6,7-dimethyl-8-ribityllumazine synthase 2.5.1.78

1 Nomenclature

EC number
2.5.1.78

Systematic name
5-amino-6-(D-ribitylamino)uracil butanedionetransferase

Recommended name
6,7-dimethyl-8-ribityllumazine synthase

Synonyms
6,7-dimethyl-8-ribityllumazine synthase <7,8,13,29> [6,14,17,20]
6,7-dimethyl-8-ribityllumazine synthase 1 <58,75> [39]
6,7-dimethyl-8-ribityllumazine synthase 2 <24,74> [39]
BLS <11> [21]
MJ0303 <25> [13]
MbtLS <9> [10]
Pbls <15> (<15> gene name [37]) [37]
RIB4 <29> (<29> gene name [17]) [17]
RibH <1,18,27> (<1,18> gene name [5,29]) [5,29,39]
RibH1 protein <11> [16]
RibH2 <11,24,39,74> [6,31,39]
lumazine synthase <4,5,6> [19]
lumazine synthase 1 <40,75> [39]
lumazine synthase 2 <39,74> [39]
lumazine synthase/riboflavin synthase complex <1> (<1> bifunctional enzyme complex [24]) [24]
ribE (gene name) <2> [18]
ribH1 <11,40,58,75> (<11> gene name [16]) [6,16,39]
type I lumazine synthase <11> [16]

2 Source Organism

<1> *Bacillus subtilis* [1,2,3,7,9,24,29,32,33,34]
<2> *Escherichia coli* [18]
<3> *Saccharomyces cerevisiae* [11,18,26]
<4> *Spinacia oleracea* [19,39]
<5> *Nicotiana tabacum* [19]
<6> *Arabidopsis thaliana* [19]
<7> *Brucella abortus* [6]

D. Schomburg and I. Schomburg (eds.), *Class 2–3.2 Transferases, Hydrolases: EC 2–3.2*,
Springer Handbook of Enzymes S9, DOI 10.1007/978-3-642-36240-8_66,

<8> *Schizosaccharomyces pombe* [7,9,14,28,35,36]
<9> *Mycobacterium tuberculosis* [7,8,9,10,15,32,33,34,36]
<10> *Candida albicans* [8,22]
<11> *Brucella sp.* [16,21,31,38,40,41]
<12> *Magnaporthe grisea* [8]
<13> *Aquifex aeolicus* [13,20,27,30]
<14> *Photobacterium leiognathi* (UNIPROT accession number: Q01994) [39]
<15> *Paracoccidioides brasiliensis* [37]
<16> *Brucella melitensis* [6]
<17> *Bacillus subtilis* (UNIPROT accession number: P11998) [4,39]
<18> *Streptomyces davawensis* [5]
<19> *Xanthomonas axonopodis pv. citri* (UNIPROT accession number: Q8PPD6) [39]
<20> *Xanthomonas campestris* (UNIPROT accession number: Q8PCM7) [39]
<21> *Xylella fastidiosa* (UNIPROT accession number: Q9PES4) [39]
<22> *Yersinia pestis* (UNIPROT accession number: Q8ZC41) [39]
<23> *Brucella sp.* (UNIPROT accession number: P61711) [23]
<24> *Mesorhizobium loti* (UNIPROT accession number: Q983B0) [6,39]
<25> *Methanocaldococcus jannaschii* (UNIPROT accession number: Q57751) [13,39]
<26> *Brucella abortus* (UNIPROT accession number: P61711) [25,39]
<27> *Actinobacillus pleuropneumoniae* (UNIPROT accession number: P50856) [39]
<28> *Agrobacterium tumefaciens* (UNIPROT accession number: Q8UG70) [39]
<29> *Saccharomyces cerevisiae* (UNIPROT accession number: P50861) [17,39]
<30> *Aquifex aeolicus* (UNIPROT accession number: O66529) [39]
<31> *Arabidopsis thaliana* (UNIPROT accession number: O80575) [39]
<32> *Archaeoglobus fulgidus* (UNIPROT accession number: O28152) [39]
<33> *Bacillus amyloliquefaciens* (UNIPROT accession number: Q44681) [39]
<34> *Bacillus halodurans* (UNIPROT accession number: Q9KCL4) [39]
<35> *Bartonella henselae* (UNIPROT accession number: Q9REF4) [39]
<36> *Buchnera aphidicola* (UNIPROT accession number: Q8K9A6) [39]
<37> *Buchnera aphidicola* (UNIPROT accession number: Q9ZNM0) [39]
<38> *Campylobacter jejuni* (UNIPROT accession number: Q9PIB9) [39]
<39> *Caulobacter vibrioides* (UNIPROT accession number: Q9A8J4) [39]
<40> *Caulobacter vibrioides* (UNIPROT accession number: Q9A9S4) [39]
<41> *Chlamydia muridarum* (UNIPROT accession number: Q9PLJ4) [39]
<42> *Chlamydia trachomatis* (UNIPROT accession number: O84737) [39]
<43> *Chlamydophila pneumoniae* (UNIPROT accession number: Q9Z733) [39]
<44> *Chlorobaculum tepidum* (UNIPROT accession number: Q8KAW4) [39]
<45> *Clostridium acetobutylicum* (UNIPROT accession number: Q97LG8) [39]
<46> *Clostridium perfringens* (UNIPROT accession number: Q8XMW9) [39]
<47> *Corynebacterium ammoniagenes* (UNIPROT accession number: O24753) [39]
<48> *Corynebacterium glutamicum* (UNIPROT accession number: Q8NQ53) [39]
<49> *Sulfurospirillum multivorans* (UNIPROT accession number: O68250) [39]

<50> *Deinococcus radiodurans* (UNIPROT accession number: Q9RXZ8) [39]
<51> *Fusobacterium nucleatum* (UNIPROT accession number: Q8RIR4) [39]
<52> *Haemophilus influenzae* (UNIPROT accession number: P45149) [39]
<53> *Halobacterium salinarum* (UNIPROT accession number: Q9HRM5) [39]
<54> *Helicobacter pylori* (UNIPROT accession number: O24854) [39]
<55> *Helicobacter pylori J99* (UNIPROT accession number: Q9ZN56) [39]
<56> *Lactococcus lactis subsp. lactis* (UNIPROT accession number: Q9CGU6) [39]
<57> *Magnaporthe grisea* (UNIPROT accession number: Q9UVT8) [39]
<58> *Mesorhizobium loti* (UNIPROT accession number: Q986N2) [6,39]
<59> *Methanopyrus kandleri* (UNIPROT accession number: Q8TYL5) [39]
<60> *Methanosarcina acetivorans* (UNIPROT accession number: Q8TPT7) [39]
<61> *Methanosarcina mazei* (UNIPROT accession number: Q8Q093) [39]
<62> *Methanothermobacter thermautotrophicus* (UNIPROT accession number: O27443) [39]
<63> *Mycobacterium leprae* (UNIPROT accession number: Q9CCP3) [39]
<64> *Schizosaccharomyces pombe* (UNIPROT accession number: Q9UUB1) [12,39]
<65> *Nicotiana tabacum* (UNIPROT accession number: Q9XH13) [39]
<66> *Anabaena sp.* (UNIPROT accession number: Q8YQ43) [39]
<67> *Pasteurella multocida* (UNIPROT accession number: P57869) [39]
<68> *Photobacterium leiognathi* (UNIPROT accession number: Q93E92) [39]
<69> *Photobacterium phosphoreum* (UNIPROT accession number: P51963) [39]
<70> *Pseudomonas aeruginosa* (UNIPROT accession number: Q9HWX5) [39]
<71> *Pyrobaculum aerophilum* (UNIPROT accession number: Q8ZTE3) [39]
<72> *Ralstonia solanacearum* (UNIPROT accession number: Q8Y1H8) [39]
<73> *Rhodococcus erythropolis* (UNIPROT accession number: Q53107) [39]
<74> *Sinorhizobium meliloti* (UNIPROT accession number: Q92NI1) [39]
<75> *Sinorhizobium meliloti* (UNIPROT accession number: Q92QU0) [39]
<76> *Streptomyces coelicolor* (UNIPROT accession number: Q9EWJ9) [39]
<77> *Sulfolobus tokodaii* (UNIPROT accession number: Q975M5) [39]
<78> *Synechocystis sp.* (UNIPROT accession number: P73527) (NYC_1 [39]) [39]
<79> *Thermotoga maritima* (UNIPROT accession number: Q9X2E5) [39]
<80> *Vibrio cholerae* (UNIPROT accession number: Q9KPU4) [39]

3 Reaction and Specificity

Catalyzed reaction

1-deoxy-L-glycero-tetrulose 4-phosphate + 5-amino-6-(D-ribitylamino)uracil = 6,7-dimethyl-8-(D-ribityl)lumazine + 2 H_2O + phosphate

Natural substrates and products

S 5-amino-6-(1-D-ribitylamino)pyrimidine-2,4(1H,^{3}H)-dione + (S)-2-hydroxy-3-oxobutyl dihydrogen phosphate <1,2,3,4,5,6,8,9,10,11,13,17,18,19,20,21,22,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,41,42,44,46,47,48,49,50,51,52,53,56,57,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,

