List of RGJ advisors 2023/2024
The Trilateral Research (TRF, RGJ and Sweden)

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Keywords: Peptides, Superbug, Fluorescent sensor

Summary of research:

My research is based at medicinal chemistry research unit, Thammasat University (Thailand). Particularly, I am interested in seeking both bioactive peptides and small molecules potentially applicable as therapeutic candidates for treating infectious diseases, particularly against superbugs and malaria. I am personally known as peptide chemist that is my main expertise. To date, we concentrated on the design and syntheses of a number of cyclic peptides with a wide range of biological activities. This further included the development of synthetic approach for the syntheses of novel peptides. Apart from peptide research, I also interested in the discovery of novel fluorescent probes for biomarker detection via PET and FRET mechanism, and showed the great application for biomarker detection in cancer cell lines. Some of our synthesized probes could be used for detection of heavy metal as well. As living in Thailand, it has been known as the country which is rich of biodiversity and natural resources. Natural product research is also one of my research interest, and we discovered a number of novel compounds with great biological activities from Thai medicinal herbs and applications are on the way. Importantly, results from all of my research disciplines (peptide, fluorescent sensor and natural product research) were published in high ranking international journals.

Publications

1. A novel γ-lactone isolated from the leaves of Pithecellobium dulce (Roxb.) Benth. and its xanthine oxidase activity. Wichaidit, W. and Thongyoo, P. *Natural Product Research*, **2023**, 37(7), 1168-1176.
2. A highly selective "Turn On" fluorescent probe based on FRET mechanism for hydrogen sulfide detection in living cells. Sontisiri, P., Yingyuad, P., Thongyoo, P. *Journal of Photochemistry and Photobiology A: Chemistry*, **2020**, 391, 112401.
3. NaV 1.6 regulates excitability of mechanosensitive sensory neurons. Irael M.R., Tanaka B.S., Castro J., Thongyoo P., Robinson S.D., Zhao P., Deuis J.R., Craik D.J., Durek T., Brierley S.M., Waxman S.G., Dib-Hajj S.D., Vetter I. *The Journal of Physiology*, **2019**, 597(14), 3751-3768.
4. Preparation, characterization and in vitro evaluation of calothrixin B liposomes. Yingyuad, P., Sinthuvanich, C., Leepasert, T., Thongyoo, P., Boonrungsiman, S. *Journal of Drug Delivery Science and Technology*, **2018**, 44, 491-497.
5. The E15R Point Mutation in Scorpion Toxin Cn2 Uncouples Its Depressant and Excitatory Activities on Human NaV1.6. Israel, M.R.*, Thongyoo, P.*, Deuis, J.R., Craik, D.J., Vetter, I., Durek, T. *Journal of Medicinal Chemistry*, **2018**, 61(4), 1730-1736. (**Joint first authors*)
6. Phytochemical Constituents from the Root of Luvunga Scandens and Biological Activity Evaluation. Prangchanok Sirinut, Awanwee Petchkongkeaw, Jariya Romsaiyud, Saisuree Prateeptongkum and Panumart Thongyoo. *Natural Product Communications,* **2017**, 12(9), 1483-1484.
7. Interaction of Synthetic Human SLURP-1 with the Nicotinic Acetylcholine Receptors. Durek T., Shelukhina, I.V., Tae, H.-S., Thongyoo, P., Spirova, E.N., Kudryavtsev, D.S., Kasheverov, I.E., Faure, G., Corringer, P.J., Craik, D.J., Adams, D.J., Tsetlin, V.I. *Scientific Reports*, **2017**, 7(1), 16606.
8. Interaction of Tarantula Venom Peptide ProTx-II with Lipid Membranes Is a Prerequisite for Its Inhibition of Human Voltage-gated Sodium Channel NaV1.7. Sónia Troeira Henriques, Evelyne Deplazes, Nicole Lawrence, Olivier Cheneval, Stephanie Chaousis, Marco Inserra, Panumart Thongyoo, Glenn F. King, Alan E. Mark, Irina Vetter, David J. Craik and Christina I. Schroeder. *Journal of Biological Chemistry*, **2016**, 291(33), 17049-17065.

