condition, KKU-213A-FR had slower growth rate than its parental cell line. Conversely, cell migration and invasion ability were increased significantly. Moreover, the proteomic analysis using LC/MS/MS identified the total of 5,680 proteins. In which, 431 were identified only in parental cells, 1,105 were found only in FR cells, whereas 4,144 were expressed in both cell lines. Heatmap analysis showed the 25 most up- and down-regulated proteins in KKU-213A-FR. The up-regulated proteins were further analyzed. Gene ontology revealed that most of these proteins were classified in cellular process group. The protein-protein interaction analysis showed 3 clustering groups that involve in N-linked glycosylation, ubiquitin-dependent protein catabolic process, and organic substance catabolic process. In conclusion, the KKU-213A-FR exhibits aggressive phenotypes and a unique proteomic profile as compared to KKU-213A. More in-depth study on the differentially expressed proteins may lead to better understanding of drug resistance mechanism and more effective treatment for CCA.

PP043

Label-free quantitative proteomic analysis of plasma protein fractionation using multiple immunoaffinity chromatography revealed biomarker candidates of patients with colorectal cancer

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ABSTRACT

Colorectal cancer (CRC) is one of the most common cancer worldwide. Globocan reports in 2020 reveal that it is third ranked incidence (10.0%) and second ranked mortality (9.4%) among all cancers. At present, carcinoembryonic antigen (CEA) is still the only blood-based biomarker approved by the U.S. FDA for colorectal cancer screening when used with other diagnostic means. This is because the CEA level can sometimes be normal in CRC patients and can be abnormal for reasons in other cancers. Nevertheless, searching specific biomarkers from liquid biopsy specimens such as plasma and serum is still an ideal non-invasive approach for primary screening of CRC. However, seeking biomarkers in plasma/serum are challenging because numerous proteins were overshadowed by high abundant proteins such as albumin and immunoglobulins. In our present work, a multiple immunoaffinity chromatography was applied to fractionate pooled plasma samples of healthy control, non-metastasis, and metastasis CRC patients into high abundant and low abundant protein fractions. Each fraction was then analysis by a label-free quantitative proteomics approach using LC-MS/MS and Progenesis QI software. Among 167 MS/MS-identified proteins, 10 proteins showed accumulated changes in a stage-dependent manner and 5 prominent candidates were validated by western blotting and will be subjected for further validation in an independent cohort study. It is hoped that our techniques will be useful in order to find more specific biomarker candidates of CRC patients.

This study was supported by the Chulabhorn Research Institute (Grant no. 302-2098).

PP044

Identification of Potential Key (Hub) Genes Associated with Prognosis of Triple-Negative Breast Cancer in Asian versus non-Asian Populations Based on Bioinformatics Analysis

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ABSTRACT

Introduction: Triple negative breast cancer (TNBC) is a heterogeneous disease associated with advanced stage and poor prognosis. There are no prognostic biomarkers of TNBC, especially in Asian women. We aimed to identify the potential key genes and their prognosis association in Asian TNBC patients through bioinformatics. **Materials and Methods:** Microarray datasets of TNBC patients were downloaded from gene expression omnibus (GEO) database. Differentially expressed genes (DEGs) between Asian and non-Asian populations were analyzed. The upregulated DEGs were selected as inputs for Gene Ontology and pathway enrichment analyses using Metascape. The significant modules from protein–protein interaction (PPI) network were screened by MCODE. UALCAN and GEPIA databases were analyzed the prognostic values of the hub genes. **Results:** In Asian population, 686 up-regulated genes were identified and mainly enriched in various pathways including vesicle-mediated transport and membrane trafficking, apoptotic signaling and T cell activation pathways. A PPI network was constructed with 588 nodes and 2,351 edges. Eleven hub genes from the significant module were related to the metabolism of RNA and mRNA splicing which highly expressed in breast cancer patients. High expression levels of *SEC61G, MRPL13* and *LSM5* genes were significantly associated with poor overall survival.
Conclusion: These genes might be beneficial for prognostic markers development TNBC patients.

