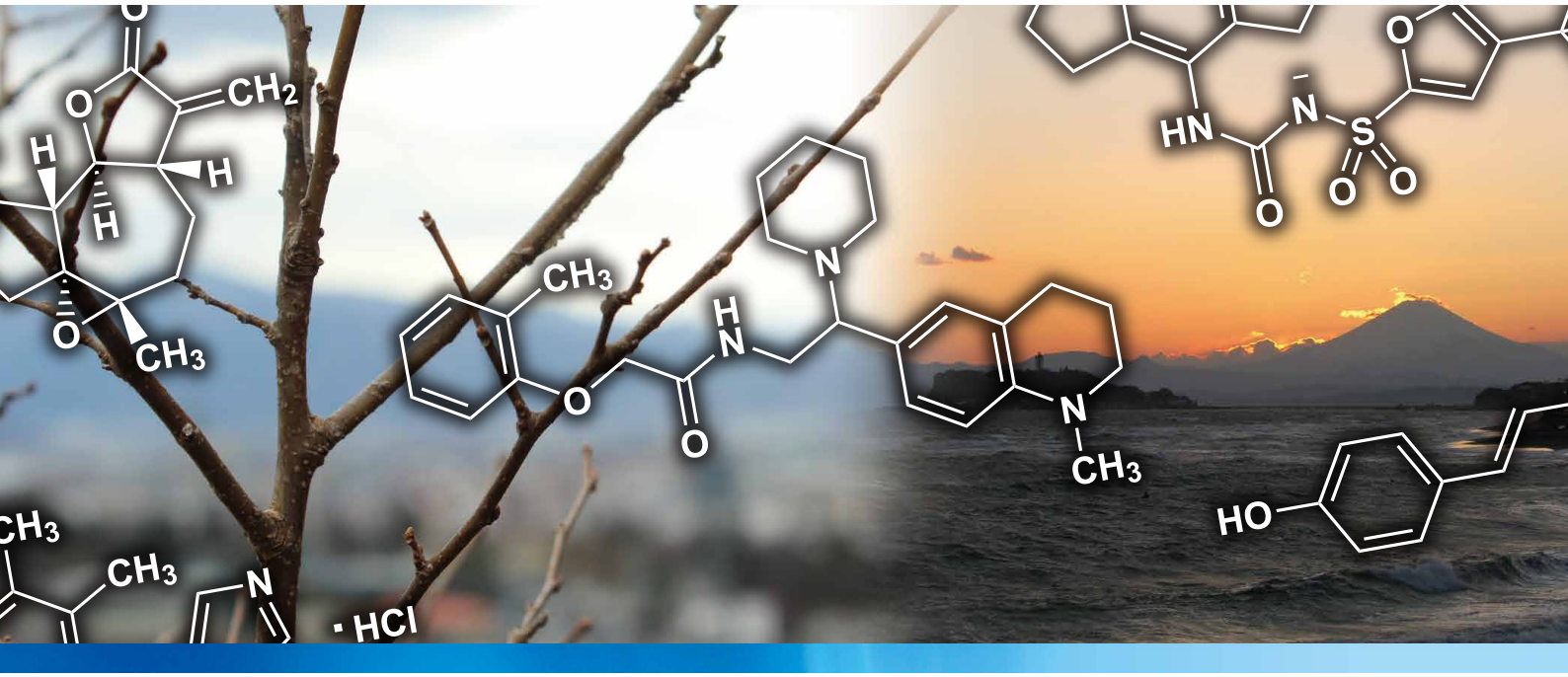
TCIMAIL

No. 194 I AUTUMN 2023



Contents

ISSN 1349-4848 CODEN:TCIMDW

Research Article

Inflammasome Regulation and Inflammasome Inhibitors

Department of Pathology, Ehime University Proteo-Science Center and Graduate School of Medicine
Junya Masumoto
Naoe Kaneko

Keywords: NLRP3, inflammasome, IL-1β, inflammasomopathy, inflammasome inhibitor

Introduction

Interleukin-1β (IL-1β) is an inflammatory cytokine that contributes to homeostasis in the human body through its diverse physiological functions. IL-1β also has pathological significance and plays an important role in a broad spectrum of diseases, including cancer and inflammatory diseases. IL-1β is initially produced as an inactive precursor in the process of "priming" by activation of the transcription factor NF-κB. The cleavage of pro-IL-1β, called "processing", is required for the activation of IL-1β. The inflammasome, a large molecular weight multi-protein complex, plays an important role in "processing". In this article, we focus on the functions of inflammasomes involved in the activation of IL-1β and consider inflammasome-associated diseases and IL-1β inhibitors.

1. IL-1β and its activation

IL-1β has diverse functions and plays important roles in the adaptation of cells and in cellular and tissue repair following damage by various physical, chemical, and biological factors.[1] On the other hand, inappropriate activation of IL-1β leads to the onset and/or progression of diseases. For example, IL-1β, mostly derived from macrophages, is implicated in the progression of atherosclerosis.[2] In a tumor microenvironment, IL-1β can induce the recruitment of tumor-associated macrophages (TAMs) and tumor immunosuppressive myeloid-derived suppressor cells (MDSCs), which promote tumor development in breast cancer.[3]

The release of IL-1β is regulated by two steps. IL-1β is first synthesized as biologically inactive pro-IL-1β (Step 1), then is activated by inflammasomes as the second step and subsequently released into the external milieu[4] (**Fig. 1**). In Step 1, 1L-1β is produced as a 269-AA precursor protein by NF-κB, then is processed by caspase-1, also known as IL-1β-converting enzyme (ICE), to release the mature IL-1β. Step 2 is called "processing". The IL-1β precursor is also processed by other serine proteases such as elastase, chymases, granzyme A, cathepsin G, and proteinase-3, then binds to IL-1 receptors.[5,6] Caspase-1 activation involves inflammasome activation machinery. Briefly, upon the recognition of pathogens or cellular damage by upstream pattern-recognition receptors, pro-caspase-1 binds to an adopter protein ASC (apoptosis-associated speck-like protein containing a caspase-recruitment domain (CARD)) via a caspase-recruitment domain: CARD), to initiate enforced-proximity activation of caspase-1 in the inflammasome (**Fig. 1**).[7,8]