76,77,78,79,80> (<2,3> the enzyme is involved in riboflavin biosynthesis [18]; <4,5> lumazine synthase catalyzes the penultimate step of riboflavin biosynthesis [19]; <11> penultimate step in the biosynthesis of riboflavin. The type II lumazine synthase is an immunodominant antigen of Brucella abortus [16]; <1,4,6,9,11,19,20,21,22,24,25,26,27,28,29,30,31,32,35,36,37, 38,39,41,42,44,46,47,48,49,50,51,52,53,56,57,59,60,61,62,63,64,65,66,67,68, 69,70,71,72,73,74,75,76,77,78> penultimate step of riboflavin biosynthesis [10,19,21,34,39]; <13,25> the enzyme is involved in biosynthesis of riboflavin [13]; <29> the enzyme is involved in biosynthesis of riboflavin. Gene disruption of the chromosomal copy of RIB4 leads to riboflavin auxotrophy and loss of enzyme activity [17]; <10> the enzyme is involved in riboflavin biosynthesis in many plants and microorganisms [22]; <18> the enzyme is part of the riboflavin biosynthesis gene cluster [5]; <11> the pathogen Brucella spp. expresses two proteins that exhibit lumazine synthase activity, RibH1 and RibH2. RibH1 appears to be the functional lumazine synthase in Brucella spp., whereas RibH2, an enzyme of lower catalytic activity, is a virulence factor presumably acting in response to oxidative stress [31]) (Reversibility: ?) [5,10,11,12,13,14,16,17,18,19,21,22, 25,31,34,39]

P 6,7-dimethyl-8-(1-D-ribityl)lumazine + phosphate + 2 H_2O
S 5-amino-6-(1-D-ribitylamino)pyrimidine-2,4(1H,3H)-dione + (S)-2-hydroxy-3-oxobutyl dihydrogen phosphate <55> (<55> penultimate step of riboflavin biosynthesis [39]) (Reversibility: ?) [39]
P 6,7-dimethyl-8-(1-D-ribityl)lumazine + phosphate + 2 H_2O
S 5-amino-6-(1-D-ribitylamino)pyrimidine-2,4(1H,3H)-dione + (S)-2-hydroxy-3-oxobutyl dihydrogen phosphate <58> (<58> penultimate step of riboflavin biosynthesis [39]) (Reversibility: ?) [39]
P 6,7-dimethyl-8-(1-D-ribityl)lumazine + phosphate
S 5-amino-6-ribitylamino-2,4(1H,^{3}H)-pyrimidinedione + L-3,4-dihydroxybutan-2-one 4-phosphate <13> (Reversibility: ?) [20]
P 6,7-dimethyl-8-(1-D-ribityl)lumazine + 2 H_2O + phosphate

Substrates and products

S 5-amino-6-(1-D-ribitylamino)pyrimidine-2,4(1H,3H)-dione + (3R)-3,4-dihydroxy-2-butanone 4-phosphate <1> (Reversibility: ?) [1]
P 6,7-dimethyl-8-(1-D-ribityl)lumazine + 2 H_2O + phosphate
S 5-amino-6-(1-D-ribitylamino)pyrimidine-2,4(1H,3H)-dione + (3S)-3,4-dihydroxy-2-butanone <1> (Reversibility: ?) [1]
P ?
S 5-amino-6-(1-D-ribitylamino)pyrimidine-2,4(1H,3H)-dione + (S)-2-hydroxy-3-oxobutyl dihydrogen phosphate <1,2,3,4,5,6,8,9,10,11,13,14,16,17, 18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42, 44,45,46,47,48,49,50,51,52,53,54,56,57,58,59,60,61,62,63,64,65,66,67,68,69, 70,71,72,73,74,75,76,77,78,79,80> (<2,3> the enzyme is involved in riboflavin biosynthesis [18]; <4,5> lumazine synthase catalyzes the penultimate step of riboflavin biosynthesis [19]; <11> penultimate step in the biosynthesis of riboflavin. The type II lumazine synthase is an immuno-

dominant antigen of Brucella abortus [16]; <1,4,6,9,11,14,18,19,20,21,22, 24,25,26,27,28,29,30,31,32,35,36,37,38,39,40,41,42,44,45,46,47,48,49,50,51, 52,53,54,56,57,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78> penultimate step of riboflavin biosynthesis [5,10,19,21,24,34,39]; <13,25> the enzyme is involved in biosynthesis of riboflavin [13]; <29> the enzyme is involved in biosynthesis of riboflavin. Gene disruption of the chromosomal copy of RIB4 leads to riboflavin auxotrophy and loss of enzyme activity [17]; <10> the enzyme is involved in riboflavin biosynthesis in many plants and microorganisms [22]; <18> the enzyme is part of the riboflavin biosynthesis gene cluster [5]; <11> the pathogen Brucella spp. expresses two proteins that exhibit lumazine synthase activity, RibH1 and RibH2. RibH1 appears to be the functional lumazine synthase in Brucella spp., whereas RibH2, an enzyme of lower catalytic activity, is a virulence factor presumably acting in response to oxidative stress [31]; <1> lumazine synthase/riboflavin synthase complex, the β subunit carries lumazine synthase activity. Product channeling for subsequent synthesis of riboflavin by riboflavin synthase present in the complex [1]; <3> NMR studies of the binding of phosphonate reaction intermediate analogues to Saccharomyces cerevisiae lumazine synthase. The Lys92 side chain could facilitate the exchange of inorganic phosphate eliminated from the substrate in one reaction, with the organic phosphate-containing substrate necessary for the next reaction [11]; <1> the rate enhancement by the enzyme is predominantly achieved by establishing a favourable topological relation of the two substrates, whereas acid/base catalysis may play a secondary role [29]) (Reversibility: ?) [1,2,5,6,10,11,12,13,14,16,17,18,19,21,22,23, 24,25,29,31,32,33,34,36,39,40]

P 6,7-dimethyl-8-(1-D-ribityl)lumazine + phosphate + 2 H_2O
S 5-amino-6-(1-D-ribitylamino)pyrimidine-2,4(1H,3H)-dione + (S)-2-hydroxy-3-oxobutyl dihydrogen phosphate <55> (<55> penultimate step of riboflavin biosynthesis [39]) (Reversibility: ?) [39]
P 6,7-dimethyl-8-(1-D-ribityl)lumazine + phosphate + 2 H2
S 5-amino-6-(1-D-ribitylamino)pyrimidine-2,4(1H,3H)-dione + (S)-2-hydroxy-3-oxobutyl dihydrogen phosphate <58> (<58> penultimate step of riboflavin biosynthesis [39]) (Reversibility: ?) [39]
P 6,7-dimethyl-8-(1-D-ribityl)lumazine + phosphate
S 5-amino-6-(1-D-ribitylamino)pyrimidine-2,4(1H,3H)-dione + (S)-2-hydroxy-3-oxobutyl dihydrogen phosphate <9> (<9> penultimate step of riboflavin biosynthesis [36]) (Reversibility: ?) [36]
P 6,7-dimethyl-8-(1-D-ribityl)lumazine + phosphat
S 5-amino-6-(1-D-ribitylamino)pyrimidine-2,4(1H,3H)-dione + 5-nitro-6-ribitylamino-2,4(1H,^{3}H)-pyrimidinedione <1> (Reversibility: ?) [1]
P ?
S 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione + L-3,4-dihydroxybutan-2-one 4-phosphate <8,13,24> (<13> an early optical transient absorbing around 330 nm is interpreted as a Schiff base intermediate obtained by reaction of the position 5 amino group of the heterocyclic substrate with the carbonyl group of 3,4-dihydroxy-2-butanone 4-phosphate.

A second transient with an absorption maximum at 445 nm represents an intermediate resulting from the elimination of phosphate from the Schiff base. The rate-determining step is the subsequent formation of the 7-exo-methylene type anion of 6,7-dimethyl-8-ribityllumazine [20]) (Reversibility: ?) [6,20,35]

P 6,7-dimethyl-8-(1-D-ribityl)lumazine + 2 H_2O + phosphate

Inhibitors

(1R)-1,2-dideoxy-1-fluoro-1-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-D-ribo-hexitol <9> [33]
(1S)-1,2-dideoxy-1-fluoro-1-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-D-ribo-hexitol <1,9> [33]
(E)-3-hydroxy-4-(2-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-yl)vinyl)benzoic acid <8,9> [36]
(E)-4-(2-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-yl)vinyl)benzoic acid <8> [36]
(E)-4-(2-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-yl)vinyl)benzoic acid <9> [36]
(E)-5-nitro-6-(2-hydroxystyryl)pyrimidine-2,4(1H,3H)-dione <8,9> (<8,9> competitive [36]) [36]
(E)-5-nitro-6-(3-(pyridin-3-yl)vinyl)pyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-5-nitro-6-(3-nitrostyryl)pyrimidine-2,4(1H,3H)-dione <8> [36]
(E)-5-nitro-6-(4-bromostyryl)pyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-5-nitro-6-(4-nitrostyryl)pyrimidine-2,4(1H,3H)-dione <8,9> [36]
(E)-5-nitro-6-[2-(1H-pyrrol-2-yl)vinyl]pyrimidine-2,4(1H,3H)-dione <8,9> [36]
(E)-6-(2,3,4-trimethoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(2,3-dihydroxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <8,9> [36]
(E)-6-(2,3-dimethoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <8,9> [36]
(E)-6-(2-(^{3}H-indol-3-yl)vinyl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(2-(naphthalen-2-yl)vinyl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(2-fluoro-3-methoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(2-fluorostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(2-hydroxy-3-nitrostyryl)-3-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(2-hydroxy-5-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(2-methoxy-5-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <8,9> [36]
(E)-6-(2-methoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <8,9> [36]
(E)-6-(2-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(3,4,5-trimethoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(3,4-dimethoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(3-hydroxy-4-methoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(3-hydroxy-4-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <8,9> [36]
(E)-6-(3-hydroxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> (<9> competitive [36]) [36]
(E)-6-(4-chlorostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(4-fluorostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
(E)-6-(4-hydroxy-3-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]