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PP045

Analysis of the molecular mechanism in metastasis osteosarcoma using gene expression re-analysis

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ABSTRACT

Background: Survival rate of osteosarcoma has remained plateaued for the past three decades and mechanism of progression remain unclear. Therefore, our aim is to explore the mechanism and identify new therapeutic targets for metastasis osteosarcoma by using gene expression profile.
Materials and Methods: Metastasis osteosarcoma gene expression microarray data were retrieved from available database. Differential gene expression with ratio ≥ 4 and adjusted *p-value* < 0.05 were identified as primary candidate genes (PCG). PCG were further used to find secondary candidate genes (SCG) which involved in pathways. PCG and SCG were matched with genes target from the Drug repurposing hub. Finally, expression of potential genes and pathway were explored by western blotting.
Results: Eighty-one genes were identified as PCG which 4 genes were able to match with 6 drugs. Sixty genes corresponding to top ten pathways were identified as SCG. However, only 3 pathways (negative regulation of anoikis, regulation of anoikis, surfactant metabolism) with 8 genes were matched with 77 Drugs. CAV1 and CAV2 were identified as PCG and found in anoikis resistance pathway which may play an important role in metastasis disease including osteosarcoma. We found that the CAV1 and CAV2 protein were down regulated in 143B osteosarcoma cell seeded on poly-Hema treated wells (as anoikis resistance condition) and reversed back to the normal levels when the anoikis resistance cells were re-attached.
Conclusion: CAV1 and CAV2 might play role in anoikis resistance in osteosarcoma. However, further validation of downstream molecular mechanism and drug inhibition is needed.

This research was financially supported by the Research Fund of the Faculty of Medicine, Prince of Songkla University (grant no. REC 63-351-4-2); partially supported by the Musculoskeletal Science and Translational Research Center (MSTR), Chiang Mai University; and the National Science and Technology Development Agency (NSTDA), code P-18-5199.

PP046

Frequency distribution of triple negative breast cancer subtype according FUSCC classification using immunohistochemistry and correlation with survival outcome

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ABSTRACT

Introduction: Triple negative breast cancer (TNBC) is a heterogeneous disease and aggressive behavior. Recently, the molecular subtype of TNBC has been studied by transcriptome profiling identified into 4 subtypes by FUSCC classification as follow: luminal androgen receptor (LAR), mesenchymal-like (MES), basal-like immune-activated (BLIA), and immunomodulatory (IM) subtypes. The purpose of this study was to identify the expression of the subtype-specific-protein markers in order to classify the subtype of TNBC patients. **Materials and Methods:** We included patients diagnosed with TNBC at Songklanakarind hospital between 2016 and 2019. The tissue microarray was constructed and performed IHC stained for androgen receptor (AR), doublecortin Like Kinase 1 (DCLK1), cluster of differentiation 8 (CD8), and forkhead Box C1(FOXC1) antibodies to classify the TNBC subtype regarding FUSCC classification. **Results:** We included 50 TNBC patients, with a mean age of 50 years old (33-77 years). The histological subtype was infiltrating duct carcinoma, not otherwise specified (NOS) (62.0%), and ductal carcinoma, NOS (32.0%). Most of the TNBC patients had high grade (3 grade = 72.0%). The distribution of 50 TNBC samples according to FUSCC classification were classified as LAR subtype (AR^+, n = 9; 18.0%), MES subtype (DCLK1 positive, n = 3; 6.0%), IM subtype (CD8 positive, n = 14; 28.0%), BLIS subtype (FOXC1 positive, n = 23, 46.0%) and unclassifiable type (n = 1; 2%). **Conclusion**: This study revealed the distribution of TNBC subtype in southern Thai population. Molecular subtypes obtained may be beneficial for targeted therapeutic guideline in TNBC patients.

This research was supported by grant from the Research Fund of the Faculty of Medicine, Prince of Songkla University, and Health Systems Research Institute, Thailand (grant no, HSRI 63-107).