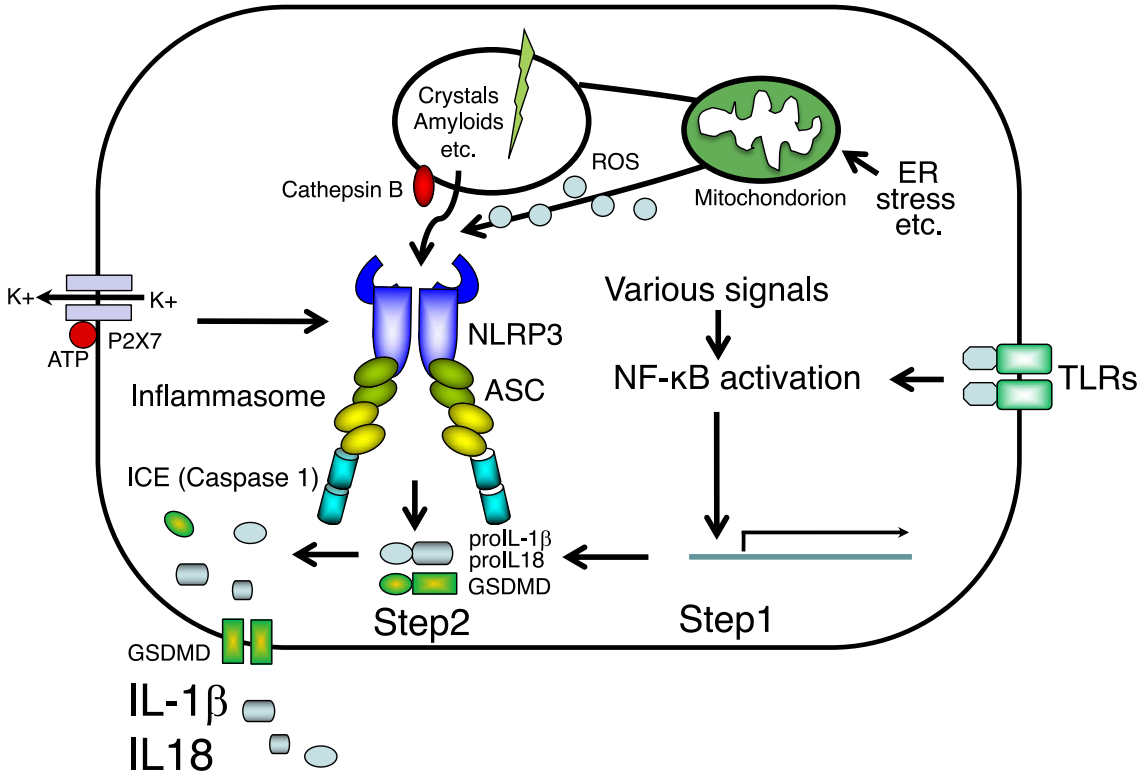


Figure 1. Schematic representation of NLRP3 inflammasome activation

2. Inflammasomes

The inflammasome is a large protein complex consisting of pattern recognition receptors (PRR)s, the ASC adaptor protein, and pro-caspase-1. The NLRP3 inflammasome is well-characterized and is reportedly activated by a wide range of pathogen-associated molecular patterns (PAMP)s and damage-associated molecular patterns (DAMP)s. The NLRP3 inflammasome can oligomerize by sensing changes in the intracellular environment through DAMPs and/or PAMPs: i.e., efflux of potassium, mitochondrial and phagosomal injury-induced reactive oxygen species (ROS), lysosomal damage and the release of cathepsin B, all of which induce the oligomerization of inflammasomes, leading to the processing of pro-IL-1β into active IL-1β (Step 2).[9,10)]

Various NLRP3-activating PAMPs have been reported; i.e., bacteria- or virus-derived RNAs, DNAs, or lethal toxins, flagellin/rod proteins, muramyl dipeptide (MDP), fungus-derived β-glucans, hypha mannan, zymosan, and protozoon-derived hemozoin.[11)] NLRP3-activating DAMPs have also been reported, i.e., self-derived glucose, β-amyloid, hyaluronan, ATP, cholesterol crystals, monosodium urate (MSU) crystals, calcium pyrophosphate dihydrate (CPPD) crystals, environment-derived alum, asbestos, silica, alloy particles, UV radiation, and skin irritants.[11)] The activation of caspase-1 induces pyroptosis and the cleavage of IL-1β precursors. Pyroptosis occurs by the cleavage of gasdermin D (GSDMD) by activated caspase-1, generating a plasma membrane pore due to the polymerization of GSDMD.[12-15)]

Non-canonical pathways of NLRP3 inflammasome activation by inflammatory caspase-4 (human), caspase-5 (human), and caspase-11 (mouse) have also been characterized. LPS is recognized by the CARD of caspase-4 (human) or caspase-11 (mouse), resulting in activation of the NLRP3 inflammasome.

3. Inflammasome activation and diseases

3-1. Autoinflammatory diseases

Variation in the *CIAS1* gene encoding NLRP3 (also called cryopyrin) of only a single amino acid can constitutively activate the NLRP3 inflammasome, leading to cryopyrin-associated periodic syndrome (CAPS). CAPS was first identified after the discovery of MLRP3 and is an autoinflammatory disease with various manifestations but with the same causative gene. Examples include familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), neonatal-onset multisystemic inflammatory disease

(NOMID)/chronic infantile neurological, cutaneous, and arthritis syndrome (CINCA). Secretion of IL-1β is increased without stimulation or by small amounts of DAMPs or PAMPs.[16-21)]

Familial Mediterranean fever (FMF) is the most common autoinflammatory disease and is an inherited autosomal recessive disorder characterized by activation of the pyrin inflammasome due to mutations in the *MEFV* gene encoding the mutant pyrin.[22)] Other autoinflammatory diseases characterized by increased IL-1β secretion include NLRP12 autoinflammatory syndrome and high IgD syndrome (HIDS)/mevalonate kinase deficiency (MKD).[23,24)]

3-2. Metabolic syndrome

The NLRP3 inflammasome is also involved in low-grade subclinical inflammation induced by chronic exposure to high concentrations of free fatty acids and glucose, which reportedly cause increased β-cell apoptosis and insulin resistance in type 2 diabetic patients.[25-27)] *In vivo* experiments showed that islet amyloid polypeptide oligomers are co-produced with insulin and activate the NLRP3 inflammasome.[28,29)] High concentrations of glucose activate intracellular NF-κB, leading to the production of IL-1β precursors.[25)] Amyloid-β can induce IL-1β via NLRP3 inflammasomes in a process involving the phagocytosis of amyloid-β and subsequent lysosomal damage and release of cathepsin B in glial cells in patients with Alzheimer's disease (FAD).[30)] We have shown that direct interaction of amyloid peptides with NLRP3 in a cell-free system using a wheat germ cell-free synthetic system can promote the formation of NLRP3 inflammasomes.[31)] Several crystals also activate the NLRP3 inflammasome. For example, cholesterol crystals cause atherosclerosis, and sodium urate crystals cause gout.[32)] The above diseases activate inflammasomes and thus they can be referred to as inflammasomopathies.[33)]

3-3. Chronic inflammation and malignant tumors

Chronic inflammation increases the risk of malignant tumor.[34)] High expression of IL-1β is associated with human breast cancer tumor progression and prognosis.[35)] The expression of IL-1β and of its receptors in human breast carcinoma tissues leads to the activation of malignant cells, contributing to angiogenesis, tumor growth, and tumor invasion in the cancer microenvironment.[36)] Thus, inflammasome activation could be an important therapeutic target for malignant tumors.

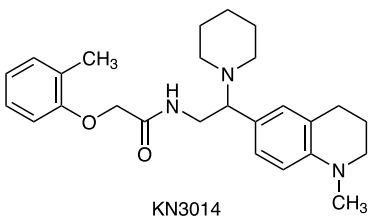
4. IL-18 and inflammasome activation

IL-18 is an IL-1 family cytokine and can be processed by caspase-1. The pathogenesis of IL-1-related diseases suggests the involvement of IL-18.[37)] IL-18 was originally identified as an interferon (IFN)-γ-inducing factor.[38)] IL-18 is the factor most structurally related to IL-1β. IL-18 is synthesized as a 24 kDa protein, which is cleaved by activation of caspase-1 through inflammasome formation to a mature, biologically active 17 kDa form.[39,40)] IL-1β is biologically active in the pg/mL order, whereas IL-18 functions at levels of 10–20 ng/mL or higher for *in vitro* activation.[41,42)]

5. Inflammasome inhibitors

5-1. KN3014

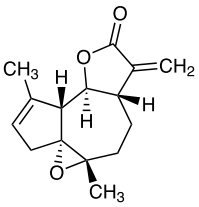
KN3014: *N*-(2-(1-Methyl-1,2,3,4-tetrahydroquinolin-6-yl)-2-(piperidin-1-yl)ethyl)-2-(*o*-tolyloxy)acetamide ($C_{26}H_{35}N_3O_2$) has a molecular weight of 422 and contains a piperidine ring. KN3014 was selected from compounds that directly inhibit the interaction between the PYD domains of NLRP3 and ASC by protein-protein interaction inhibition screening of wheat germ cell-free synthesized proteins. The interaction between the PYD domains of AIM2 and ASC was also inhibited, suggesting that the compound has affinity for the PYD domain of ASC.[43)] KN3014 at a concentration of 50 μM completely inhibits the secretion of IL-1β by auto-inflammation from peripheral blood mononuclear cells derived from patients with Muckle-Wells syndrome and does not inhibit the production of TNFα, indicating that it does not act in the Step 1 priming of IL-1β activation.[43)]