(E)-6-(4-hydroxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione <9> [36]
1,3,6,8-tetrahydroxynaphthyridine <8> [35]
1,3,7-trihydro-9-D-ribityl-2,4,8-purinetrione <9,10> (<9> association constants and thermodynamic parameters of binding [15]; <10> association constants and thermodynamic parameters of binding of different inhibitors to lumazine synthase [22]) [15,22]
1-[(5-amino-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)(methyl)amino]-1-deoxy-D-ribitol hydrochloride <1,9> [33]
1-deoxy-1-(2,4,6,7-tetraoxo-1,3,4,5,6,7-hexahydropteridin-8(2H)-yl)-D-ribitol <1,9> [34]
1-deoxy-1-(2,6,8-trioxo-1,2,3,6,7,8-hexahydro-9H-purin-9-yl)-D-ribitol <1,9> [32]
1-deoxy-1-[(2,6-dioxo-5-[[5-(phosphonooxy)pentanoyl]amino]-1,2,3,6-tetrahydropyrimidin-4-yl)amino]-D-ribitol <1> [32]
1-deoxy-1-[2,4,6,7-tetraoxo-5-[4-(phosphonooxy)butyl]-1,3,4,5,6,7-hexahydropteridin-8(2H)-yl]-D-ribitol <9> [34]
1-deoxy-1-[2,4,6,7-tetraoxo-5-[5-(phosphonooxy)pentyl]-1,3,4,5,6,7-hexahydropteridin-8(2H)-yl]-D-ribitol <9> [34]
1-deoxy-1-[2,6,8-trioxo-7-[4-(phosphonooxy)butyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol <1,9> [34]
1-deoxy-1-[2,6,8-trioxo-7-[5-(phosphonooxy)pentyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol <1,9> [32,34]
1-deoxy-1-[2,6,8-trioxo-7-[6-(phosphonooxy)hexyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol <1,9> [32]
1-deoxy-1-[[2,6-dioxo-5-(4-phosphonobutyl)-1,2,3,6-tetrahydropyrimidin-4-yl]amino]-D-ribitol <1> [32]
1-deoxy-1-[[2,6-dioxo-5-(5-phosphonopentyl)-1,2,3,6-tetrahydropyrimidin-4-yl]amino]-D-ribitol <1> [32]
1-deoxy-1-[[2,6-dioxo-5-(6-phosphonohexyl)-1,2,3,6-tetrahydropyrimidin-4-yl]amino]-D-ribitol <1> [32]
2,4-dioxo-6-[[(2R,3R,4R)-2,3,4,5-tetrahydroxypentyl]sulfanyl]-1,2,3,4-tetrahydropyrimidin-5-aminium chloride <1,8,9> (<1,8,9> inhibition of both lumazine synthase and riboflavin synthase [7]) [7]
2,5,8,11-tetraaza-5,11-dihydro-4,10-dihydroxyperylene-1,3,6,7,9,12-hexaone <8> [35]
3-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)-1-propanol <9> [32]
3-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)propane 1-phosphate <1,9> [32]
3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrion-7-yl)butane 1-phosphate <9> (<9> highly specific binding of the purinetrione inhibitor to the Mycobacterium tuberculosis enzyme with dissociation constants in micromolar range [10]) [10]
3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)butane 1-phosphate <9, 10,12> (<9,10,12> competitive [8]) [8]
3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)pentane 1-phosphate <9, 10,12> (<9,10,12> competitive [8]) [8]

3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)propane 1-phosphate <9> (<9> highly specific binding of the purinetrione inhibitor to the Mycobacterium tuberculosis enzyme with dissociation constants in micromolar range [10]) [10]
3-(1,3-dihydro-9-D-ribityl-2,4,8-purinetrione-7-yl)propane 1-phosphate <10> (<10> association constants and thermodynamic parameters of binding of different inhibitors to lumazine synthase [22]) [22]
3-(7-hydroxy-8-ribityllumazine-6-yl)propionic acid <13> [30]
3-[4,6-dioxo-4,5,6,7-tetrahydro-1-D-ribityl-1H-pyrazolo[3,4-d]pyrimidin-3-yl]propyl 1-phosphate <9,10,12> (<9,10> competitive [8]; <12> mixed type inhibition [8]) [8]
4-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)butane 1-phosphate <1, 9> [32]
4-(1,5,6,7-tetrahydro-6,7-dioxo-8-D-ribityllumazin-5-yl)butane 1-phosphate <1, 9> [34]
4-(6,7(5H,8H)-dioxo-8-D-ribityllumazine-5-yl)butane 1-phosphate <10> (<10> association constants and thermodynamic parameters of binding of different inhibitors to lumazine synthase [22]) [22]
4-(6-chloro-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-yl)-butyl <10> (<10> association constants and thermodynamic parameters of binding of different inhibitors to lumazine synthase [22]) [22]
4-(6-chloro-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-yl)butyl 1-phosphate <9> (<9> association constants and thermodynamic parameters of binding [15]) [15]
4-[2,4,7-trioxo-8-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-1,2,3,4,7,8-hexahydropteridin-6-yl]butanoic acid <1> (<1> uncompetitive, comparison with inhibition of Escherichia coli riboflavin synthase [2]) [2]
4-[2,4,7-trioxo-8-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-1,2,3,4,7,8-hexahydropteridin-6-yl]butyl dihydrogen phosphate <1> (<1> competitive, comparison with inhibition of Escherichia coli riboflavin synthase [2]) [2]
4-[2,4,7-trioxo-8-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-1,2,3,4,7,8-hexahydropteridin-6-yl]propyl dihydrogen phosphate <1> (<1> uncompetitive, comparison with inhibition of Escherichia coli riboflavin synthase [2]) [2]
4-[4,6-dioxo-4,5,6,7-tetrahydro1-D-ribityl-1H-pyrazolo[3,4-d]-pyrimidin-3-yl]butyl 1-phosphate <9,10> (<9,10> competitive [8]) [8]
5-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)-1,1-difluoropentane 1-phosphonate <1,9> [32]
5-(1,3,7-trihydro-9-D-ribityl-2,4,8-purinetrione-7-yl)pentane1-phosphate <9> (<9> association constants and thermodynamic parameters of binding [15]) [15]
5-(1,3,7-trihydro-9-D-ribityl-2,4,8-purinetrione-7-yl)1,1-difluoropentane-1-phosphate <9> (<9> association constants and thermodynamic parameters of binding [15]) [15]
5-(1,5,6,7-tetrahydro-6,7-dioxo-8-D-ribityllumazin-5-yl-)pentane 1-phosphate <1,9> [34]
5-(4-phosphonobutyryl)amino-6-D-ribitylaminouracil <1> (<1> comparison with inhibition of Escherichia coli riboflavin synthase [3]) [3]

5-(4-phosphonopentyl)amino-6-D-ribitylaminouracil <1> (<1> comparison with inhibition of Escherichia coli riboflavin synthase [3]) [3]
5-(5-phosphonoxyvaleryl)amino-6-D-ribitylaminouracil <1> (<1> mixed inhibition, comparison with inhibition of Escherichia coli riboflavin synthase [3]) [3]
5-(6-D-ribitylamino-2,4(1H,3H)pyrimidinedione-5-yl)-1-pentyl-phosphonic acid <13> [30]
5-(hexyl 6-dihydrogen phosphate)-6-([(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl] amino)pyrimidine-2,4(1H,3H)-dione <1> (<1> mixed inhibition, comparison with inhibition of Escherichia coli riboflavin synthase [2,3]) [2,3]
5-(pentyl 6-dihydrogen phosphate)-6-([(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl] amino)pyrimidine-2,4(1H,3H)-dione <1> (<1> mixed inhibition, comparison with inhibition of Escherichia coli riboflavin synthase [2]) [2]
5-[4,6-dioxo-4,5,6,7-tetrahydro-1-D-ribityl-1H-pyrazolo[3,4-d]pyrimidin-3-yl]pentyl 1-phosphate <9,10> (<9,10> competitive [8]) [8]
5-nitro-6-(D-ribitylamino)-2,4(1H,3H)-pyrimidinedione <1,8> [14,24,28]
5-nitro-6-[(D-ribityl)methyl]pyrimidine-2,4-dione <1> [33]
5-nitro-6-[[(2R,3R,4R)-2,3,4,5 tetrahydroxypentyl]sulfanyl]pyrimidine-2,4(1H, 3H)-dione <1,8,9> (<1,8,9> inhibition of both lumazine synthase and riboflavin synthase [7]) [7]
5-nitro-6-ribitylamino-2,4(1H,3H)-pyrimidinedione <7,16,24,58> (<7,16,24,58> crystallization data [6]) [6]
5-nitro-6-styryluracil <8,9> [36]
5-nitroso-6-(D-ribitylamino)-2,4(1H,3H)-pyrimidinedione <8> [14]
5-nitroso-6-ribityl-amino-2,4(1H,3H)pyrimidinedione <13> [30]
6-(1,3,7-trihydro-9-d-ribityl-2,4,8-purinetrione-7-yl)hexane 1-phosphate <9> (<9> association constants and thermodynamic parameters of binding [15]) [15]
6-carboxyethyl-7-oxo-8-ribityllumazine <8> [14,28]
6-methyl-7-methylidene-8-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-7,8-dihydropyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione <1> (<1> mixed inhibition, comparison with inhibition of Escherichia coli riboflavin synthase [2]) [2]
7-dioxo-5H-8-ribitylaminolumazine <13> [30]
9-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-7,9-dihydro-1H-purine-2,6,8(3H)-trione <1> (<1> comparison with inhibition of Escherichia coli riboflavin synthase [2]) [2]
N^6-(ribitylamino)pyrimidine-2,4(1H,3H)-dion-5-ylpropionamide <1,8,9> (<8> competitive, inhibition of both lumazine synthase and riboflavin synthase [9]; <1> partial inhibition, inhibition of both lumazine synthase and riboflavin synthase [9]; <9> competitive, inhibition of both lumazine synthase and riboflavin synthase (EC 2.5.1.9) [9]) [9]
N^6-(ribitylamino)pyrimidine-2,4(1H,3H)-dione-5-ylisobutyramide <1,8,9> (<8> competitive, inhibition of both lumazine synthase and riboflavin synthase [9]; <1> partial inhibition, inhibition of both lumazine synthase and riboflavin synthase [9]; <9> partial inhibition, inhibition of both lumazine synthase and riboflavin synthase (EC 2.5.1.9) [9]) [9]