PP047

Investigation of multidrug resistance protein expression profile in HepG2 cells and microparticles induced by hypoxia

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ABSTRACT

Tumor microenvironment such as hypoxia impact tumor aggressiveness and treatment outcome. Hepatocellular carcinoma remains one of the most challenging cancers to treat, as indicated by its high mortality and recurrence rate. One of the main obstacles to treatment is decrease efficacy of chemotherapeutic drugs due to increase multidrug resistance (MDR). As the tumor grows, the mass is subjected to temporal and spatial hypoxia – a low O_2 condition. Cells experiencing hypoxia can release cellular vesicle mediators called extracellular vesicles (EV) that imparts cell survival mechanisms to nearby cells. We hypothesize that cell survival mechanism such as MDR may be transferable through release of EV induced under hypoxic condition.

HepG2 cells were cultured under hypoxic (1% O_2) or normoxic (20% O_2) conditions for 24 to 72 hours. Expression of HIF-1α as a hypoxic marker was determined by Western blotting. Increased survival rate of HepG2 cells when treated with Sorafenib, Doxorubicin and Curcumin were observed under hypoxic condition. Interestingly, hypoxic conditioned media was able to increase survival rate of normoxic cells by roughly 20%. We, therefore, believe hypoxic-induced drug resistance of the cells are transferred to conditioned media through EV and set out to elucidate the underlying mechanism(s). Profile of drug transporters including MDR1, MRP1 and MRP3 were analyzed on cells under normoxic and hypoxic conditions and on extracted microparticles, using flow cytometry. We found that both cells and microparticles displayed differential expression of drug transporter profile, suggesting that hypoxia may enhance transfer of MDR transporters from cells to microparticles.

PP048

In silico repurposing and side effect studies of first-and second-generation antipsychotic drugs in methamphetamine addiction treatment

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ABSTRACT

Methamphetamine (METH) is an illicit psychostimulant that is widely abused and associated with psychological morbidity. METH performs its function directly through the central nervous system, especially on the brain reward circuitry. METH passes through a dopamine transporter and induces excess dopamine discharging. Consistently, antipsychotic drugs can efficiently bind with D2 receptors. Thus, these drugs may be able to prevent the effects of dopamine overstimulation caused by METH. Previous studies in mice indicated that antipsychotic drugs may be used as an intervention for METH-induced sensitization and hyperlocomotion. Therefore, this project is focused on the repurposing of antipsychotic drugs including prochlorperazine, haloperidol, olanzapine, zotepine, and aripiprazole in addiction using computational methods to estimate the binding affinity between drugs and binding sites of the dopaminergic D2 and serotonergic 5-HT2A receptors. Molecular docking scores were calculated using the Scoring Function of the AutoDock Vina. According to the results, five tested antipsychotic drugs displayed a higher binding affinity to the D2 receptor when compared to dopamine. They can inhibit the binding of dopamine towards the D2 receptor and thus suppress neuronal overstimulation. Considering appropriate medications, the most suitable antipsychotic drug is determined by the lower side effects indicated by lower D2 and higher 5-HT2A binding affinity. In this study, the first most appropriate drug is prochlorperazine; a typical antipsychotic. The second is olanzapine; an atypical antipsychotic. In addition, the relationship between molecular interactions and binding affinities was also studied, and found that H-bonds, hydrophobic contacts, and amino sequences are associated with the binding affinity values.

Keywords: Antipsychotic drugs; Dopamine D2 receptor; Methamphetamine; Molecular docking; Serotonin 5-HT2A receptor

PP049

Develop *in vivo* and *in vitro* coupling strategies to produce nicotinamide mononucleotide