9. A highly selective 'turn-on' fluorescent sensor for Zn^{2+} based on fluorescein conjugates. Chantalakana, K., Choengchan, N., Yingyuad, P., Thongyoo, P. *Tetrahedron Letters*, **2016**, 57 (10), 1146-1149.
10. Facile iron(III) chloride hexahydrate catalyzed synthesis of coumarins. Prateeptongkum, S., Duangdee, N., Thongyoo, P. *Arkivoc*, **2015**, 5, 248-258.
11. Potent and specific inhibition of the biological activity of the type-II transmembrane serine protease matriptase by the cyclic microprotein MCoTI-II. Gray K., S. Elghadban S., Thongyoo P., Owen K.A., Szabo R., Bugge T.H., Tate E.W., Leatherbarrow R.J., Ellis V. *Thrombosis and Haemostasis*, **2014**, 112 (2), 402-411.
12. Anti-inflammatory effects of the ethyl acetate extract of Aquilaria crassna inhibits LPS-induced tumour necrosis factor-alpha production by attenuating P38 MAPK activation. Kumphune. S., Prompunt. E., Phaebuaw. K., Sriudwong. P., Pankla. R., Thongyoo. P.. *Int J Green Pharm*, **2011**, 5 (1), 43-48.
13. Potent Inhibitors of β-Tryptase and Human Leukocyte Elastase Based on the MCoTI-II Scaffold. Panumart Thongyoo, Camille Bonomelli, Robin J. Leatherbarrow and Edward W. Tate. *J.Med.Chem.*, **2009**, 52(20), 6197-6200
14. Chemical and biomimetic total syntheses of natural and engineered MCoTI cyclotides. Panumart Thongyoo, Nuria Roque-Rosell, Robin J. Leatherbarrow and Edward W. Tate. *Org.Biomol.Chem.*, **2008**, *6*, 1462-1470 (***This publication is highlighted in 2012 by the Royal Society of Chemistry as one of their 10 most highly cited articles within the last 10 years in the area of natural products***)
15. Immobilized protease-assisted synthesis of engineered cysteine knot microproteins. Panumart Thongyoo, Agnes M. Jaulent, Edward W. Tate and Robin J. Leatherbarrow. *ChembioChem*, **2007**, *8*, 1107-1109
16. Total synthesis of the macrocyclic cysteine knot microprotein MCoTi-II. Panumart Thongyoo, Edward W. Tate and Robin J. Leatherbarrow. *Chem. Comm.*, **2006**, 2848-2850
17. Generation and Reactions of Novel Oxiranyl "Remote" Anions. A. Chaiyanurakkul, R. Jitchati, M. Kaewpet, S. Rajviroongit, Y. Thebtaranonth, P. Thongyoo and W. Watcharin. *Tetrahedron*, **2003**, *59*, 9825-9837
18. Stereoselective Synthesis of Naturally Occurring α- methylenebis -γ-butyrolactones: an Application of Novel Oxiranyl "Remote" Anions. Jittiwud Lertvorchon, Yodhathai, Thebtaranonth, Tienthong Thongpanchang and Panumart Thongyoo. *J.Org.Chem.***2001**, *66*, 4692-4694.

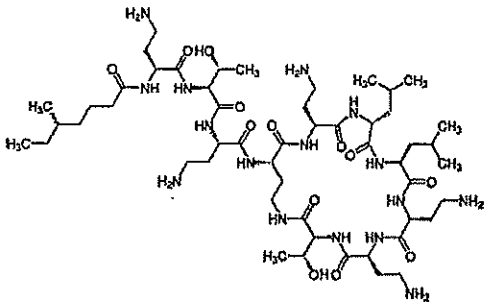
Research Project

The Trilateral Research fund (TRF, RGJ and Sweden): Fiscal year 2024

1. **Project title:** The Discovery of Anti-multidrug Resistant Bacteria Candidates based on Lugdunin and Mortiamide Scaffold
2. **Principal investigator / Department / Faculty / Institute:** Asst. Prof. Panumart Thongyoo, Department of Chemistry, Faculty of Science and Technology, Thammasat University, Tel: 0870711249, email: tpanumas@tu.ac.th
3. **Keyword:** Lugdunin, Anti-superbug activity, Antibiotic, Cyclic peptide, Mortiamide
4. **Subject Catagory (Select 1 discipline)**

   ☐ Biology ■ Chemistry ☐ Physics ☐ Mathematics
5. **Problem statement and significance of research:**