N-[2,4-dioxo-6-(ribitylamino)-1,2,3,4-tetrahydropyrimidin-5-yl]oxalamic acid ethyl ester <1,8,9> (<1> competitive, inhibition of both lumazine synthase and riboflavin synthase [9]; <8> mixed type inhibition, inhibition of both lumazine synthase and riboflavin synthase [9]; <9> partial inhibition, inhibition of both lumazine synthase and riboflavin synthase (EC 2.5.1.9) [9]) [9]
riboflavin <64> [12]
Additional information <1> (<1> incorporation of an amide into 5-phosphonoalkyl-6-D-ribitylaminopyrimidinedione lumazine synthase inhibitors results in an unexpected reversal of selectivity for riboflavin synthase versus lumazine synthase [3]; <1> certain purinetriones bearing phosphate side chains can inhibit both lumazine synthase as well as riboflavin synthase, and molecular modeling with 3-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)propane 1-phosphate suggests possible binding modes to each enzyme. Antibiotics that would inhibit both lumazine synthase and riboflavin synthase would be less likely to suffer from the development of antibiotic resistance by the organisms that they are supposed to treat, since pathogenic microorganisms would have to simultaneously select for mutations in both enzymes in order to escape the cytotoxic effects of the antibiotics [32]; <1> no inhibition: 5-amino-6-[(D-ribityl)methyl]pyrimidine-2,4-dione hydrochloride, 5-nitro-6-(N-methyl)ribitylpyrimidine-2,4-dione [33]) [3,32,33]

Turnover number (s^{-1})

0.0003 <24> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <24> isoform RibH2, pH 7.0, 37°C [6]) [6]
0.0022 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme H88K [29]) [29]
0.0022 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme H88K [29]) [29]
0.003 <16> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <16> pH 7.0, 37°C [6]) [6]
0.0038 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F113S [29]) [29]
0.0038 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F113S [29]) [29]
0.0054 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme K131N [29]) [29]
0.0054 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme K131N [29]) [29]
0.0067 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme H88A [29]) [29]
0.0067 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme H88A [29]) [29]
0.0081 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F22D [29]) [29]
0.0081 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F22D [29]) [29]

0.0122 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme K135A [29]; <1> pH 7.0, 37°C, mutant enzyme N23S [29]) [29]
0.0122 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme K135A [29]; <1> pH 7.0, 37°C, mutant enzyme N23S [29]) [29]
0.0147 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F22V [29]) [29]
0.0147 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F22V [29]) [29]
0.0166 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme K131R [29]) [29]
0.0166 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme K131R [29]) [29]
0.0244 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F22W [29]; <1> pH 7.0, 37°C, mutant enzyme F57S [29]) [29]
0.0244 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F22W [29]; <1> pH 7.0, 37°C, mutant enzyme F57S [29]) [29]
0.0263 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F22S [29]) [29]
0.0263 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F22S [29]) [29]
0.0307 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme T80V [29]) [29]
0.0307 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme T80V [29]) [29]
0.0347 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme S142L [29]) [29]
0.0347 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme S142L [29]) [29]
0.0389 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme R127H [29]) [29]
0.0389 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme R127H [29]) [29]
0.0391 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme E58Q [29]) [29]
0.0391 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme E58Q [29]) [29]
0.04 <58> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <58> isoform RibH1, pH 7.0, 37°C [6]) [6]
0.0453 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme A56S [29]) [29]
0.0453 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme A56S [29]) [29]
0.051 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme D138A [29]) [29]

0.051 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme D138A [29]) [29]
0.0557 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, wild-type enzyme [29]) [29]
0.0557 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, wild-type enzyme [29]) [29]
0.056 <1> (5-nitro-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> native enzyme complex, pH 7.0, 37°C [1]) [1]
0.056 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> native enzyme complex, pH 7.0, 37°C [1]) [1]
0.076 <1> (5-nitro-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> hollow $\beta_6$0 capsid, pH 7.0, 37°C [1]) [1]
0.076 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> hollow $\beta_6$0 capsid, pH 7.0, 37°C [1]) [1]

Specific activity (U/mg)

0.011 <25> (<25> 37°C, pH 7.0 [13]) [13]
0.018 <11> [16]
0.031 <13> (<13> 37°C, pH 7.0 [13]) [13]
0.113 <8,9> [36]
0.166 <10> [22]
0.266 <8> [35]
11.8 <2> [18]
13 <64> [12]
15.4 <3> [18]
16.5 <4> [19]

K_m-Value (mM)

0.0025 <58> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <58> isoform RibH1, pH 7.0, 37°C [6]) [6]
0.003 <64> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <64> pH 7.0, 37°C, mutant enzyme W27F [12]; <64> pH 7.0, 37°C, mutant enzyme W27Y [12]) [12]
0.004 <3,16> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <16> pH 7.0, 37°C [6]) [6,18]
0.0041 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme T80V [29]) [29]
0.0042 <1,2> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme A56S [29]) [18,29]
0.005 <64> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <64> pH 7.0, 37°C, wild-type enzyme [12]) [12]
0.005 <1> (5-nitro-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> native enzyme complex, pH 7.0, 37°C [1]) [1]
0.0052 <1,9> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1,9> pH 7.0, 37°C, Tris buffer [32]) [32]
0.00612 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F22W [29]) [29]

0.0067 <1,9> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1,9> pH 7.0, 37°C, MOPS buffer [32]) [32]
0.00857 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, wild-type enzyme [29]) [29]
0.01 <1,13> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <13> 37°C, pH 7.0 [13]; <1> pH 7.0, 37°C, mutant enzyme D138A [29]) [13,29]
0.0107 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F22V [29]) [29]
0.011 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme S142L [29]) [29]
0.0115 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme K135A [29]) [29]
0.0119 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme N23S [29]) [29]
0.0125 <25> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <25> 37°C, pH 7.0 [13]) [13]
0.0138 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme K131R [29]) [29]
0.015 <58> (L-3,4-dihydroxybutan-2-one 4-phosphate, <58> isoform RibH1, pH 7.0, 37°C [6]) [6]
0.02 <4,24> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <24> isoform RibH2, pH 7.0, 37°C [6]) [6,19]
0.026 <4,13> (L-3,4-dihydroxybutan-2-one 4-phosphate, <13> 37°C, pH 7.0 [13]) [13,19]
0.0345 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F57S [29]) [29]
0.0355 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme K131N [29]) [29]
0.042 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme H88K [29]; <1> pH 7.0, 37°C, mutant enzyme T80V [29]) [29]
0.05 <1,9> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme K131R [29]; <1,9> pH 7.0, 37°C, phosphate buffer [32]) [29,32]
0.052 <25> (L-3,4-dihydroxybutan-2-one 4-phosphate, <25> 37°C, pH 7.0 [13]) [13]
0.0546 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, wild-type enzyme [29]) [29]
0.056 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme H88K [29]) [29]
0.0581 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme N23S [29]) [29]
0.0598 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F22W [29]) [29]
0.06 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme H88A [29]) [29]
0.062 <2> (L-3,4-dihydroxybutan-2-one 4-phosphate) [18]
0.0636 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme D138A [29]) [29]