KN3014

5-2. Arglabin

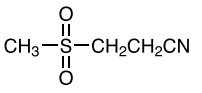
Arglabin is a sesquiterpene-γ-lactone extracted from the herb *Artemisia glabella*. Mouse peritoneal macrophages were pretreated with LPS (10 ng/mL) for 2 hours, incubated in the presence or absence of arglabin (50 nmol/L) for 1 hour, then cholesterol crystals (1 mg/mL) were added to all of the samples. After 6 hours of incubation, the expression of NLRP3, an IL-1β precursor, as well as caspase-1 precursor, and active caspase-1 in all of the cell lysates were analyzed. There was no change in the amount of IL-1β precursor, and activation of active caspase-1 from caspase-1 precursor was inhibited, indicating that this inhibitor specifically inhibits Step 2.[44]



Arglabin

5-3. Dapansutrile (OLT1177)

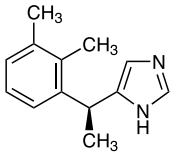
Dapansutrile is an orally available sulfonyl compound that inhibits the release of IL-1β and IL-18, but not of TNFα. Dapansutrile thus inhibits Step 2 without affecting Step 1 in the presence of LPS (1 μg/mL) for 4 hours and inhibits IL-1β release after stimulation with 10 μM nigericin or 5 mM ATP. There was no significant difference in the mRNA levels of the *nlrp3*, *asc*, *caspase-1*, *il1β*, and *il18* genes, suggesting it does not affect priming (Step 1) in the formation of the NLRP3 inflammasome.[45]



Dapansutrile (OLT1177)

5-4. Dexmedetomidine

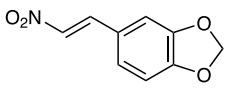
Dexmedetomidine is an α2-adrenergic receptor agonist effective in reducing IL-1β in lung parenchyma and in alveolar lavage fluid due to hyperoxic conditions in the lungs. Culturing RAW 264.7 mouse macrophage cells in a medium containing 100 ng/mL LPS for 1 hour, then with 1 nM dexmedetomidine and 5 mM ATP for 1 hour, inhibited IL-1β release into the culture medium and suppressed the expression of NLRP3, pro-IL-1β, and caspase-1 precursor. These results suggest that the inhibition of IL-1β release is not due to direct inhibition of the inflammasome but rather to the inhibition of priming (Step 1).[46]



Dexmedetomidine

5-5. 3,4-Methylenedioxy-β-nitrostyrene (MNS)

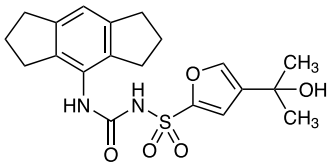
3,4-Methylenedioxy-β-nitrostyrene (MNS) is a β-nitrostyrene derivative exhibiting tyrosine kinase inhibitory activity. Mouse bone marrow-derived macrophages primed with LPS (100 ng/mL) for 4 hours, treated with MNS in the range of 0.5-10 μM for 15 minutes, and incubated with ATP (5 mM) for 30 minutes, inhibited IL-1β release into the culture supernatant in a dose-dependent manner. MNS inhibits only Step 2 because it does not affect TNFα production, an indicator of Step 1. MNS targets NLRP3 directly and inhibits oligomerization of the NLRP3 inflammasome (Step 2) by suppressing ATPase activity.[47]



3,4-Methylenedioxy-ß-nitrostyrene (MNS)

5-6. MCC950/CRID3/CP456,773

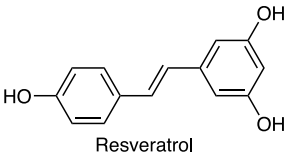
MCC950 was selected from the compounds from which diallyl sulfonylurea, a diabetes drug, was identified. MCC950 inhibits IL-1β precursor processing (Step 2).[48,49] Initial studies showed that the priming of mouse bone marrow-derived macrophages with 10 ng/mL LPS or human peripheral blood monocyte-derived macrophages with 1 μg/mL LPS for 3 hours, followed by 5 mM ATP for 30 minutes or 10 μM nigericin for 1 hour, suppressed the release of IL-1β in a dose-dependent manner within the range of 0.01-10 μM MCC950. MCC950 did not affect TNFα production, suggesting that MCC950 inhibits NLRP3 inflammasome oligomerization (Step 2) by affecting the ATP binding site of NLRP3.[50,51]



MCC950/CRID3/CP456,773

5-7. Resveratrol

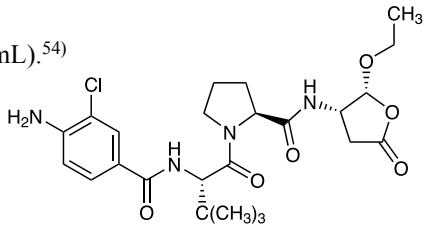
Resveratrol is a polyphenol found in grape peels.[52] Pretreatment of mesenchymal stem cells (MSCs) with 200 μM resveratrol for 1 hour before irradiation reduced the expression of NLRP3 and IL-1β induced by 4 Gy of radiation, suggesting that inflammasome inhibition occurs at Step 1.[53]



Resveratrol

5-8. VX-765

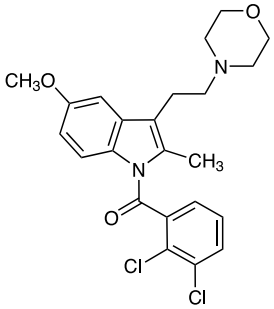
VX-765 is an orally ingestible pro-drug that is metabolized to VRT-043198, a competitive inhibitor of caspase-1. IL-1β release from PBMCs (isolated from FCAS patients) was inhibited following incubation with LPS (0.01-10 ng/mL).[54)]



VX-765

5-9. GW-405833

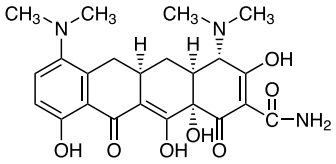
GW-405833 is an agonist of the cannabinoid (CB) receptor 2 (CB2) for tetrahydrocannabidiol, the main component of cannabis.[55)] GW-405833 affects the P2X7 ATP receptor and inhibits activation of the NLRP3 inflammasome.[56)]



GW-405833

5-10. Minocycline

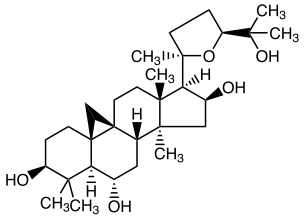
Minocycline is a tetracycline antibiotic. Minocycline inhibits the production of IL-1β and IL-18 from the BV2 mouse microglia cell line in a dose-dependent manner, as determined using the ischemia-reperfusion model. The production of TNFα and IL-6 was also suppressed, suggesting that the suppression of IL-1β production occurs at priming (Step 1) rather than inhibition of the inflammasome.[57)]



Minocycline

5-11. Cycloastragenol

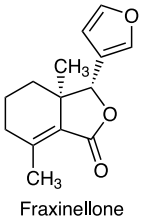
Cycloastragenol is an aglycon derived from the hydrolysis of astragaloside IV. Cycloastragenol suppresses ER stress-induced ROS production from cells stimulated by palmitate in a dose-dependent manner.[58)]



Cycloastragenol

5-12. Fraxinellone

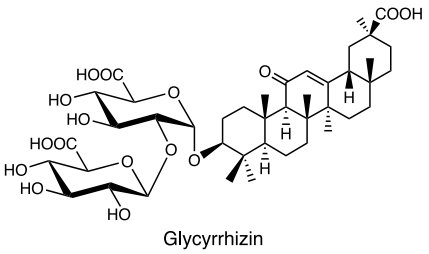
Fraxinellone inhibits NF-κB in the macrophage cell line RAW264.7.[59)] Fraxinellone (10-30 μM) inhibits LPS (500 ng/mL)-induced IL-1β production from THP-1-derived macrophages in a dose-dependent manner and inhibits Step 1 IL-6 and TNF production. Caspase-1 cleavage was also inhibited but the expression levels of the inflammasome components NLRP3, ASC, and caspase-1 were unaffected, suggesting a direct action on inflammasomes.[60)]