The resistance of bacteria has now become one of the most important health issues affecting people globally. Great amount of budgets for medication and research have been allocated to discover the new antibiotics to the drug market to fight against "Superbug" or multidrug resistance bacteria. Antibiotics have been widely known as effective medicines which were used for the treatment and prevention of bacterial infection for over decades. Unfortunately, a number of antibiotics have now been decreased its antibacterial efficacy mainly due to the bacterial resistance crises. To date, antibacterial resistance has caused patients to overstay in hospital, to pay higher medicine cost and to increase mortality. The Centers for Disease Control and Prevention in United State of American (CDC) reported that at least 2 million people infected by anti-biotic resistance bacteria and at least 23,000 people died each year from this infection (https://www.cdc.gov/drugresistance). There was a report that American people died from new *E.coli* species infection called "Superbug" resistant to colistin (http://www.cidrap.umn.edu/news-perspective/2016/08/us-first-e-coli-resistant-both-colistin-carbapenems), the last resort of antibiotic drugs used in human. This clearly indicated that the antibiotic resistance problem has now become one of the most catastrophic crises for humanity. Therefore, the discovery and development of new antibiotic drugs to fight against a panel of superbugs has been of great importance. To date, peptide-based pharmaceuticals have attracted of great attention, mainly due to their intrinsic properties, ranging from great potency, very high specificity and low toxicity.[1, 2] So far, there have been only a few peptide-based pharmaceuticals which have finally been approved, and commercialized as approved pharmaceuticals for the treatment of bacterial infection. The great example is colistin as previously mentioned.



Colistin

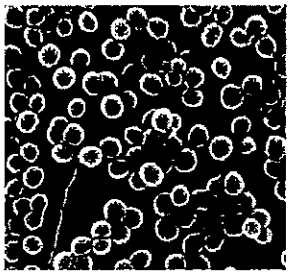


Figure 1 The chemical structure of colistin (left), The picture of methicillin-resistant *Staphylococcus aureus* (right). (http://edition.cnn.com/2013/06/28/us/mrsa-fast-facts/index.)

In the past 40 year, there have been only four classes of compounds which have been developed as effective antibiotic drugs.[3] Importantly, a number of drug companies have ceased the development of novel antibiotic drugs to the market mainly due to the rapid bacterial resistant problem, and it is not economically profitable for the research and development investment. It was found that only a few new anti-biotic drugs have been developed, and reached to the market. Notably, the bacterial resistance in human can be ascribed from the misconception, the discontinuous uses and excessive uses of anti-biotic drugs.

6. Hypothesis

Today, the development of new antibiotic drug is of great importance, many of which are generally required for the surgery operation since a number of antibiotic drugs have been routinely used for surgery operation to prevent the blood stream infection, possibly causing of the death in patients. In 2016, Alexander Zipperer and his research group discovered the macrocyclic thiazolidine peptide antibiotics named "Lugdunin" which was successfully isolated from *Staphylococcus lugdunensis* found in human nose.[4] Lugdunin is a cyclic peptide containing a thiazolidine group, which is comprised of six amino acids with a head-to-tail cyclization. Lugdunin demonstrated a very effective anti-microbial activity against methicillin-resistant *S. aureus* (MIC = 1.9-15.3 μM). The mechanism of inhibition is believed that when bacterial cells were exposed by Lugdunin, which resulted in the cease of incorporating call-wall precursor, causing the death of bacteria almost simultaneously even at concentrations below the MIC. Also, lugdunin has led to the rapid breakdown of bacterial energy resources. However, the development of resistance is not observed in *Staphylococcus aureus*. Mortiamide A is a novel class of cyclic peptides with seven amino acid residues isolated from marine sediment Mortierella sp. in the North Pole, obviously demonstrating a significantly inhibitory effect against P. falciparum strain 3D7 and Dd2 with IC_{50} value of 7.85±0.97 μM and 5.31±0.24 μM,

respectively.[5] The hybridization of lugdunin and mortiamide structure will potentially increase their biological activities.

7. Objectives

- To develop a novel synthetic strategy for the syntheses of Lugdunin-Mortiamide hybrid.
- To synthesize Lugdunin-Mortiamide hybrid by replacing a thiazolidine moiety according from the lugdunin scaffold to various "war head" units, ranging from penicillin, trimethoprim, cephalosporin, cysteine to methionine
- All lugdunin-Mortiamide hybrid will be further examined anti-bacterial activities against a panel of multidrug resistant bacteria, such as *Methicillin-Resistance Staphylococcus Aureus* and etc.

8. Output, Outcome and Impact

- Poster/Oral presentation at International Scientific Conference
- Publication in high impact factor journal (Paper at least 3 papers with Q1 journal)

9. Literature Review

Here are some of bioactive peptides potentially used as therapeutic candidates, Honggang Hu[6] reported the synthesis of Tyrocidine A and glycosylated derivative of Tyrocidine A, a cyclic peptide isolated from *Bacillus* bacteria. Tyrocidine A is a macrocyclic decapeptide, consisting of tyrosine, valine, ornithine, leucine, D-phenylanine, L-phenylanine, proline, asparagine and glutamine. The bactericidal activity of Tyrocidine A was tested against gram-positive bacterium *Bacillus subtilis* (strain CMCC-B 63501) with MIC value of 31 μg/mL.