0.065 <64> (L-3,4-dihydroxybutan-2-one 4-phosphate, <64> pH 7.0, 37°C, mutant enzyme W27F [12]) [12]
0.0665 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F113S [29]) [29]
0.067 <64> (L-3,4-dihydroxybutan-2-one 4-phosphate, <64> pH 7.0, 37°C, wild-type enzyme [12]) [12]
0.0702 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme A56S [29]) [29]
0.08 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F57S [29]) [29]
0.0849 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme R127H [29]) [29]
0.086 <64> (L-3,4-dihydroxybutan-2-one 4-phosphate, <64> pH 7.0, 37°C, mutant enzyme W27Y [12]) [12]
0.09 <11> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione) [16]
0.09 <3> (L-3,4-dihydroxybutan-2-one 4-phosphate) [18]
0.0905 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme S142L [29]) [29]
0.124 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F22V [29]) [29]
0.125 <11> (L-3,4-dihydroxybutan-2-one 4-phosphate) [16]
0.13 <1> ((3S)-3,4-dihydroxy-2-butanone, <1> native enzyme complex, pH 7.0, 37°C [1]) [1]
0.137 <1,64> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F22S [29]; <64> pH 7.0, 37°C, mutant enzyme W27I [12]) [12,29]
0.14 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F22S [29]) [29]
0.145 <64> (L-3,4-dihydroxybutan-2-one 4-phosphate, <64> pH 7.0, 37°C, mutant enzyme W27H [12]) [12]
0.146 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme E58Q [29]) [29]
0.147 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme H88A [29]) [29]
0.167 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme K135A [29]) [29]
0.168 <64> (L-3,4-dihydroxybutan-2-one 4-phosphate, <64> pH 7.0, 37°C, mutant enzyme W27G [12]) [12]
0.173 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme E58Q [29]) [29]
0.187 <64> (L-3,4-dihydroxybutan-2-one 4-phosphate, <64> pH 7.0, 37°C, mutant enzyme W27S [12]) [12]
0.225 <16> (L-3,4-dihydroxybutan-2-one 4-phosphate, <16> pH 7.0, 37°C [6]) [6]
0.23 <64> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <64> pH 7.0, 37°C, mutant enzyme W27I [12]) [12]

0.278 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F113S [29]) [29]
0.283 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme K131N [29]) [29]
0.4 <64> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <64> pH 7.0, 37°C, mutant enzyme W27H [12]) [12]
0.43 <64> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <64> pH 7.0, 37°C, mutant enzyme W27G [12]) [12]
0.45 <24> (L-3,4-dihydroxybutan-2-one 4-phosphate, <24> isoform RibH2, pH 7.0, 37°C [6]) [6]
0.46 <64> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <64> pH 7.0, 37°C, mutant enzyme W27S [12]) [12]
0.675 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme F22D [29]) [29]
0.72 <1> (5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, <1> pH 7.0, 37°C, mutant enzyme F22D [29]) [29]
3.14 <1> (L-3,4-dihydroxybutan-2-one 4-phosphate, <1> pH 7.0, 37°C, mutant enzyme R127H [29]) [29]

K_i-Value (mM)

0.0000008 8e-007 <12> (3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)pentane 1-phosphate, <12> pH 7.0, 27°C [8]) [8]
0.0000009 9e-007 <10> (3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)pentane 1-phosphate, <10> pH 7.0, 27°C [8]) [8]
0.0000013 1.3e-006 <10> (4-[4,6-dioxo-4,5,6,7-tetrahydro1-D-ribityl-1H-pyrazolo[3,4-d]-pyrimidin-3-yl]butyl 1-phosphate, <10> pH 7.0, 27°C [8]) [8]
0.000002 <12> (3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)butane 1-phosphate, <12> pH 7.0, 27°C [8]) [8]
0.0000037 <10> (3-[4,6-dioxo-4,5,6,7-tetrahydro-1-D-ribityl-1H-pyrazolo[3,4-d]pyrimidin-3-yl]propyl 1-phosphate, <10> pH 7.0, 27°C [8]) [8]
0.0000041 <9> (1-deoxy-1-[2,6,8-trioxo-7-[4-(phosphonooxy)butyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol, <9> pH 7.0, 37°C [34]) [34]
0.0000041 <9> (4-[4,6-dioxo-4,5,6,7-tetrahydro1-D-ribityl-1H-pyrazolo[3,4-d]-pyrimidin-3-yl]butyl 1-phosphate, <9> pH 7.0, 27°C [8]) [8]
0.0000047 <9> (1-deoxy-1-[2,6,8-trioxo-7-[5-(phosphonooxy)pentyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol, <9> pH 7.0, 37°C [34]) [34]
0.0000047 <9> (5-[4,6-dioxo-4,5,6,7-tetrahydro-1-D-ribityl-1H-pyrazolo[3,4-d]pyrimidin-3-yl]pentyl 1-phosphate, <9> pH 7.0, 27°C [8]) [8]
0.0000077 <10> (5-[4,6-dioxo-4,5,6,7-tetrahydro-1-D-ribityl-1H-pyrazolo[3,4-d]pyrimidin-3-yl]pentyl 1-phosphate, <10> pH 7.0, 27°C [8]) [8]
0.000012 <9> (1-deoxy-1-[2,4,6,7-tetraoxo-5-[5-(phosphonooxy)pentyl]-1,3,4,5,6,7-hexahydropteridin-8(2H)-yl]-D-ribitol, <9> pH 7.0, 37°C [34]) [34]
0.000012 <9> (5-(1,5,6,7-tetrahydro-6,7-dioxo-8-D-ribityllumazin-5-yl-)pentane 1-phosphate, <9> pH 7.0, 37°C [34]) [34]
0.000014 <10> (3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)butane 1-phosphate, <10> pH 7.0, 27°C [8]) [8]

0.000015 <9> (3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)butane 1-phosphate, <9> pH 7.0, 27°C [8]) [8]
0.00003 <9> (3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)pentane 1-phosphate, <9> pH 7.0, 27°C [8]) [8]
0.000036 <9> (1-deoxy-1-[2,4,6,7-tetraoxo-5-[4-(phosphonooxy)butyl]-1,3,4,5,6,7-hexahydropteridin-8(2H)-yl]-D-ribitol, <9> pH 7.0, 37°C [34]) [34]
0.000036 <9> (4-(1,5,6,7-tetrahydro-6,7-dioxo-8-D-ribityllumazin-5-yl)butane 1-phosphate, <9> pH 7.0, 37°C [34]) [34]
0.00004 <9> (3-[4,6-dioxo-4,5,6,7-tetrahydro-1-D-ribityl-1H-pyrazolo[3,4-d]pyrimidin-3-yl]propyl 1-phosphate, <9> pH 7.0, 27°C [8]) [8]
0.000101 <12> (3-[4,6-dioxo-4,5,6,7-tetrahydro-1-D-ribityl-1H-pyrazolo[3,4-d]pyrimidin-3-yl]propyl 1-phosphate, <12> pH 7.0, 27°C [8]) [8]
0.00016 <8> (2,4-dioxo-6-[[(2R,3R,4R)-2,3,4,5-tetrahydroxypentyl]sulfanyl]-1,2,3,4-tetrahydropyrimidin-5-aminium chloride, <8> pH 7.0, 27°C [7]) [7]
0.0014 <9> (1-deoxy-1-(2,4,6,7-tetraoxo-1,3,4,5,6,7-hexahydropteridin-8(2H)-yl)-D-ribitol, <9> pH 7.0, 37°C [34]) [34]
0.002 <8> (5-nitro-6-[[(2R,3R,4R)-2,3,4,5 tetrahydroxypentyl]sulfanyl]pyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [7]) [7]
0.0026 <1> (2,4-dioxo-6-[[(2R,3R,4R)-2,3,4,5-tetrahydroxypentyl]sulfanyl]-1,2,3,4-tetrahydropyrimidin-5-aminium chloride, <1> pH 7.0, 27°C [7]) [7]
0.0026 <9> (3-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)-1-propanol, <9> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.0037 <9> ((E)-5-nitro-6-(4-nitrostyryl)pyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.0041 <9> (4-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)butane 1-phosphate, <9> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.0045 <9> (3-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)propane 1-phosphate, <9> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.0047 <9> (1-deoxy-1-[2,6,8-trioxo-7-[5-(phosphonooxy)pentyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol, <9> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.0049 <8> ((E)-6-(2,3-dihydroxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.0071 <9> ((E)-6-(3-hydroxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.0078 <9> ((E)-6-(2-fluorostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.0078 <1> (1-deoxy-1-(2,4,6,7-tetraoxo-1,3,4,5,6,7-hexahydropteridin-8(2H)-yl)-D-ribitol, <1> pH 7.0, 37°C [34]) [34]
0.0091 <9> (1-deoxy-1-(2,6,8-trioxo-1,2,3,6,7,8-hexahydro-9H-purin-9-yl)-D-ribitol, <9> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.0096 <9> ((E)-6-(2,3-dimethoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]

0.011 <8> ((E)-5-nitro-6-(3-nitrostyryl)pyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.011 <9> ((E)-6-(2-(naphthalen-2-yl)vinyl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.011 <9> (5-nitro-6-[[(2R,3R,4R)-2,3,4,5 tetrahydroxypentyl]sulfanyl]pyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [7]) [7]
0.012 <9> ((E)-6-(2,3-dihydroxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.012 <9> ((E)-6-(3-hydroxy-4-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.012 <9> ((E)-6-(4-hydroxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.013 <8> ((E)-5-nitro-6-(4-nitrostyryl)pyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.013 <8> ((E)-5-nitro-6-[2-(1H-pyrrol-2-yl)vinyl]pyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.015 <9> (5-nitro-6-styryluracil, <9> pH 7.0, 27°C [36]) [36]
0.016 <9> ((E)-6-(2-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.017 <9> ((E)-6-(3,4,5-trimethoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.017 <64> (riboflavin, <64> pH 7.0, 37°C, wild-type enzyme [12]) [12]
0.022 <8> (2,5,8,11-tetraaza-5,11-dihydro-4,10-dihydroxyperylene-1,3,6,7,9,12-hexaone, <8> pH 7.0, 37°C, phosphate buffer [35]) [35]
0.023 <9> ((E)-6-(2-methoxy-5-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.024 <9> ((E)-6-(2-fluoro-3-methoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.026 <9> ((E)-5-nitro-6-(3-(pyridin-3-yl)vinyl)pyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.026 <9> ((E)-5-nitro-6-(4-bromostyryl)pyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.026 <1> (5-nitro-6-[[(2R,3R,4R)-2,3,4,5 tetrahydroxypentyl]sulfanyl]pyrimidine-2,4(1H,3H)-dione, <1> pH 7.0, 27°C [7]) [7]
0.027 <1> (5-(1,5,6,7-tetrahydro-6,7-dioxo-8-D-ribityllumazin-5-yl-)pentane 1-phosphate, <1> pH 7.0, 37°C [34]) [34]
0.028 <9> ((E)-6-(2-methoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.03 <9> ((E)-5-nitro-6-[2-(1H-pyrrol-2-yl)vinyl]pyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.031 <9> ((E)-6-(2-hydroxy-5-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.031 <9> (2,4-dioxo-6-[[(2R,3R,4R)-2,3,4,5-tetrahydroxypentyl]sulfanyl]-1,2,3,4-tetrahydropyrimidin-5-aminium chloride, <9> pH 7.0, 27°C [7]) [7]
0.032 <9> ((E)-6-(3,4-dimethoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]