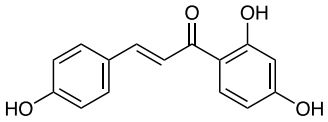
Fraxinellone

5-13. Glycyrrhizin and Isoliquiritigenin

Glycyrrhizin and isoliquiritigenin are the main active ingredients of Chinese herbal medicines such as Kanzo-to and Shakuyaku-kanzo-to. Glycyrrhizin and isoliquiritigenin inhibit pro-IL-1β production in mouse bone marrow-derived macrophages when incubated with LPS (1 μg/mL) and isoliquiritigenin (1-10 μM) or glycyrrhizin (0.1-1 mM) for 3 hours. When pre-incubated with LPS (200 ng/mL) for 3 hours and with glycyrrhizin or isoliquiritigenin and ATP, proIL-1β is not processed to IL-1β, suggesting that both active ingredients may affect Steps 1 and 2.[61)]



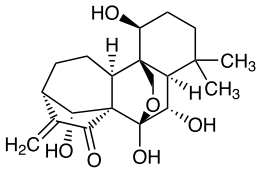
Glycyrrhizin



Isoliquiritigenin

5-14. Oridonine

Oridonine is a component of the herb yama-hakka, found in Chinese herbal medicines such as Kami-shoyo-san and Bofu-tsusho-san. Oridonine inhibits caspase-1 cleavage, IL-1β release, and pyroptosis in mouse bone marrow-derived macrophages incubated with LPS (50 ng/mL) for 3 hours, followed by nigericin and 0.5-2 μM oridonine for 30 minutes. Oridonine binds to the NOD domain of NLRP3 and inhibits the NLRP3 inflammasome by blocking the binding of NLRP3 to NEK7.[62)]



Oridonine

In summary

We reviewed inflammasome activation, related diseases, and inflammasome inhibitors. The regulation of inflammasome activation is more complicated than described here and the pathophysiologies of the resulting disease are even more complicated. The greater the types of inhibitors available for study, the greater the number of clinical application studies possible. We hope that this paper will help the development of therapeutic agents for inflammasome research and related diseases.

Acknowledgements

This work was supported by JSPS Grants-in-Aid for Scientific Research 17H00599, 18H00464, 20H03719, and 23H02944. We also thank the "AMED Basic Initiative for Life Science and Drug Discovery Research Support (BINDS)" (coordinated by Dr. Hirotatsu Kojima, Institute for Drug Discovery, The University of Tokyo)

References

1) N. Kaneko, M. Kurata, T. Yamamoto, S. Morikawa, J. Masumoto, *Inflamm. Regen*. **2019**, *39*, 12.
2) B. Z. Shao, H. Y. Xu, Y. C. Zhao, X. R. Zheng, F. Wang, G. R. Zhao, *Inflammation* **2023**, *46*, 35.
3) B. Guo, S. Fu, J. Zhang, B. Liu, Z. Li, *Sci. Rep*. **2016**, *6*, 36107.
4) C. Eder, *Immunobiology* **2009**, *214*, 543.
5) C. J. March, B. Mosley, A. Larsen, D. P. Cerretti, G. Braedt, V. Price, S. Gillis, C. S. Henney, S. R. Kronheim, K. Grabstein, P. J. Conlon, T. P. Hopp, D. Cosman, *Nature* **1985**, *315*, 641.
6) M. G. Netea, F. L. van de Veerdonk, J. W. van der Meer, C. A. Dinarello, L. A. Joosten, *Annu. Rev. Immunol*. **2015**, *33*, 49.
7) S. M. Srinivasula, J. L. Poyet, M. Razmara, P. Datta, Z. Zhang, E. S. Alnemri, *J. Biol. Chem*. **2002**, *277*, 21119.
8) F. Martinon, K. Burns, J. Tschopp, *Mol. Cell*. **2002**, *10*, 417.
9) E. Latz, T. S. Xiao, A. Stutz, *Nat. Rev. Immunol*. **2013**, *13*, 397.
10) M. Lamkanfi, V. M. Dixit, *Cell* **2014**, *157*, 1013.
11) B. K. Davis, H. Wen, J. P. Ting, *Annu. Rev. Immunol*. **2011**, *29*, 707.
12) N. Kayagaki, M. T. Wong, I. B. Stowe, S. R. Ramani, L. C. Gonzalez, S. Akashi-Takamura, K. Miyake, J. Zhang, W. P. Lee, A. Muszyński, L. S. Forsberg, R. W. Carlson, V. M. Dixit. *Science* **2013**, *341*, 1246.
13) J. A. Hagar, D. A. Powell, Y. Aachoui, R. K. Ernst, E. A. Miao, *Science* **2013**, *341*, 1250.
14) J. Shi, Y. Zhao, Y. Wang, W. Gao, J. Ding, P. Li, L. Hu, F.