Yang-Min Ma[7] reported malformin E, a cyclic peptide isolated from the culture broth of endophytic fungus FR02 from the roots of Ficus carica. Malformin E was a cyclic pentapeptide, consisting of leucine, valine, isoleucine and cysteine. It showed cytotoxic activities against human cancer cell strains MCF-7 and A549 with IC_{50} values of 0.65 and 2.42 uM, respectively and anti-microbial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Penicillium chrysogenum*, *Candida albicans*, and *Fusarium solani* with MIC values of 0.91, 0.45, 1.82, 0.91, 3.62, 7.24, and 7.24 uM, respectively.

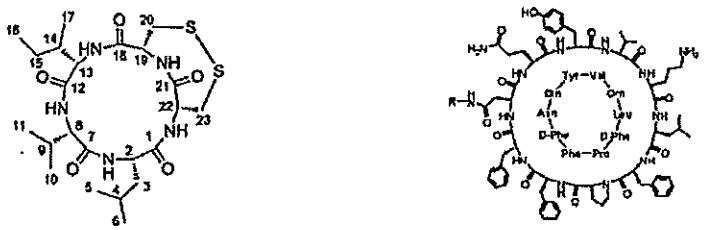


Figure 15 The chemical structure of malformin E (left), Chemical structure of Tyrocidine A (right)

According to these literature reviews, the cyclic peptide showed a very promising antibacterial activity, especially sulfur containing peptides, which demonstrated a potent antibacterial activity against a panel of

pathogenic bacteria. Therefore, Lugdunin-Mortiamide hybrid were designed by replacing with a wide range of war-head pharmacophores, ranging from a thiazolidine ring, penicillin, cephalosporin and cysteine to order to increase their antibacterial efficacy.

10. Methodology

This research focused on the syntheses of Lugdunin-Mortiamide hybrid. The syntheses of Lugdunin-Mortiamide hybrid are designed by replacing a thiazolidine moiety with a number of pharmacophore, ranging from penicillin, trimethoprim, cephalosporin and cysteine residues. To the synthetic strategy, Lugdunin-Mortiamide hybrid can be categorized into two main steps. (1) The amino acid assembly of linear peptide using solid phase peptide synthesis (SPPS). (2) The cyclization of linear peptide chain under an optimized high dilution condition. The Lugdunin-Mortiamide hybrid will be further examined anti-bacterial activities against a panel of multidrug resistant bacteria, such as *Methicillin-resistance Staphylococcus aureus*, *Vancomycin-resistant Enterococcus faecium*, *Cephalosporin-resistant Neisseria gonorrhoeae* and etc.

11. Scope of the study

Today, the development of new antibiotic drug is of great importance, many of which are generally required for the surgery operation since a number of antibiotic drugs have been routinely used for surgery operation to prevent the blood stream infection, possibly causing of the death in patients. Lugdunin is a cyclic peptide containing a thiazolidine group and demonstrated a very effective anti-microbial activity against methicillin-resistant *S. aureus* (MIC = 1.9-15.3 μM). Importantly, the development of resistance is not observed in *Staphylococcus aureus*.

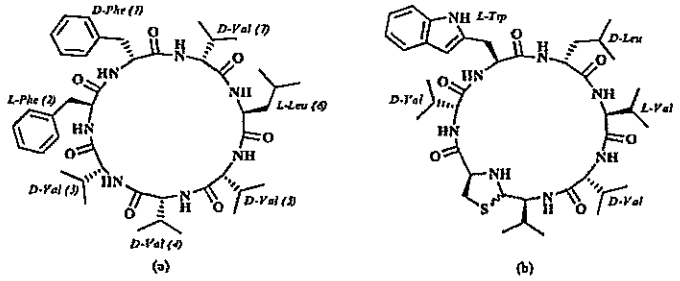


Figure 3 Chemical structures of Mortiamide (left), Lugdunin (right)

Therefore, the syntheses and development of novel antibiotics based on the "Lugdunin-Mortiamide hybrid" are of great interest. To this research, we focused on the syntheses of Lugdunin-Mortiamide hybrid. Lugdunin-Mortiamide hybrid were synthesized by replacing a thiazolidine moiety by a wide range of war-head pharmacophores, ranging from penicillin, trimethoprim, cephalosporin to cysteine residues. Penicillin and Cephalosporin are widely regarded as a group of β-lactam antibiotics which potentially inhibited the formation of peptidoglycan cross-linked in bacterial cell wall syntheses. To

this mechanism, the β-lactam ring interacts with DD-transpeptidase enzyme, which ceases the formation of cross-linked peptidoglycan in bacteria cell wall, resulting, in the death of bacteria.

