



Title	Purine nucleotide biosynthesis pathway as a drug target : Identification of novel IMPDH and GMPR from Trypanosoma congolense, and an inhibitor screening study of Cryptosporidium parvum and human type II IMPDH [an abstract of dissertation and a summary of dissertation review]
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学位論文内容の要旨

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学位論文題名

Purine nucleotide biosynthesis pathway as a drug target: Identification of novel IMPDH and GMPR from *Trypanosoma congolense*, and an inhibitor screening study of *Cryptosporidium parvum* and human type II IMPDH

(薬物標的としてのプリンヌクレオチド生合成：*Trypanosoma congolense* 由来の新規 IMPDH 及び GMPR の同定,並びに *Cryptosporidium parvum* 及びヒト II 型 IMPDH 阻害剤の探索研究)

The purine nucleotide biosynthesis is an essential biological system for all organisms. The guanosine and adenosine nucleotide produced from the pathway are important precursors for cellular nucleic acid synthesis. In both *de novo* and salvage pathway, nucleotides are synthesized from a common precursor, inosine 5'-monophosphate (IMP). Two enzymes regulate the intracellular concentration of IMP: IMP dehydrogenase (IMPDH) and GMP reductase (GMPR). IMPDH catalyzes the conversion of IMP to xanthosine monophosphate (XMP). XMP is subsequently converted to GMP by another enzyme, GMP synthase. GMPR maintains the intracellular balance of purine nucleotides by converting GMP back to IMP.

The activity of these enzymes has been proved to be therapeutically important, such as in the salvage purine nucleotide biosynthesis of various pathogenic protozoa and in guanine pool maintenance of rapidly dividing cells like cancer cells. Therefore, these enzymes have been considered as rational chemotherapeutic targets. Inhibitors of IMPDH and GMPR have been proposed as antiprotozoal, antineoplastic, antiviral, and immunosuppressive agents.

In this study, novel GMPR and IMPDH from pathogenic protozoa *Trypanosoma congolense* were identified and characterized. Furthermore, a high-throughput screening study was conducted to identify inhibitors against IMPDH of pathogen *Cryptosporidium parvum* and human.

1. Identification of novel GMPR and IMPDH from *T. congolense*

T. congolense is a protozoan parasite that cause *nagana* disease in African animal. The depletion in the number of domesticated cattle due to infections has resulted in a major economic drawback in tropical area of Africa. While naturally

T. congolense does not infect human, several cases of human infection have been reported. Unfortunately, the current chemotherapeutic agents for *nagana* consist of outdated or toxic compounds. Therefore, it is important to discover new drugs and drug targets against *T. congolense*.

For its nucleotide biosynthesis, *T. congolense* relies solely on the streamlined salvage pathway, which consisted of IMPDH and GMPR. Therefore, the disruption of the enzyme activity could inhibit the growth of the parasite.

The author first identified a novel GMPR from *T. congolense* (TcGMPR).. The kinetics of novel TcGMPR was characterized, and was found to be significantly different from that of mammalian GMPR. Therefore, it is promising to develop selective TcGMPR inhibitors as chemotherapeutic compounds against *nagana* disease. TcGMPR was also found to be able to carry out a reverse reaction, the direct conversion of IMP to GMP, in the presence of ammonia.

Furthermore, an orthologue of TcGMPR was also found in the genome of *T. congolense*. Subsequent enzymatic characterization unveiled its IMPDH activity (TcIMPDH). The characterization of the TcIMPDH is currently ongoing.

Mycophenolic acid (MPA), a potent mammalian IMPDH inhibitor, showed an inhibitory activity against both TcGMPR and TcIMPDH. This implied that MPA could be a lead compound that targets both enzymes in the purine nucleotide biosynthesis of *T. congolense*.

2. High-Throughput Screening of IMPDH inhibitors

Among the enzymes in the purine nucleotide biosynthesis pathway, IMPDH and their inhibitors have been studied extensively as a therapeutic strategy for immunosuppression, antiviral, anti-infection, and antineoplastic.

To discover novel IMPDH inhibitors, a luminescence-based high-throughput screening was performed on a chemical library of 3,200 compounds. The screening resulted in the identification of several inhibitors: disulfiram (**1**), bronopol (**2**), and ebselen (**3**) from the known-compound library, and a series of synthesized adenosine derivatives (**4**, **5**, and **6**) from the synthetic compound library.

The known compounds (**1–3**) are cysteine inhibitors of various enzymes; these compounds reacted irreversibly to IMPDH type II of human (hIMPDH II) and IMPDH of *C. parvum* (CpIMPDH), with an exception of reversible inhibition of **3** to CpIMPDH. Compounds **1**, **2**, and **3** showed potent inhibition to both enzymes. However, inhibition selectivity by **1** and **2** was low. Furthermore, an excessive IMP concentration and the presence of reducing agents reduced the inhibitory activity of all inhibitors, indicating the interaction of inhibitors with the catalytic cysteine residue in the IMP binding site of IMPDH. Interestingly, the inhibitors (**1–3**) reversibly inhibit TcGMPR, which suggested that the compounds might be dual IMPDH-GMPR inhibitors.

On the other hand, the adenosine derivatives (**4–6**) exerted a potent inhibitory activity to IMPDHs, as expected from nucleosides. Further inhibitory characterizations are ongoing.