Shao, *Nature* **2014**, *514*, 187.
15) J. L. Schmid-Burgk, M. M. Gaidt, Schmidt T, T. S. Ebert, E. Bartok, V. Hornung, *Eur. J. Immunol*. **2015**, *45*, 2911.
16) H. M. Hoffman, J. L. Mueller, D. H. Broide, A. A. Wanderer, R. D. Kolodner, *Nat. Genet*. **2001**, *29*, 301.
17) I. Aksentijevich, M. Nowak, M. Mallah, J. J. Chae, W. T. Watford, S. R. Hofmann, L. Stein, R. Russo, D. Goldsmith, P. Dent, H. F. Rosenberg, F. Austin, E. F. Remmers, J. E. Balow Jr, S. Rosenzweig, H. Komarow, N. G. Shoham, G. Wood, J. Jones, N. Mangra, H. Carrero, B.S. Adams, T. L. Moore, K. Schikler, H. Hoffman, D. J. Lovell, R. Lipnick, K. Barron, J. J. O'Shea, D. L. Kastner, R. Goldbach-Mansky, *Arthritis Rheum*. **2002**, *46*, 3340.
18) J. Feldmann, A. M. Prieur, P. Quartier, P. Berquin, S. Certain, E. Cortis, D. Teillac-Hamel, A. Fischer, G. de Saint Basile, *Am. J. Hum. Genet*. **2002**, *1*, 198.
19) R. Goldbach-Mansk, N. J. Dailey, S. W. Canna, A. Gelabert, J. Jones, B. I. Rubin, H. J. Kim, C. Brewer, C. Zalewski, E. Wiggs, S. Hill, M. L. Turner, B. I. Karp, I. Aksentijevich, F. Pucino, S. R. Penzak, M. H. Haverkamp, L. Stein, B. S. Adams, T. L. Moore, R. C. Fuhlbrigge, B. Shaham, J. N. Jarvis, K. O'Neil, R. K. Vehe, L. O. Beitz, G. Gardner, W. P. Hannan, R. W. Warren, W. Horn, J. L. Cole, S. M. Paul, P. N. Hawkins, T. H. Pham, C. Snyder, R. A. Wesley, S. C. Hoffmann, S. M. Holland, J. A. Butman, D. L. Kastner, *N. Engl. J. Med*. **2006**, *355*, 581.
20) M. Gattorno, S. Tassi, S. Carta, L. Delfino, F. Ferlito, M. A. Pelagatti, A. D'Osualdo, A. Buoncompagni, M. G. Alpigiani, M. Alessio, A. Martini, A. Rubartelli, *Arthritis Rheum*. **2007**, 56, 3138.
21) H. M. Hoffman, S. D. Brydges, *J. Biol. Chem*. **2011**, *286*, 10889.
22) H. Xu, J. Yang, W. Gao, L. Li, P. Li, L. Zhang, Y. Gong, X. Peng, J. J. Xi, S. Chen, F. Wang, F. Shao, *Nature* **2014**, *513*, 237.
23) S. Borghini, S. Tassi, S. Chiesa, F. Caroli, S. Carta, R. Caorsi, M. Fiore, L. Delfino, D. Lasiglié, C. Ferraris, E. Traggiai, M. Di Duca, G. Santamaria, A. D'Osualdo, M. Tosca, A. Martini, I. Ceccherini, A. Rubartelli, M. Gattorno, *Arthritis Rheum*. **2011**, *63*, 830.
24) J. Frenkel, G. T. Rijkers, S. H. L. Mandey, S. W. M. Buurman, S. M. Houten, R. J. A. Wanders, H. R. Waterham, W. Kuis, *Arthritis Rheum*. **2002**, *46*, 2794.
25) K. Maedler, P. Sergeev, F. Ris, J. Oberholzer, H. I. Joller-Jemelka, G. A. Spinas, N. Kaiser, P. A. Halban, M. Y. Donath, *J. Clin. Invest*. **2002**, *110*, 851.
26) R. Zhou, A. Tardivel, B. Thorens, I. Choi, J. Tschopp, *Nat. Immunol*. **2010**, *11*, 136.
27) M. Böni-Schnetzler, S. Boller, S. Debray, K. Bouzakri, D. T. Meier, R. Prazak, J. Kerr-Conte, F. Pattou, J. A. Ehses, F. C. Schuit, M. Y. Donath, *Endocrinology* **2009**, *150*, 5218.
28) S. L. Masters, A. Dunne, S. L. Subramanian, R. L. Hull, G. M. Tannahill, F. A. Sharp, C. Becker, L. Franchi, E. Yoshihara, Z. Chen, N. Mullooly, L. A. Mielke, J. Harris, R. C. Coll, K. H. G. Mills, K. H. Mok, P. Newsholme, G. Nuñez, J. Yodoi, S. E. Kahn, E. C. Lavelle, L. A. J. O'Neill, *Nat. Immunol*. **2010**, *11*, 897.
29) T. Mandrup-Poulsen, *Nat. Immunol*. **2010**, *11*, 881.
30) A. Halle, V. Hornung, G. C. Petzold, C. R. Stewart, B. G. Monks, T. Reinheckel, K. A. Fitzgerald, E. Latz, K. J. Moore, D. T. Golenbock, *Nat. Immunol*. **2008**, *9*, 857.
31) N. Kaneko, W. Mori, M. Kurata, T. Yamamoto, T. Zako, J. Masumoto, *Int. J. Immunopathol. Pharmacol*. **2022**, *36*, 3946320221104554.
32) B. Z. Shao, H. Y. Xu, Y. C. Zhao, X. R. Zheng, F. Wang, G. R. Zhao, *Inflammation* **2023**, *46*, 35.
33) S. L. Masters, A. Simon, I. Aksentijevich, D. L. Kastner, *Annu. Rev. Immunol*. **2009**, *27*, 621.
34) A. Mantovani, P. Allavena, A. Sica, F. Balkwill, *Nature* **2008**, *454*, 436.
35) L. Jin, R. Q. Yuan, A. Fuchs, Y. Yao, A. Joseph, R. Schwall, S. J. Schnitt, A. Guida, H. M. Hastings, J. Andres, G. Turkel, P. J. Polverini, I. D. Goldberg, E. M. Rosen, *Cancer* **1997**, *80*, 421.
36) A. G. Pantschenko, I. Pushkar, K. H. Anderson, Y. Wang, L. J. Miller, S. H. Kurtzman, G. Barrows, D. L. Kreutzer, *Int. J. Oncol*. **2003**, 23, 269.
37) C. A. Dinarello, D. Novick, S. Kim, G. Kaplanski, *Front. Immunol*. **2013**, *4*, 289.
38) K. Nakamura, H. Okamura, M. Wada, K. Nagata, T. Tamura, *Infect. Immun*. **1989**, *57*, 590.
39) Y. Gu, K. Kuida, H. Tsutsui, G. Ku, K. Hsiao, M. A. Fleming, N. Hayashi, K. Higashino, H. Okamura, K. Nakanishi, M. Kurimoto, T. Tanimoto, R. A. Flavell, V. Sato, M. W. Harding, D. J. Livingston, M. S. Su, *Science* **1997**, *275*, 206.
40) T. Ghayur, S. Banerjee, M. Hugunin, D. Butler, L. Herzog, A. Carter, L. Quintal, L. Sekut, R. Talanian, M. Paskind, W. Wong, R. Kamen, D. Tracey, H. Allen, *Nature* **1997**, *386*, 619.
41) J. C. Morel, C. C. Park, J. M. Woods, A. E. Koch, *J. Biol. Chem*. **2001**, *276*, 37069.
42) J. K. Lee, S. H. Kim, E. C. Lewis, T. Azam, L. L. Reznikov, C. A. Dinarello, *Proc. Natl. Acad. Sci. U.S.A*. **2004**, *101*, 8815.
43) N. Kaneko, M. Kurata, T. Yamamoto, T. Shigemuri, K. Agematsu, T. Yamazaki, H. Takeda, T. Sawasaki, T. Koga, A. Kawakami, A. Yachie, K. Migita, K. I. Yoshiura, T. Urano, J. Masumoto, *Sci. Rep*. **2020**, *10*, 13562.
44) A. Abderrazak, D. Couchie, D. F. D. Mahmood, R. Elhage, C. Vindis, M. Laffargue, V. Matéo, B. Büchele, M. R. Ayala, M. E. Gaafary, T. Syrovets, M. N. Slimane, B. Friguet, T. Fulop, T. Simmet, K. E. Hadri, M. Rouis, *Circulation* **2015**, *131*, 1061.
45) C. Marchetti, B. Swartzwelter, F. Gamboni, C. P. Neff, K. Richter, T. Azam, S. Carta, I. Tengesdal, T. Nemkov, A. D'Alessandro, C. Henry, G. S. Jones, S. A. Goodrich, J. P. St Laurent, T. M. Jones, C. L. Scribner, R. B. Barrow, R. D. Altman, D. B. Skouras, M. Gattorno, V. Grau, S. Janciauskiene, A. Rubartelli, L. A. B. Joosten, C. A. Dinarello, *Proc. Natl. Acad. Sci. U.S.A*. **2018**, *115*, E1530.
46) Q. Zhang, D. Wu, Y. Yang, T. Liu, H. Liu, *Cell Physiol. Biochem*. **2017**, *42*, 1907.
47) Y. He, S. Varadarajan, R. Muñoz-Planillo, A. Burberry, Y. Nakamura, G. Núñez, *J. Biol. Chem*. **2014**, *289*, 1142.
48) D. G. Perregaux, P. McNiff, R. Laliberte, N. Hawryluk, H. Peurano, E. Stam, J. Eggler, R. Griffiths, M. A. Dombroski, C. A. Gabel, *J. Pharmacol. Exp. Ther*. **2001**, *299*, 187.
49) R. C. Coll, A. Robertson, M. Butler, M. Cooper, L. A. J. O'Neill, *PLoS One* **2011**, *6*, e29539.
50) R. C. Coll, A. A. B. Robertson, J. J. Chae, S. C. Higgins, R. M. Planillo, M. C. Inserra, I. Vetter, L. S. Dungan, B. G. Monks, A. Stutz, D. E. Croker, M. S. Butler, M. Haneklaus, C. E. Sutton, G. Núñez, E. Latz, D. L. Kastner, K. H. G. Mills, S. L. Masters, K. Schroder, M. A. Cooper, L. A. J. O'Neill, *Nat. Med*. **2015**, *21*, 248.