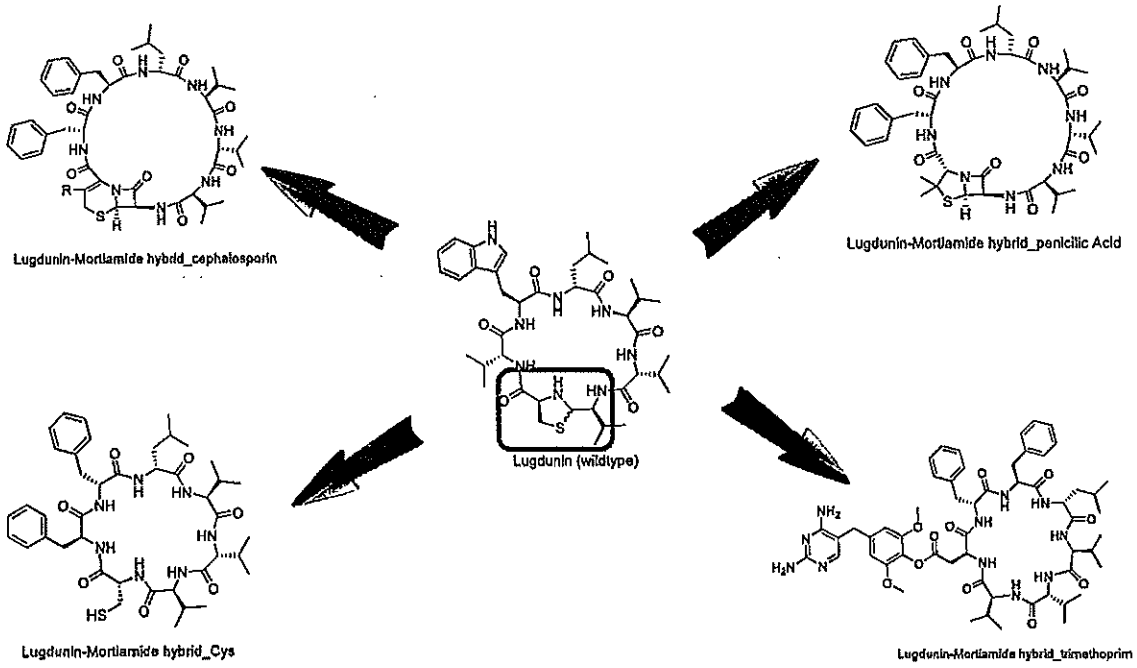


Figure 4 Chemical structure of Lugdunin (1) and their derivatives synthesized by replacing thiazolidine ring with (2) penicillin, (3) cephalosporin, (4) cysteine and (5) trimethoprim

References:

1. Wang, C.K., et al., *Effects of Cyclization on Peptide Backbone Dynamics.* Journal of Physical Chemistry B, 2015. 119(52): p. 15821-15830.
2. Durek, T. and D.J. Craik, *Therapeutic conotoxins: A US patent literature survey.* Expert Opinion on Therapeutic Patents, 2015. 25(10): p. 1159-1173.
3. Cooper, M.A. and D. Shlaes, *Fix the antibiotics pipeline.* Nature, 2011. 472(7341): p. 32.
4. Zipperer, A., et al., *Human commensals producing a novel antibiotic impair pathogen colonization.* Nature, 2016. 535(7613): p. 511-516.
5. Bérubé, C., et al., *Total synthesis and antimalarial activity of mortiamides A–D.* Chemical Communications, 2019. 55(52): p. 7434-7437.
6. Honggang, H., et al., *Synthesis and antibacterial activities of N-Glycosylated derivatives of tyrocidine a, a macrocyclic peptide antibiotic.* Journal of Medicinal Chemistry, 2009. 52(7): p. 2052-2059.
7. Ma, Y.M., et al., *Cytotoxic and Antibiotic Cyclic Pentapeptide from an Endophytic Aspergillus tamarii of Ficus carica.* Journal of Agricultural and Food Chemistry, 2016. 64(19): p. 3789-3793.