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ABSTRACT

Nicotinamide mononucleotide (NMN), a ribonucleotide, is a key intermediate in the biosynthesis of coenzyme, nicotinamide adenine dinucleotide (NAD^+). Recently, NMN has gained lots of attention for self-medication as a nutraceutical. However, the *in vitro* biosynthesis of NMN requires expensive substrates, which makes this approach is difficult for large scale production. Therefore, we tried to develop a pathway with lower cost. In this project, the biosynthesis of NMN could be divided into two modules. The first module is to produce ribose from xylose by engineered *Escherichia coli*. In the first module, we conducted CRISPR-Cas9 to knock out two transketolase genes (*tktA* and *tktB*) and one gene (*ptsG*) encoding glucose-specific PTS enzyme IIBC component in *E. coli* MG1655. The engineered *E. coli* MG1655 could produce 2.47 g/L of ribose from 5g/L of xylose in LB medium after 48 hours. The second module is to construct *in vitro* biosynthetic pathway to convert ribose to NMN. The pathway involves *E. coli* ribose kinase (*Ec*RbsK), *E. coli* PRPP synthase (*Ec*PRPP), and *Chitinophaga pinensis* nicotinamide phosphoribosyl transferase (*Cp*Nampt) to convert ribose in the supernatant of engineered *E. coli* MG1655 medium to NMN with the incubation of excess ATP and stoichiometric nicotinamide. To reduce ATP cost, polyphosphate kinase was incorporated in the reaction to regenerate ATP from AMP and ATP using *Cytophaga hutchinsonii* polyphosphate kinase (PPK2). Furthermore, to improve the yield of NMN, the *Ec*PRPP inhibitor of pyrophosphate was hydrolyzed by the addition of Ppase. With all effort, the developed system could produce NMN from Nam with about 70% yield using the supernatant of engineered *E. coli* MG1655 medium. Currently, we continue to optimize the production protocol.

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PP050

Computer Aided Molecular Design of FabI1 Inhibitors as Anti-melioidosis Agents from Natural Product Compounds of *Morinda coreia* Root

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ABSTRACT

Melioidosis caused by *Burkholderia pseudomallei* (*B. pseudomallei*) is top infection diseases of Northeastern part, Thailand. This disease is difficult to manage and often fatal in humans. Herein, we applied computer aided molecular design to evaluate thirteen natural product compounds extracted from *Morinda coreia* root as FabI1 inhibitors of *B. pseudomallei*. The obtained results demonstrated that natural product compounds extracted from *Morinda coreia* root were promising compounds for *B. pseudomallei* FabI1 inhibition. Based on docking calculation, all natural compounds from *Morinda coreia* root were favorable for binding in FabI1 binding site with the docking score ranging from -4.52 to -10.39 kcal/mol. Hydrogen bond and sigma-pi interactions were found as the crucial interaction for binding in FabI1 binding site. The anti-bacterial prediction revealed that all compounds were acted as anti-bacterial agents with the predicted values from 0.0628-0.3181 and 0.0846-0.4625 for *B. pseudomallei* and resistant-*B. pseudomallei*, respectively. Therefore, these finding results aided to identify natural compounds and plant for developing of novel anti-melioidosis agents

This research was supported by the Faculty of Science, Ubon Ratchathani University. National Electronics and Computer Technology Center (NECTEC) is gratefully acknowledged for supporting this research.

PP051

Proposal of JAK2 Inhibitors from Natural Chalcone Derivatives as Erythropoiesis Stimulant Agents for Thalassemia Therapy: Biological Predictions, Molecular Docking and Pharmacokinetic Predictions

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ABSTRACT

The ineffective erythropoiesis inhibition by JAK2 inhibitors has been developed as novel thalassemia therapy. Herein, we applied the biological predictions, molecular docking, and pharmacokinetic predictions to identify natural chalcone as JAK2 inhibitor and erythropoiesis simulating agent for developing as novel thalassemia drug candidate. Seven natural chalcone derivatives were identified as JAK2 inhibitors with erythropoiesis simulating agent property. In addition, these finding compounds were strongly bound with JAK2 binding site which the Glide XP docking score ranging from -8.27 to -7.23 kcal/mol. The main interaction of chalcone derivative is hydrogen bond interactions between an oxygen carbonyl or an oxygen atom of meta-substitution on chalcone analog with NH backbone of Leu932. The pharmacokinetic properties predictions demonstrated that collected compounds were suitable for acting as drug. Therefore, these finding results aid to collect the potential compounds for biological assay evaluations and development as novel drug for thalassemia therapy.