0.035 <9> ((E)-4-(2-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-yl)vinyl)benzoic acid, <9> pH 7.0, 27°C [36]) [36]
0.041 <8> ((E)-4-(2-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-yl)vinyl)benzoic a, <8> pH 7.0, 27°C [36]) [36]
0.0414 <1> (3-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)propane 1-phosphate, <1> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.042 <9> ((E)-5-nitro-6-(3-nitrostyryl)pyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.042 <9> ((E)-6-(2-hydroxy-3-nitrostyryl)-3-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.045 <9> ((E)-6-(4-chlorostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.046 <1> (1-deoxy-1-(2,6,8-trioxo-1,2,3,6,7,8-hexahydro-9H-purin-9-yl)-D-ribitol, <1> pH 7.0, 37°C, variable concentration of L-3,4-dihydroxybutan-2-one 4-phosphate [32]) [32]
0.046 <1> (9-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-7,9-dihydro-1H-purine-2,6,8(3H)-trione, <1> pH 7.5, 37°C, recombinant β60 capsid [2]) [2]
0.048 <9> ((E)-6-(2,3,4-trimethoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.052 <9> ((E)-6-(3-hydroxy-4-methoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.06 <9> (5-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)-1,1-difluoropentane 1-phosphonate, <9> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.066 <8> (2,5,8,11-tetraaza-5,11-dihydro-4,10-dihydroxyperylene-1,3,6,7,9,12-hexaone, <8> pH 7.0, 37°C, Tris buffer [35]) [35]
0.07 <9> ((E)-6-(2-(3H-indol-3-yl)vinyl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.074 <9> ((1S)-1,2-dideoxy-1-fluoro-1-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-D-ribo-hexitol, <9> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [33]) [33]
0.0783 <1> (1-deoxy-1-[2,6,8-trioxo-7-[6-(phosphonooxy)hexyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol, <1> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.084 <1> (4-[2,4,7-trioxo-8-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-1,2,3,4,7,8-hexahydropteridin-6-yl]butanoic acid, <1> pH 7.5, 37°C, recombinant β60 capsid [2]) [2]
0.085 <8> ((E)-6-(2-methoxy-5-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.086 <9> ((E)-6-(4-fluorostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.087 <9> ((E)-6-(4-hydroxy-3-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.094 <8> ((E)-6-(2-(naphthalen-2-yl)vinyl)-5-nitropyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]

0.094 <8> ((E)-6-(3-hydroxy-4-nitrostyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.094 <1> (6-methyl-7-methylidene-8-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-7,8-dihydropyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione, <1> pH 7.5, 37°C, recombinant β60 capsid [2]) [2]
0.095 <9> ((E)-5-nitro-6-(2-hydroxystyryl)pyrimidine-2,4(1H,3H)-dione, <9> pH 7.0, 27°C [36]) [36]
0.113 <9> ((1R)-1,2-dideoxy-1-fluoro-1-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-D-ribo-hexitol, <9> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [33]) [33]
0.12 <1> (4-[2,4,7-trioxo-8-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-1,2,3,4,7,8-hexahydropteridin-6-yl]propyl dihydrogen phosphate, <1> pH 7.5, 37°C, recombinant β60 capsid [2]) [2]
0.13 <8> ((E)-3-hydroxy-4-(2-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-yl)vinyl)benzoic acid, <8> pH 7.0, 27°C [36]) [36]
0.13 <1> (1-deoxy-1-[[2,6-dioxo-5-(6-phosphonohexyl)-1,2,3,6-tetrahydropyrimidin-4-yl]amino]-D-ribitol, <1> pH 7.0, 37°C, variable concentration of L-3,4-dihydroxybutan-2-one 4-phosphate [32]) [32]
0.13 <1> (5-(hexyl 6-dihydrogen phosphate)-6-([(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]amino)pyrimidine-2,4(1H,3H)-dione, <1> pH 7.5, 37°C, recombinant β60 capsid [2,3]) [2,3]
0.132 <1> (5-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)-1,1-difluoropentane 1-phosphonate, <1> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.141 <8> ((E)-6-(2-(3H-indol-3-yl)vinyl)-5-nitropyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.143 <8> (5-nitro-6-styryluracil, <8> pH 7.0, 27°C [36]) [36]
0.15 <1> (4-(1,5,6,7-tetrahydro-6,7-dioxo-8-D-ribityllumazin-5-yl)butane 1-phosphate, <1> pH 7.0, 37°C [34]) [34]
0.151 <8> ((E)-6-(2-fluoro-3-methoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.16 <1> (4-[2,4,7-trioxo-8-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-1,2,3,4,7,8-hexahydropteridin-6-yl]butyl dihydrogen phosphate, <1> pH 7.5, 37°C, recombinant β60 capsid [2]) [2]
0.168 <1> (4-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)butane 1-phosphate, <1> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.17 <1> (1-deoxy-1-[2,6,8-trioxo-7-[4-(phosphonooxy)butyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol, <1> pH 7.0, 37°C [34]) [34]
0.17 <9> (1-deoxy-1-[2,6,8-trioxo-7-[6-(phosphonooxy)hexyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol, <9> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.175 <1> (1-deoxy-1-[2,6,8-trioxo-7-[6-(phosphonooxy)hexyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol, <1> pH 7.0, 37°C, variable concentration of L-3,4-dihydroxybutan-2-one 4-phosphate [32]) [32]

0.18 <1> (1-[(5-amino-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)(methyl) amino]-1-deoxy-D-ribitol hydrochloride, <1> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [33]) [33]
0.18 <1> (1-deoxy-1-[[2,6-dioxo-5-(5-phosphonopentyl)-1,2,3,6-tetrahydropyrimidin-4-yl]amino]-D-ribitol, <1> pH 7.0, 37°C, variable concentration of L-3,4-dihydroxybutan-2-one 4-phosphate [32]) [32]
0.18 <1> (5-(pentyl 6-dihydrogen phosphate)-6-([(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]amino)pyrimidine-2,4(1H,3H)-dione, <1> pH 7.5, 37°C, recombinant β60 capsid [2]) [2]
0.197 <9> ((E)-3-hydroxy-4-(2-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-yl)vinyl)benzoic acid, <9> pH 7.0, 27°C [36]) [36]
0.205 <8> ((E)-6-(2-methoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.21 <8> ((E)-5-nitro-6-(2-hydroxystyryl)pyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.221 <1> ((1S)-1,2-dideoxy-1-fluoro-1-(5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-D-ribo-hexitol, <1> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [33]) [33]
0.243 <8> ((E)-6-(2,3-dimethoxystyryl)-5-nitropyrimidine-2,4(1H,3H)-dione, <8> pH 7.0, 27°C [36]) [36]
0.264 <1> (5-nitro-6-[(Δ-ribityl)methyl]pyrimidine-2,4-dione, <1> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [33]) [33]
0.27 <1> (1-deoxy-1-[2,6,8-trioxo-7-[5-(phosphonooxy)pentyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol, <1> pH 7.0, 37°C [34]) [34]
0.271 <1> (1-deoxy-1-[2,6,8-trioxo-7-[5-(phosphonooxy)pentyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol, <1> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [32]) [32]
0.341 <9> (1-[(5-amino-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)(methyl) amino]-1-deoxy-D-ribitol hydrochloride, <9> pH 7.0, 37°C, variable concentration of 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [33]) [33]
0.35 <8> (1,3,6,8-tetrahydroxynaphthyridine, <8> pH 7.0, 37°C [35]) [35]
0.44 <1> (1-deoxy-1-[[2,6-dioxo-5-(4-phosphonobutyl)-1,2,3,6-tetrahydropyrimidin-4-yl]amino]-D-ribitol, <1> pH 7.0, 37°C, variable concentration of L-3,4-dihydroxybutan-2-one 4-phosphate [32]) [32]
0.492 <1> (1-[(5-amino-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)(methyl) amino]-1-deoxy-D-ribitol hydrochloride, <1> pH 7.0, 37°C, variable concentration of L-3,4-dihydroxybutan-2-one 4-phosphate [33]) [33]
0.83 <1> (1-deoxy-1-[(2,6-dioxo-5-[[5-(phosphonooxy)pentanoyl]amino]-1,2,3,6-tetrahydropyrimidin-4-yl)amino]-D-ribitol, <1> pH 7.0, 37°C, variable concentration of L-3,4-dihydroxybutan-2-one 4-phosphate [32]) [32]
0.83 <1> (5-(5-phosphonoxyvaleryl)amino-6-D-ribitylaminouracil, <1> pH 7.5, 37°C, recombinant β60 capsid [3]) [3]
0.852 <1> (1-deoxy-1-[2,6,8-trioxo-7-[5-(phosphonooxy)pentyl]-1,2,3,6,7,8-hexahydro-9H-purin-9-yl]-D-ribitol, <1> pH 7.0, 37°C, variable concentration of L-3,4-dihydroxybutan-2-one 4-phosphate [32]) [32]