51) U. Ohto, Y. Kamitsukasa, H. Ishida, Z. Zhang, K. Murakami, C. Hirama, S. Maekawa, T. Shimizu, *Proc. Natl. Acad. Sci. U.S.A*. **2022**, 119, e2121353119.
52) R. Nakata, S. Takahashi, H. Inoue, *Biol. Pharm. Bull*. **2012**, 35, 273.
53) Y. Fu, Y. Wang, L. Du, C. Xu, J. Cao, T. Fan, J. Liu, X. Su, S. Fan, Q. Liu, F. Fan, *Int. J. Mol. Sci*. **2013**, *14*, 14105.
54) J. H. Stack, K. Beaumont, P. D. Larsen, K. S. Straley, G. W. Henkel, J. C. R. Randle, H. M. Hoffman, *J. Immunol*. **2005**, *175*, 2630.
55) K. J. Valenzano, L. Tafesse, G. Lee, J. E. Harrison, J. M. Boulet, S. L. Gottshall, L. Mark, M. S. Pearson, W. Miller, S. Shan, L. Rabadi, Y. Rotshteyn, S. M. Chaffer, P. I. Turchin, D. A. Elsemore, M. Toth, L. Koetzner, G. T. Whiteside, *Neuropharmacology* **2005**, *48*, 658.
56) C. Liu, H. Ma, A. L. Slitt, N. P. Seeram, *J. Nat. Prod*. **2020**, *83*, 2025.
57) Y. Lu, G. Xiao, W. Luo, *Neuroimmunomodulation* **2016**, *23*, 230.
58) Y. Zhao, Q. Li, W. Zhao, J. Li, Y. Sun, K Liu, B. Liu, N. Zhang, *J. Ethnopharmacol*. **2015**, *169*, 210.
59) J. H. Kim, Y. M. Park, J. S. Shin, S. J. Park, J. H. Choi, H. J. Jung, H. J. Park, K. T. Lee, *Biol. Pharm. Bull*. **2009**, *32*, 1062.
60) X. F. Wu, Z. J. Ouyang, L. L. Feng, G. Chen, W. J. Guo, Y. Shen, X. D. Wu, Y. Sun, Q. Xu, *Toxicol. Appl. Pharmacol*. **2014**, *281*, 146.
61) H. Honda, Y. Nagai, T. Matsunaga, N. Okamoto, Y. Watanabe, K. Tsuneyama, H. Hayashi, I. Fujii, M. Ikutani, Y. Hirai, A. Muraguchi, K. Takatsu, *J. Leukoc. Biol*. **2014**, *96*, 1087.
62) H. He, H. Jiang, Y. Chen, J. Ye, A. Wang, C. Wang, Q. Liu, G. Liang, X. Deng, W. Jiang, R. Zhou, *Nat. Commun*. **2018**, *9*, 2550.

Author Information

Junya Masumoto, Professor and Chairman
Ehime University Proteo-Science Center and Graduate School of Medicne
Department of Pathology

Naoe Kaneko, Technical Specialist Staff
Ehime University Proteo-Science Center and Graduate School of Medicne
Department of Pathology

Related Products

KN3014		10mg	50mg	K0077
Arglabin			5mg	A3449
Dapansutrile		100mg	1g	D5955
Dexmedetomidine Hydrochloride		20mg	100mg	D5062
MNS		100mg	500mg	M3390
MCC950 Sodium Salt			10mg	M3396
Resveratrol	1g	5g	25g	R0071
VX-765			10mg	V0176
GW-405833			10mg	G0600
Minocycline Hydrochloride		1g	5g	M2288
Cycloastragenol		25mg	100mg	C3469
Astragaloside IV		250mg	1g	A3305
Fraxinellone		10mg	50mg	F1187
Glycyrrhizin		1g	25g	G0150
Isoliquiritigenin		100mg	1g	I0822
Oridonine			50mg	O0387

Chemistry Chat

My Familiar Compound Family

– Heterocyclic Compounds –

Nagatoshi Nishiwaki

Kochi University of Technology, School of Engineering Science

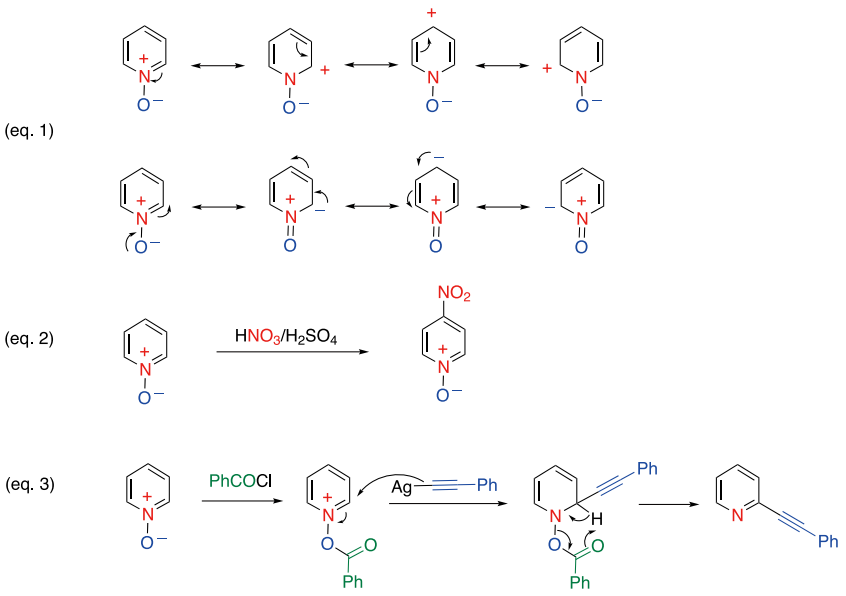
Heterocyclic compounds are lightly treated in textbooks, even though they account for one-third of all organic compounds. In addition, many people feel that the mere mention of the term "heterocyclic chemistry" makes it sound difficult. For me, benzene chemistry is more difficult than heterocyclic chemistry because I have no clue about it. There are two reasons why people feel heterocyclic chemistry is difficult; first, because it has a long history and, as in the field of fatty acids, common names are widely used. It is natural that people cannot understand a compound name unless they can recall its structure. Another is that different ring sizes and different types, positions, and numbers of heteroatoms can cause completely different behavior.

When I was young, I once attended an international conference on heterocyclic chemistry held in Taiwan. I thought that the same expressions would be used since the Chinese characters were the same as those used in Taiwan, but the signboard read " 雑環化学 ", which made me feel a sense of incongruity. This is because the character for " 雑 " is rarely used to mean something good, such as " 雑 誌 (magazine)", " 雑 巾 (duster)", " 雑 菌 (harmful germs)", or " 煩雑 (troublesome)". However, after much deliberation, I have come to think that this expression is more appropriate for describing the miscellaneous nature of heterocyclic chemistry. Perhaps this field suited my " 雑 (sloppy)" nature, and I have been involved in this field ever since.

Dual Reactivity

As with the imprint of the baby bird, I still find myself drawn to papers that describe pyridine *N*-oxides, which I dealt with in my first topic. I am attracted to carbonyl and nitro groups due to their dual reactivity, but their origin may lie in pyridine *N*-oxides.

As can be seen from the resonance structures, the ring carbon is electron-deficient due to the positive charge of the ring nitrogen. On the other hand, one can also draw



Scheme 1. Reactions of pyridine *N*-oxide with electrophiles and nucleophiles

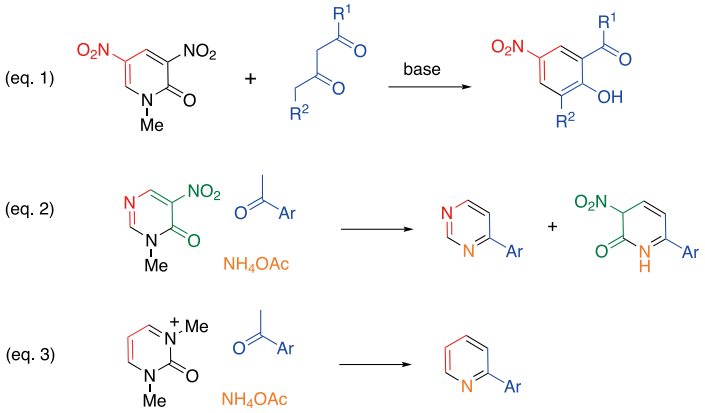
resonance structures in which a negative charge appears on the ring carbon due to the back donation from oxygen (**Scheme 1**, eq. 1). In other words, pyridine *N*-oxides can react with both nucleophiles and electrophiles, changing their positions depending on the partner. The nitration of pyridine is very difficult and proceeds only in low yield under severe conditions, whereas nitration easily proceeds even under mild conditions when it is converted to *N*-oxide because of the back donation (eq. 2). On the other hand, *O*-acylated *N*-oxide improves the electrophilicity of pyridines and readily react with nucleophiles accompanied by aromatization to afford substituted pyridines (eq. 3).[1]

Nucleophilic Ring Transformation

In the laboratory where I was first hired as an assistant professor, we traditionally developed nucleophilic ring transformation reactions (**Scheme 2**, eq. 1)[2] using dinitropyridones. The theme I received was a ring transformation reaction using its aza-analog, nitropyrimidinone. I had easily thought that the nitro group was simply replaced with a ring of nitrogen, but the difference was unexpectedly large, and I was unable to reach a favorable result. In searching for the causes, I discovered that the substrate was decomposed by ammonia, which was used as a nitrogen source. Then I used less nucleophilic ammonium acetate instead of ammonia, which led to a great improvement of the yield (eq. 2).[3] Additionally, a new ring transformation was also discovered that proceeds in a reaction mode not seen in the past.