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PP052

Screening of JAK2 Inhibitors from Natural Curcumin and Its Derivatives as Erythropoiesis Stimulant Agents for Thalassemia Therapy: Computer Aided Molecular Design Approaches

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ABSTRACT

JAK2 inhibitors has been validated as novel therapeutic process for thalassemia. This research aimed to developed curcumin and its derivatives as JAK2 inhibitors and erythropoiesis stimulant agents. The biological predictions, molecular docking, and pharmacokinetic predictions were applied on curcumin derivatives and these processes revealed that eleven curcumin derivatives will be act as JAK2 inhibitors with the high binding affinity in JAK2 binding site based on Glide XP docking score ranging from –9.65 to -12.09 kcal/mol. Hydrogen bond interaction with NH backbone of Leu932. In addition, sigma-pi interaction of aromatic ring on curcumin analog with Leu983 sidechain. The ADMET predictions suggested that curcumin derivatives were suitable for acting as drug. Therefore, these finding results aid to collect the potential compounds for biological assay evaluations and development as novel drug for thalassemia therapy based on JAK2 inhibition mechanism.

This research was supported by National Science, Research and Innovation Fund (NSRF), the Faculty of Science, Ubon Ratchathani University and Faculty of Science, Nakhon Phanom University. National Electronics and Computer Technology Center (NECTEC) is gratefully acknowledged for supporting this research.

PP053

Flavans from *Desmos cochinchinensis* as Highly Potent FabI1 Inhibitors of *Burkholderia pseudomallei*: *In silico* based Rational Design Approaches

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ABSTRACT

Burkholderia pseudomallei (*B. pseudomallei*) is the etiologic agent of melioidosis. Herein, we applied *in silico* based rational design approaches to evaluate natural flavans extracted from *Desmos cochinchinensis* as FabI1 inhibitors. The obtained results suggested that three natural flavans, (2*S*,4*R*)-2-Phenyl-5,7-dimethoxy-6,8-dimethylchroman-4-ol (**1**), (2*S*)-5-Hydroxy-7-methoxy-6,8-dimethylflavanone (**2**) and (2*S*)-5-hydroxy-7-methoxy-8-methyl-2-phenyl-2,3-dihydrochromen-4-one (**3**) extracted from *Desmos cochinchinensis* were promising compounds with the anti-bacterial prediction against resistant-*B. pseudomallei* ranging from 0.0204-0.0912. The highest binding affinity was found between flavan compound **2** (-10.07 kcal/mol) with *B. pseudomallei* FabI1 and hydrogen bond interaction between hydroxyl (OH) with an oxygen carbonyl backbone of Gly93 and pi-pi interaction of phenyl ring with Phe203 sidechain were suggested as the crucial interaction for binding. Therefore, these results aided to identify natural compounds and medicinal plant for developing of novel anti-melioidosis agents.

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PP054

Potential FabI1 Inhibitors of *B. pseudomallei* from the heartwoods of *Mansonia gagei* Drumm.: Biological prediction and molecular docking calculations

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ABSTRACT

In this research, we applied biological prediction and molecular docking calculation to evaluate natural compounds extracted from *Mansonia gagei* Drumm.as FabI1 inhibitors of *B. pseudomallei,* the novel and promising target for melioidosis treatment. Six compounds, Mansorin B, Mansorin C, Mansonone C, Mansonone G, Mansonone E and Mansonone H were identified as potential FabI1 Inhibitors of *B. pseudomallei* based on anti-bacterial prediction and molecular docking calculations. All compounds were active against resistant-*B. pseudomallei* with the prediction value ranging from 0.0329 to 0.2859. Mansorin B was highest binding affinity with -4.93 kcal/mol for binding in FabI1 binding site. The crucial interaction is hydrogen bond interaction of an oxygen carbonyl on Mansorin B with hydroxyl (OH) of Tyr156 sidechain. In addition, hydrophobic interaction was improved the binding affinity in the FabI1 binding site of *B. pseudomallei*. Therefore, these results aided to identify natural compounds for developing of novel anti-melioidosis agents.