0.86 <1> (5-(4-phosphonobutyryl)amino-6-D-ribitylaminouracil, <1> pH 7.5, 37°C, recombinant β60 capsid [3]) [3]
1 <1> (5-(4-phosphonopentyl)amino-6-D-ribitylaminouracil, <1> pH 7.5, 37°C, recombinant β60 capsid [3]) [3]

pH-Optimum

7 <1,8,9,10,12,13,16,23,25,64> (<1,8,9,10,12,13,16,23,25,64> assay at [6,7,8,9,12,13,23,29,32,33,34,36]) [6,7,8,9,12,13,23,29,32,33,34,36]
7.5 <1,29> (<1,29> assay at [3,17]) [1,3,17]

pi-Value

6.6 <15> (<15> calculated from sequence [37]) [37]

Temperature optimum (°C)

27 <1,8,9,12> (<1,8,9,12> assay at [7,8,9,36]) [7,8,9,36]
37 <1,9,10,13,16,23,25,64> (<1,9,10,13,16,23,25,64> assay at [1,3,6,7,8,12,13,23,29,32,33,34]) [1,3,6,7,8,12,13,23,29,32,33,34]

4 Enzyme Structure

Molecular weight

75300 <16> (<16> isoform RibH1, light scattering experiments [6]) [6]
80100 <7> (<7> light scattering experiments, isoform RibH1 [6]) [6]
85500 <58> (<58> isoform RibH1, light scattering experiments [6]) [6]
87000 <64> (<64> sedimentation equilibrium centrifugation [12]) [12]
88000 <11> (<11> sedimentation equilibrium experiments [16]) [16]
90000 <3,23> (<23> gel filtration [23]; <3> sedimentation equilibrium analysis [18]) [18,23]
158500 <24> (<24> isoform RinH2, light scattering experiments [6]) [6]
850000 <4> (<4> gel filtration [19]) [19]
973000-991000 <4> (<4> sedimentaion equilibrium studies [19]) [19]
977000 <2> (<2> sedimentation equilibrium analysis [18]) [18]
Additional information <2> (<2> the protein is an icosahedral capsid of 60 subunits with a mass of about 1 MDa as shown by hydrodynamic studies and by electron microscopy. The lumazine synthase of Escherichia coli is not physically associated with another enzyme of the riboflavin pathway, and the core of the icosahedral capsid is empty [18]) [18]

Subunits

60-mer <2,4,13,17,25,30> (<4> 60 * 15500, subunit of mature enzyme, SDS-PAGE [19]; <25> 60 * 15645, the enzyme can form capsids with icosahedral 532 symmetry consisting of 60 subunits, electrospray mass spectrometry [13]; <25> 60 * 16000, the enzyme can form capsids with icosahedral 532 symmetry consisting of 60 subunits, SDS-PAGE [13]; <2> 60 * 16156, calculated from sequence [18]; <4> 60 * 16534, subunit of mature enzyme, calculated from sequence [19]; <4> 60 * 16536, subunit of mature enzyme, electrospray ionization mass spectrometry [19]; <4,17,30> sequence determinants responsible for the icosahedral quaternary structure [39]; <13> the spherical

protein consists of 60 identical subunits with strict icosahedral 532 symmetry [27]) [13,18,19,27,39]
? <15,23> (<23> x * 18000, SDS-PAGE [23]; <15> x * 19000, calculated from sequence [37]) [23,37]
decamer <11,24> (<24> 10 * 17300, calculated, isoform RibH2 [6]; <11> 10 * 18000, it is demonstrated by means of solution light scattering and X-ray structural analyses that the enzyme assembles as a very stable dimer of pentamers. A mechanism for dissociation/unfolding of this macromolecular assembly is postulated [21]; <11> a head-to-head oriented dimer of pentamers. pH plays a critical role in the structure of the interface between pentamers in Brucella spp. RibH2 [31]) [6,21,31]
pentamer <3,7,8,11,16,26,29,57,58,64> (<7,16> 5 * 16800, calculated, isoform RibH1 [6]; <58> 5 * 17200, calculated, isoform RibH1 [6]; <64> 5 * 17188, calculated from sequence [12]; <64> 5 * 17189, electrospray MS [12]; <11> 5 * 17599, calculated from sequence [16]; <3> 5 * 18598, calculated from sequence [18]; <29> 5 * 18600, calculated from sequence [17]) [6,12,16,17,18,25,26,28,39]
Additional information <1,4,14,17,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80> (<23> Brucella spp. lumazine synthase arranges in icosahedric capsids similar to those formed by the lumazine synthases of other bacteria [23]; <4,14,17,19,20,21,22,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80> lumazine synthase is found in different species in two different quaternary structures, pentameric and icosahedral, built from practically the same structural monomeric unit. The icosahedral structure is best described as a capsid of twelve pentamers. Despite this noticeable difference, the active sites are virtually identical in all structurally studied members. The main regions involved in the catalysis are located at the interface between adjacent subunits in the pentamer. Combined analysis that includes sequence-structure and evolutionary studies to find the sequence determinants of the different quaternary assemblies of this enzyme. The positions involved in icosahedral contacts suffer a larger increase in constraints than the rest. Eight sequence sites that would be the most important icosahedral sequence determinants are identified [39]; <1> the bifunctional lumazine synthase/riboflavin synthase (EC 2.5.1.9) complex is composed of 3 α (riboflavin synthase) subunits and 60 β (lumazine synthase) subunits and has a relative mass of 1 MDa. The 60 β subunits are arranged in an icosahedral capsid enclosing the α trimer in the central core. Hollow, icosahedral capsids consisting of 60 β subunits can be obtained by inhibitor-driven renaturation of isolated β subunits. They catalyse the formation of 6,7-dimethyl-8-ribityllumazine at the same rate as the native $\alpha 3\beta_6 0$ complex and can be crystallised in two different hexagonal and one monoclinic form [24]) [23,24,39]

Posttranslational modification

proteolytic modification <4,5,6> (<4,5,6> plant lumazine synthase is synthesized in the cytosol as a larger molecular weight precursor protein, which is post-translationally imported into chloroplasts where it is proteolytically cleaved to its mature size [19]) [19]

Additional information <41> (<41> lumazine synthase is found in different species in two different quaternary structures, pentameric and icosahedral, built from practically the same structural monomeric unit. The icosahedral structure is best described as a capsid of twelve pentamers. Despite this noticeable difference, the active sites are virtually identical in all structurally studied members. The main regions involved in the catalysis are located at the interface between adjacent subunits in the pentamer. Combined analysis that includes sequence-structure and evolutionary studies to find the sequence determinants of the different quaternary assemblies of this enzyme. The positions involved in icosahedral contacts suffer a larger increase in constraints than the rest. Eight sequence sites that would be the most important icosahedral sequence determinants are identified [39]) [39]

5 Isolation/Preparation/Mutation/Application

Localization

chloroplast <4> (<4> constitutes less than 0.02% of the total chloroplast protein. The enzyme is exclusivelylocated in the chloroplast stroma [19]) [19]

cytoplasm <23> [23]

Purification

<1> [29]

<2> (recombinant) [18]

<3> (recombinant) [18]

<8> [14,28]

<9> [10]

<10> [22]

<11> [16,21]

<13> [13,27]

<23> [23]

<25> (recombinant enzyme) [13]

<29> (overexpression of the RIB4 coding sequence in yeast cells under the control of the strong TEF1 promoter allowed ready purification of 6,7-dimethyl-8-ribityllumazine synthase to apparent homogeneity by a simple procedure) [17]

<64> (recombinant enzyme) [12]

Crystallization

<1> (crystal structure analysis of reconstituted, icosahedral β-subunit capsids with bound substrate analogue inhibitor (5-nitro-6-(D-ribitylamino)-2,4(1H,3H)-pyrimidinedione) at 2.4 A resolution) [24]