When I established the ring transformation using nitropyrimidinone, I considered that a similar reaction could proceed by changing the ring nitrogen to onium without making the ring electron-deficient with a nitro group. However, I never told anyone about it, never actually tried it, and the time passed as it was. One day, a few years later, I was flipping through a magazine and saw an advertisement for a company with the exact reaction I was thinking of at the time. Although the idea was not stolen or anything, I vividly remember feeling regretful (with myself).

Lesson learned, "It is better to act and have regrets than not to act and have regrets."



Scheme 2. Synthesis of heterocyclic compounds by ring transformation

Pyridines Substituted with Five Aryl Groups

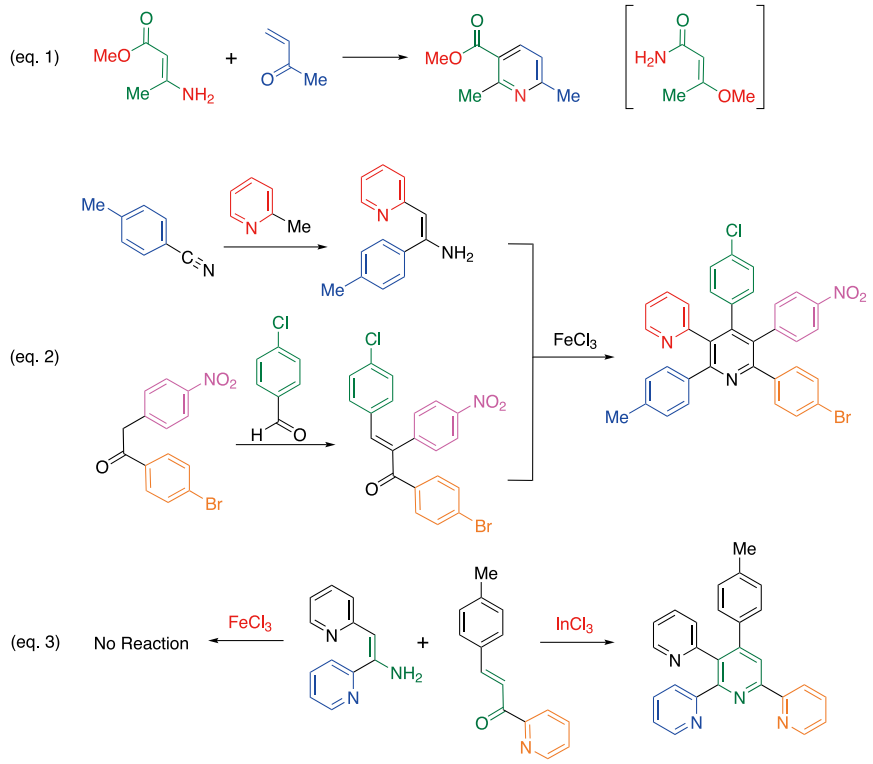
We were conducting a reaction with an unsaturated carbonyl compound using a push-pull alkene (β-methoxy acrylamide) with a biased electron density, which unexpectedly yielded pyridine. When I submitted a paper about finding the interesting reaction that nitrogen and oxygen were switched, one of the referees pointed out that the starting material was an enamino ester. Embarrassingly, I did not realize that the nitrogen and oxygen were switched during the synthesis of the starting material, not during the reaction (**Scheme 3**, eq. 1).[4] At the same time, I was relieved that the paper was not published in a journal.

We performed the same reaction using a pyridyl group instead of the ester functional group as an electron-withdrawing group and found that polysubstituted pyridines could be synthesized, although in some cases the substrates had to be activated by $FeCl_3$. The advantage of this reaction is that the pyridine ring can be easily modified by simply altering the enamine or unsaturated ketone. The conventional synthesis of pyridines with five different aryl groups requires a considerable number of steps,[5] but the new method successfully achieved only in three steps, including the synthesis of the starting materials (eq. 2).[6]

In this method, when a pyridine ring was introduced on the ketone side, a bipyridyl derivative could be easily synthesized, but when a pyridine ring was introduced on the enamine side, the reaction did not proceed at all because of formation of a complex with $FeCl_3$. At that time, we thought that we could solve this problem by using $InCl_3$, which has low coordination property, as Lewis acid. I felt very good when the reaction actually proceeded as predicted and I succeeded in synthesizing terpyridine (eq. 3). I tried to convey this feeling by explaining that it was "like when you get a perfect Kanchan* in mahjong", but recent students did not understand me at all.

* Drawing a 3 between the 2 and 4 tiles.

Lesson learned, "You had better turn any situation to your advantage."



Scheme 3. Short step synthesis of pyridines by condensation of enamines and unsaturated ketones

References

1. N. Nishiwaki, S. Minakata, M. Komatsu, Y. Ohshiro, *Chem. Lett*. **1989**, *18*, 773.
2. S. T. Le, H. Asahara, N. Nishiwaki, *Molecules* **2021**, *26*, 639.
3. N. Nishiwaki, T. Adachi, K. Matsuo, H.-P. Wang, T. Matsunaga, Y. Tohda, M. Ariga, *J. Chem. Soc., Perkin Trans. 1* **2000**, 27.
4. S. Hirai, Y. Horikawa, H. Asahara, N. Nishiwaki, *Chem. Commun*. **2017**, *53*, 2390.
5. A. I. Reza, K. Iwai, N. Nishiwaki, *Chem. Rec*. **2022**, *22*, e202200099.
6. M. Arita, S. Yokoyama, H. Asahara, N. Nishiwaki, *Eur. J. Org. Chem*. **2020**, 466.

Author Information

Professor Nagatoshi Nishiwaki received a Ph.D. in 1991 from Osaka University. He worked in Professor Ariga's group in the Department of Chemistry, Osaka Kyoiku University, as an assistant professor (1991-2000) and associate professor (2001-2008). From 2000 to 2001, he was with Karl Anker Jørgensen's group at Århus (Aarhus) University in Denmark. He worked at the Center for Collaborative Research, Anan National College of Technology as an associate professor from 2008 to 2009. Then, he moved to the School of Environmental Science and Engineering, Kochi University of Technology in 2009, where he has been a professor since 2011. His research interests comprise synthetic organic chemistry using nitro compounds, heterocycles (synthesis, ring transformation, 1,3-dipolar cycloaddition, application as tools in organic synthesis), pseudo-intramolecular reactions.

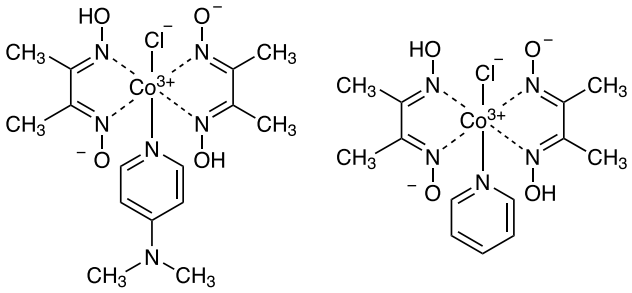
New Products Information

Cobalt Complexes to Catalyze the Dehydrogenative Cross-Coupling Reactions

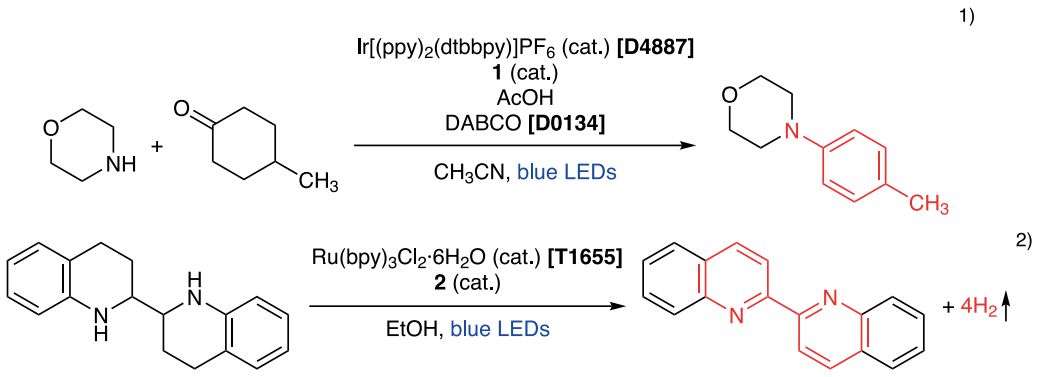
Chlorobis(dimethylglyoximato)[4-(dimethylamino)pyridine] cobalt(III) (= $Co(dmgH)_2(DMAP)Cl$) (1) — Product Number: **C3711** **1g**