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PP055

Molecular Modeling Analysis for Purposing Depsidones and Diaryl Ethers from The Endophytic Fungus *Corynespora cassiicola* L36 Against *Burkholderia pseudomalle* FabI1

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ABSTRACT

An infectious disease, melioidosis caused by the bacterium *Burkholderia pseudomallei* is public health problem of people from Northeastern part of Thailand. Herein, we applied molecular modeling approaches to evaluate depsidones and diaryl ethers from the endophytic fungus *Corynespora cassiicola* L36 as FabI1 inhibitors. Five compounds based on were identified as potential FabI1 inhibitors which displayed the biological activity prediction against resistant- *Burkholderia pseudomallei* (0.0294 - 0.2629). The binding affinity based on docking score raining from -5.15 to -6.92 kcal/mol. 2,3',4-Trihydroxy-5',6-dimethyldiphenyl ether was highest binding affinity which shown H-bond interaction with NH and an oxygen carbonyl of Gly96 backbone in FabI1 binding site. In addition, H-bond interaction of OH of diphenyl ether with OH of NAD^+ was obtained. Consequently, these results aided to identify natural compounds and understand the binding mechanism for developing of novel anti-melioidosis agents based on FabI1 Inhibitors.

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PP056

Discovery of novel JAK2 inhibitors as erythropoiesis stimulant agents for thalassemia therapy through virtual screening approaches

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ABSTRACT

JAK2 is an enzyme responsible for regulating erythropoiesis associated with the ineffective erythropoiesis process. This is currently a new key target for developing drugs with the new therapeutic option for thalassemia. Herein, we applied the ligand and structure based virtual screening to identify novel JAK2 inhibitors for erythropoiesis stimulating agent (ESA). The obtained results demonstrate that seven compounds were predicted to be active against both JAK2 and ESA and showed strongly bound with JAK2 binding site. In addition, the binding mode, and binding interactions in the ATP binding site of JAK2 were investigated. The obtained results revealed that the hydrogen bond interactions with Leu932 backbone in the ATP binding site of JAK2 are key interaction for binding of new finding compounds. Therefore, the finding compounds were proposed as novel and potential JAK2 inhibitors as ESA for thalassemia therapy.

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PP057

Understanding binding modes and binding interactions of 4-oxocrotonic acid as highly potential PknB inhibitors through computer aided drug design

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ABSTRACT

The *Mycobacterium tuberculosis* Ser/Thr protein kinases B or PknB is essential for growth and survival of the pathogen in vitro and in vivo. 4-oxocrotonic acid derivatives which is majority anti-TB activity of minimum inhibitory concentrations (MICs) is falling in micromolar range and significantly higher than other reported PknB inhibitors selected to study. Molecular docking calculations of 4-oxocrotonic acid derivatives were performed via Autodock 4.2 program to get better understanding binding modes and binding interactions in PknB binding site. The result illustrated the hydrogen bond interaction was found between oxygen atom of ligand with carbonyl group of Val95 residue. Another hydrogen bond interaction was formed between carbonyl group of core structure of ligand with carbonyl backbone of Val72 residue. In addition, hydrophobic interaction between ligand and Leu17, Val25, Ala38, Val72, Met92, Tyr94, Met155 and Asp156 residues were found. The obtained result provides beneficially information for further modification of 4-oxocrotonic acid derivatives with highly and more potent against *mycobacterium tuberculosis*.

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PP058

A protein engineering to create the high thermostable formate dehydrogenase enzyme for biocatalysis applications

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ABSTRACT

Formate dehydrogenase (FDH) catalyzes the generation of NADH from NAD^+ by oxidation of formate to carbondioxide. The enzyme is useful for coenzyme regenerating systems, in which NADH is continuously generated by the FDH enzyme to support the main synthesis enzyme reaction in biotransformations. The novel FDH from *Bacillus simplex* (BsFDH) was recently characterized and revealed the highest catalytic efficiency. However, the major painpoint of BsFDH is low thermostability. Therefore, in this study, we employed a FireProt computational prediction to rational engineer to improve the thermostability of BsFDH. The program suggested 61 variants that may have more thermostability than the wild-type. By complement three criteria, we selected 9 candidate variants to perform site-directed mutagenesis and 7 variants were successfully overexpressed and purified. The enzyme activity and thermostability were investigated in comparison with wild-type enzyme. The results showed that the Q125L, A216I, S50L, and T213C exhibited comparable activity to the wild-type enzyme, while only Q125L had higher thermostability than the wild-type. At 55ºC incubation, the Q125L was able to retain more than 50% after 6 h of incubation, while the wild-type activity dropped 35% activity. This result suggests that the Q125L variant of the BsFDH might be suitable for biosynthesis.

Keyword: Thermostability, Formate dehydrogenases, Biocatalysis, Coenzyme regenerating system, FireProt

PP059

Characterization of a cellobiohydrolase from the thermophilic bacterium *Thermothelomyces thermophilus* (TtCel7) produced in recombinant *Escherichia coli*

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ABSTRACT

Cellulose is a major source of biomass monosaccharide, which may be applied as a feedstock for industrial biorefinery. Cellulose is a linear polymer with glucose monomers linked exclusively by β-1,4 glycosidic bonds, tethered to other molecules by a hydrogen bonding network that joins individual cellulose polymers to form crystalline cellulose. Cellobiohydrolases play an important role in hydrolysis of cellulose to smaller molecules, along with endoglucanases and β-glucosidases. In this study, we produced cellobiohydrolase from thermophilic *Thermothelomyces thermophilus* and its recombinant protein TtCel7A, which is a member of glycoside hydrolase family 7. TtCel7A was simply expressed in E. coli system (Origami(DE3)), purified and biochemically characterized. TtCel7 exhibited cellobiohydrolase activity against cellooligosaccharides (C3 to C6), microcrystalline cellulose from commercial and natural sources, respectively, under optimal conditions of 40-50 °C and pH 5.5. It retained over 80% residual activity after incubation at 60◦C for 24 hours. TtCel7A has ability to work in high methanol and ethanol concentrations, and displayed over 80% residual activity after incubated at 20% ethanol or methanol for 30 min. TtCel7A is also active to release glucose and cellooligosaccharides in the presence of various metal ions. TtCel7A hydrolysis of cellotetraose releases glucose, cellobiose, and cellotriose, suggesting that cellotetraose can bind in multiple positions to produce different products. However, the breaking down of cellooligosaccharide and pre-treated agricultural biomass (rice straw, rice husk, and sugarcane leaf) confirmed that cellobiose is the main product of TtCel7 hydrolysis. The properties of TtCel7 make it a potential biocatalyst for the conversion of biomass in contaminated conditions for practical industrial applications and simultaneous saccharification and fermentation conditions to convert agricultural wastes to valuable compounds.

This work is supported by the Thailand Research Fund, the Synchrotron Light Research Institute and Suranaree University of Technology.

PP060

Enzymatic synthesis of glycosylated compounds by rice Os9BGlu31 transglucosidase and its mutants

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ABSTRACT

Rice Os9BGlu31 (EC 3.2.1.21) is one of enzyme in glycoside hydrolase family 1 (GH1), a family that mostly catalyzes hydrolysis reactions. Os9BGlu31, however, mainly has transglycosylation activity that can transfer a glucosyl moiety to another aglycon moiety to form new glycosylated compounds (glycoconjugates) through a retaining mechanism. This reaction may improve the bioactivity, stability, solubility, and physicochemical and physiological properties of the compounds, such as promising functional compounds and pharmaceuticals. In this study, we investigated the ability of rice Os9BGlu31 transglucosidase for glycosylation of phytosterol and phenolic acids to synthesis glycosides or glucosyl esters. Os9BGlu31 and its mutants were expressed in *Escherichia coli* strain Origami B(DE3), then purified by an immobilized metal affinity chromatography (IMAC). The glycosylated products of several glucose acceptor were obtained by transglycosylation reactions, then were detected by thin layer chromatography (TLC), measured by ultra-high performance liquid chromatography (UHPLC) and their structures verified by Nuclear Magnetic Resonance (NMR) spectroscopy. Rice Os9BGlu31 transglucosidase and its mutants transferred a glucosyl moiety from *p*-nitrophenol β-D-glucopyranoside as glucose donor to sterol compounds and phenolic acids. Rice Os9BGlu31 mutants had higher activity than wildtype on phytosterol compounds and phenolic acids to produce glucoside. However, the activity of Os9BGlu31 on phytosterols was lower than on phenolic acids. Meanwhile, Os9BGlu31 wildtype tended to produce a single product of phenolic glucosyl ester. Rice Os9BGlu31 transglucosidase is promising for glycosylation of compounds of interest, which may be improved by engineering the substrate specificity to allow production of a range glycoconjugates.