Chlorobis(dimethylglyoximato)(pyridine)cobalt(III) (= $Co(dmgH)_2PyCl$) (2) — Product Number: **C3718** **1g**

$Co(dmgH)_2(DMAP)Cl$ (**1**) and $Co(dmgH)_2PyCl$ (**2**) are utilized as catalysts for the oxidant-free dehydrogenative cross-coupling reactions and dehydrogenation reactions of nitrogen-containing heterocyclic rings. For instance, in the presence of **1** and a photoredox catalyst, cyclohexanones react with amines to give the corresponding aniline derivatives via C-N bond formation at the ketone moiety and successive aromatization of the cyclohexane ring. This reaction has the great advantage of not using any oxidants. In addition, the dehydrogenation of nitrogen-containing heterocycles proceeds under similar conditions to give aromatized heterocycles.



(**1**) (**2**)



References

1) S. Dighe, F. Juliá, A. Luridiana, J. J. Douglas, D. Leonori, *Nature* **2020**, *584*, 75.
2) K.-H. He, F.-F. Tan, C.-Z. Zhou, G.-J. Zhou, X.-L. Yang, Y. Li, *Angew. Chem. Int. Ed.* **2017**, *56*, 3080.

Related Products

$Co(dmgH)_2Cl_2$		1g	5g	D5924
$Ir[(ppy)_2(dtbbpy)]PF_6$			200mg	D4887
1,4-Diazabicyclo[2.2.2]octane (= DABCO)	25g	100g	500g	D0134
$Ru(bpy)_3Cl_2·6H_2O$		1g	5g	T1655

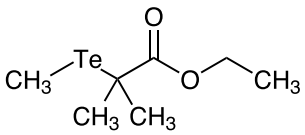
Organotellurium Compounds for Controlled Radical Polymerization

Ethyl 2-Methyl-2-(methyltellanyl)propanoate (1) Product Number: **E1508** **100mg 1g**

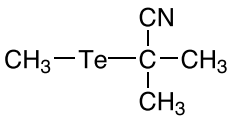
2-Methyl-2-(methyltellanyl)propanenitrile (2) Product Number: **M3520** **100mg 1g**

[1-(Methyltellanyl)ethyl]benzene (3) Product Number: **M3521** **100mg 1g**

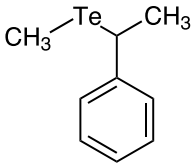
Organotellurium-mediated radical polymerization (TERP) is a type of controlled radical polymerizations, also known as reversible deactivation radical polymerization, and was developed by Yamago *et al*.[1] Ethyl 2-methyl-2-(methyltellanyl)propanoate (**1**), 2-methyl-2-(methyltellanyl)propanenitrile (**2**), and [1-(methyltellanyl)ethyl]benzene (**3**) are useful organotellurium compounds as chain transfer agents (CTAs) for TERP. **1**, **2**, and **3** effectively control the polymerization of vinyl monomers such as styrenes, acrylates, and methacrylates, even with polar functional groups like amino group and carboxy group. In addition, these CTAs have been applied to the synthesis of block copolymers including amphiphilic polymers,[2] and the synthesis of hyperbranched polymers using **1** was also reported.[3]



(**1**)



(**2**)



(**3**)

References

1) S. Yamago, *Chem. Rev*. **2009**, *109*, 5051.
2) S. Kumar, M. Changez, C. N. Murthy, S. Yamago, J.-S. Lee, *Macromol. Rapid Commun*. **2011**, *32*, 1576.
3) Y. Lu, S. Yamago, *Macromolecules* **2020**, *53*, 3209.

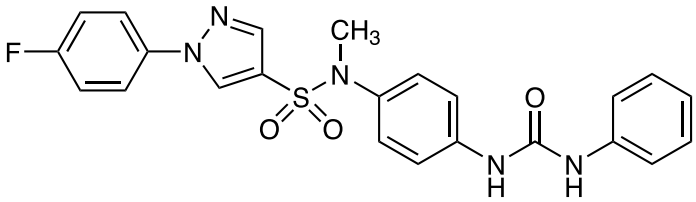
Related Products

Dimethyl Ditelluride	100mg	1g	D6090
Diphenyl Ditelluride	1g	5g	D2718

Integrin α2β1 Inhibitor

BTT 3033 (1)

Product Number: **B5415**
50mg



(**1**)

BTT 3033 (**1**) is a selective inhibitor of integrin α2β1 with EC_{50} = 130 nM on the basis of binding for the integrin to collagen I.[1] On the other hand, **1** does not inhibit other integrins like α3β1, α4β1, α5β1 and αvβ1, as shown in **Table 1**.

1 also has anti-inflammatory effects in a platelet-activating factor (PAF)-stimulated air pouch model and moderate anti-inflammatory effects in an arachidonic acid-induced ear edema model.[2]

Table 1. The effects of **1** on cell adhesion to different matrices.[1]

Integrin type	Cell & matrix	Cell adhesion (%) *
α3β1	PC-3 on laminin-332	93.7±10.6
α4β1	HL-60 on 40 kDa fibronectin	109.4±10.1
α5β1	MG-63 on 120 kDa fibronectin	102.6±7.1
αvβ1	MG-63 on vitronectin	113.7±17.0

* At EC_{50} concentration of **1** in CHO-α 2wt/collagen I assays

References

1) L. Nissinen, J. Koivunen, J. Käpylä, M. Salmela, J. Nieminen, J. Jokinen, K. Sipilä, M. Pihlavisto, O. T. Pentikäinen, A. Marjamäki, J. Heino, *J. Biol. Chem*. **2012**, *287*, 44694.
2) L. Nissinen, M. Ojala, B. Langen, R. Dost, M. Pihlavisto, J. Käpylä, A. Marjamäki, J. Heino, *Pharma. Res. Per*. **2015**, *3*, e00146.

TCIMAIL
AUTUMN 2023 I No. 194

New! TCI SpectraViewer

A convenient tool for visualizing the excitation/emmision spectra of our and other common fluorescent reagents

Select your fluorophore of interest or search for a fluorophore that matches your instrument setup to display its emission and excitation spectra in-graph. Add filters and lasers to help design experiments.

www.TCIchemicals.com

Ordering and Customer Service

TCI AMERICA
Tel : 800-423-8616 / 503-283-1681
Fax : 888-520-1075 / 503-283-1987
E-mail : Sales-US@TCIchemicals.com

TCI EUROPE N.V.
Tel : +32 (0)3 735 07 00
Fax : +32 (0)3 735 07 01
E-mail : Sales-EU@TCIchemicals.com

TCI Deutschland GmbH
Tel : +49 (0)6196 64053-00
Fax : +49 (0)6196 64053-01
E-mail : Sales-DE@TCIchemicals.com

Tokyo Chemical Industry UK Ltd.
Tel : +44 (0)1865 78 45 60
E-mail : Sales-UK@TCIchemicals.com

梯希爱(上海)化成工业发展有限公司
Tel : 800-988-0390 / 021-67121386
Fax : 021-6712-1385
E-mail : Sales-CN@TCIchemicals.com

Tokyo Chemical Industry (India) Pvt. Ltd.
Tel : 1800 425 7889 / 044-2262 0909
E-mail : Sales-IN@TCIchemicals.com

TOKYO CHEMICAL INDUSTRY CO., LTD.
Tel : +81 (0)3-5640-8878
E-mail : globalbusiness@TCIchemicals.com

TOKYO CHEMICAL INDUSTRY
Printed in Japan