Keywords: Transglycosylation, Os9BGlu31, transglucosidase, chromatography.

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PP061

Immobilization of *Thermoanaerobacterium xylanolyticum Tx*GH116 and E441G nuclophile mutant

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ABSTRACT

*Thermoanaerobacterium xylanolyticum Tx*GH116 β-glucosidase belongs to glycoside hydrolase (GH) family 116 and hydrolyzes β-1,3- and β-1,4- linked oligosaccharides. *Tx*GH116 E441G nucleophile mutant catalyzed transfer of glucose from α-glucosyl fluoride (α-GlcF) to cellobiose acceptor without hydrolysis of the products and showed broad specificity for α-glycosyl fluoride donors and *p*-nitrophenyl glycoside acceptors. Moreover, the *Tx*GH116 E441G catalyzed synthesis of α-D-glucosyl azide from sodium azide and *p*NP-β-D-glucoside (*p*NPGlc) or cellobiose for production of α-glucosyltriazoles. Enzyme immobilization promotes high catalytic activity and stability, and convenient handling of reusable enzymes. In order to economize on the enzymes, *Tx*GH116 and E441G were immobilized on Sepharose 4B activated by cyanogen bromide. The optimum pH of *Tx*GH116 was slightly reduced from 5.5 to 5.0 upon immobilization and had lower relative activity than free enzyme from pH 5.5 to 8.0, while the temperature optimum and thermal stability of immobilized *Tx*GH116 was similar to free enzyme. The immobilized *Tx*GH116 remained 94% of its initial activity after 20 cycles. The optimum pH of immobilized E441G was pH 4.5, similar to free enzyme, while immobilized E441G had lower thermal stability than free enzyme. The activity of immobilized E441G remained only 53% after 10 cycles. Small scale immobilization of E441G on immobilized metal affinity chromatography (IMAC) resin gave higher stability than on cyanogen bromide-activated Sepharose 4B, with 100% activity remaining after 10 cycles, but it was unstable in larger scale reactions. Therefore, the immobilization process could not enhance the pH and temperature stability of *Tx*GH116 and E441G, but the immobilized enzymes were reusable.

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PP062

Development of extracellular vesicle-based liquid biopsy for MYCN-amplified high-risk neuroblastoma

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ABSTRACT

MYCN amplification is an important marker for detecting high-risk neuroblastoma (NB), an aggressive neuroblast tumor with a poor prognosis. However, detecting MYCN amplification requires invasive procedures, e.g., bone marrow aspiration or tumor biopsies. Extracellular vesicles (EVs) contain molecular signatures (e.g., DNA, RNA, proteins) of originate cells that serve as an invaluable source for a less-invasive liquid biopsy. This study aimed to establish a method for detecting MYCN amplification status in EVs deriving from NB cells. Two EV subtypes, i.e., microvesicles and exosomes, were isolated from NB cell culture supernatants (3 MYCN-amplified and 3 MYCN-non-amplified). After characterizing EV protein and particle evidence, MYCN amplification status in EVs deriving from six different NB cells was detected by qRT-PCR. Interestingly, MYCN amplification status was only detectable in the microvesicles, but not exosomes, of MYCN-amplified NB. The feasibility of this method was successfully demonstrated by using simulated samples prepared by pulsing NB-derived microvesicles into the human serum. This study established a liquid biopsy workflow for detecting MYCN-amplification status in the isolated microvesicles. Further validation using the clinical specimens may enable the use of the microvesicle-based liquid biopsy for the high-risk NB diagnosis in the future.

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