<1> (molecular modeling of enzyme with inhibitor 5-nitro-6-[[(2R,3R,4R)-2,3,4,5 tetrahydroxypentyl]sulfanyl]pyrimidine-2,4(1H,3H)-dione) [7]
<1> (molecular modeling of inhibitors to the active site) [2]
<3> (sitting-drop vapour-diffusion method. Crystals of the recombinant enzyme with a size of up to 1.6 mm are obtained. The space group is $P4_12_12$ with lattice dimensions 82.9 A x 82.9 A x 300.2 A. X-ray diffraction data collected under cryogenic conditions are complete to 1.85 A resolution. The structure of the enzyme in complex with the intermediate analogue, 5-(6-D-ribitylamino-2,4-dihydroxypyrimidine-5-yl)-1-pentyl-phosphonic acid is solved via molecular replacement using the structure of the Bacillus subtilis enzyme as search model and is refined to a final R-factor of 19.8%) [26]
<7> (isoform RibH1, unliganded, to 2.2 A resolution, and bound to the substrate analogue inhibitor 5-nitro-6-ribitylamino-2,4(1H,3H)-pyrimidinedione. Comparison with structure of isoform RibH2) [6]
<8> (crystals are grown at 18°C by the sitting drop vapor diffusion method. The W27Y mutant protein in complex with riboflavin, the substrate analogue 5-nitroso-6-ribitylamino-2,4(1H,3H)-pyrimidinedione, and the product analogue 6-carboxyethyl-7-oxo-8-ribityllumazine, are determined by X-ray crystallography at resolutions of 2.7-2.8 A) [14]
<8> (sitting drop vapour diffusion method, the enzyme is crystallised either in complex with bound riboflavin (RIBO) or in complex with the substrate analogue 5-nitro-6-(D-ribitylamino)-2,4(1H,3H)-pyrimidinedione (NRAP) or the product analogue 6-carboxyethyl-7-oxo-8-ribityllumazine (CEOL). The mutant proteins W27G, W63Y and W63Y/L119F, which do not bind riboflavin, and the mutant L119F, which only weakly binds to riboflavin, are also analysed. Diffraction data are collected to resolutions of 2.4 A (RIBO), 2.4 A (NRAP), 2.6 A (CEOL), 2.0 A (W27G), 3.1 A (W63Y and L119F) and 2.7 A (W63Y/L119F), respectively. All crystals belong to space group C222(1) with one pentamer in the asymmetric unit corresponding to the solution state of the protein) [28]
<9> (crystallized in the presence of two inhibitor compounds 3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)propane 1-phosphate and 3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrion-7-yl)butane 1-phosphate. The crystals are obtained in sitting drops by the vapor diffusion technique with the following macroseeding procedure) [10]
<9> (crystals are obtained in sitting drops by the vapour diffusion technique with the macroseeding procedure) [15]
<9> (in complex with inhibitor N-6-(ribitylamino)pyrimidine-2,4(1H,3H)-dion-5-ylpropionamide and phosphate, to 2.3 A resolution. The aromatic ring of the inhibitor is packed in the hydrophobic environment in the active site formed by Trp27, Ile60, Val81 and Val82, Ile83, Phe90, and Val93 residues of one subunit. The pyrimidine ring is in stacking interaction with the indole ring of Trp27 at a distance of 4 A) [9]
<9> (molecular modeling of enzyme in complex with inhibitor 3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)pentane 1-phosphate. The pyrazolopyrimidinedione ring of the ligand is stacked with the indole ring of Trp27. The phosphate of the ligand is extensively hydrogen bonded with the one

water molecule, the side chain nitrogens of Arg128, as well as the backbone nitrogens of Gln86 and Thr87 and the side-chain hydroxyl of Thr87. The ribityl hydroxyl groups are hydrogen bonded to the backbone nitrogen and oxygen of Asn114, the side-chain oxygens of Glu_61, and the backbone nitrogen of Ile60. The pyrazolopyrimidinedione ring of the ligand is hydrogen bonded to the backbone nitrogen of Ala59, the backbone nitrogen of Ile83, backbone oxygen of Val81, and the side-chain nitrogen of Lys138) [8]
<10> (crystallized in sitting drops by vapor diffusion. The crystal structure of lumazine synthase from Candida albicans is solved by molecular replacement and refined at 2.5 A resolution. The results of crystallographic investigations and sedimentation equilibrium experiments clearly indicate the presence of pentameric assemblies of the enzyme either in crystals or in solution) [22]
<10> (molecular modeling of enzyme in complex with inhibitor 3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)pentane 1-phosphate. The pyrazolopyrimidinedione ring of the ligand is stacked with the indole ring of Trp27. The phosphate of the ligand is extensively hydrogen bonded with the one water molecule, the side chain nitrogens of Arg128, as well as the backbone nitrogens of Gln86 and Thr87 and the side-chain hydroxyl of Thr87. The ribityl hydroxyl groups are hydrogen bonded to the backbone nitrogen and oxygen of Asn114, the side-chain oxygens of Glu61, and the backbone nitrogen of Ile60. The pyrazolopyrimidinedione ring of the ligand is hydrogen bonded to the backbone nitrogen of Ala59, the backbone nitrogen of Ile83, backbone oxygen of Val81, and the side-chain nitrogen of Lys138) [8]
<11> [21]
<11> (crystals are obtained by means of the hanging-drop, vapor-diffusion method at room temperature) [31]
<13> (crystallized at room temperature by sitting-drop vapor-diffusion method, the protein is crystallized in the cubic space group I23 with the cell dimensions a = b = c = 180.8 A, diffraction data are collected to 1.6 A resolution) [27]
<13> (sitting-drop vapor diffusion method, crystal structures of the enzyme from the hyperthermophilic bacterium Aquifex aeolicus in complex with different inhibitor compounds. The structures are refined at resolutions of 1.72 A (enzyme-7-dioxo-5H-8-ribitylaminolumazine complex), 1.85 A (enzyme-3-(7-hydroxy-8-ribityllumazine-6-yl)propionic acid complex), 2.05 A (enzyme-5-nitroso-6-ribityl-amino-2,4(1H,3H)pyrimidinedione complex) and 2.2 A (enzyme-5-(6-D-ribitylamino-2,4(1H,3H)pyrimidinedione-5-yl)-1-pentylphosphonic acid complex), respectively. Structural comparisons of the native enzyme and the inhibitor complexes as well as the kinetic data of single site mutants of lumazine synthase from Bacillus subtilis show that several highly conserved residues at the active site, namely Phe22, His88, Arg127, Lys135 and Glu138 are most likely involved in catalysis. A structural model of the catalytic process, which illustrates binding of substrates, enantiomer specificity, proton abstraction/donation, phosphate elimination, formation of the Schiff base and cyclization is proposed) [30]

<16> (isoform RibH1, bound to the substrate analogue inhibitor 5-nitro-6-ribitylamino-2,4(1H,3H)-pyrimidinedione. Comparison with structure of isoform RibH2) [6]
<17> (native protein, 2.4 A resolution, space group P6322 or C2. Mutant D44G/C93S/C139S/T118A crystallizes in space group R3 and diffracts to 1.6 A resolution) [4]
<24> (isoform RibH2, bound to the substrate analogue inhibitor 5-nitro-6-ribitylamino-2,4(1H,3H)-pyrimidinedione) [6]
<26> (three-dimensional X-ray crystal structure of the enzyme solved and refined at 2.7 A resolution to a final R-value of 0.18. Structures of the enzyme from Bacillus subtilis and Bruvella abortus are compared) [25]
<64> (sitting-drop vapour diffusion method, crystallizes in space group $C222_1$. The crystals diffract to a resolution of 2.4 A) [12]

Cloning

<1> [29]
<1> (expression of β60 capsid) [2]
<1> (expression of β60 capsid carying lumazine synthase activity) [3]
<2> [18]
<3> [18]
<4> (expression in Escherichia coli) [19]
<5> (expression in Escherichia coli) [19]
<6> (expression in Escherichia coli) [19]
<7> (expression in Escherichia coli) [6]
<10> (expression in Escherichia coli) [22]
<11> [16]
<11> (expression in Escherichia coli) [21]
<13> (expression in Escherichia coli) [27]
<16> (expression in Escherichia coli) [6]
<17> (expression in Escherichia coli) [4]
<23> (expression in Escherichia coli. The recombinant protein is soluble only under reducing conditions, but alkylation with iodoacetamide renders it soluble in non-reducing media) [23]
<24> (expression in Escherichia coli) [6]
<25> (expression in Escherichia coli) [13]
<29> [17]
<58> (expression in Escherichia coli) [6]
<64> (expression in Escherichia coli) [12]

Engineering

A56S <1> (<1> k_{cat} is 81.3% of wild-type value [29]) [29]
D138A <1> (<1> k_{cat} is 91.5% of wild-type value [29]) [29]
D44G/C93S/C139S/T118A <17> (<17> mutant constructed to improve the overexpression and purification of the molecule as well as to obtain new crystal forms. Two cysteines are replaced to bypass misfolding problems and a charged surface residue is replaced to force different molecular packings. Mutant crystallizes in space group R3 and diffracts to 1.6 A resolution [4]) [4]
E58Q <1> (<1> k_{cat} is 70.2% of wild-type value [29]) [29]

F113S <1> (<1> k_{cat} is 6.8% of wild-type value [29]) [29]
F22D <1> (<1> k_{cat} is 14.5% of wild-type value [29]) [29]
F22S <1> (<1> k_{cat} is 47.2% of wild-type value [29]) [29]
F22V <1> (<1> k_{cat} is 26.4% of wild-type value [29]) [29]
F22W <1> (<1> k_{cat} is 43.8% of wild-type value [29]) [29]
F57S <1> (<1> k_{cat} is 43.8% of wild-type value [29]) [29]
H88A <1> (<1> k_{cat} is 12% of wild-type value [29]) [29]
H88K <1> (<1> k_{cat} is 39.5% of wild-type value [29]) [29]
K131N <1> (<1> k_{cat} is 9.7% of wild-type value [29]) [29]
K131R <1> (<1> k_{cat} is 29.8% of wild-type value [29]) [29]
K135A <1> (<1> k_{cat} is 21.9% of wild-type value [29]) [29]
L119F <8> (<8> weakly binds to riboflavin [28]) [28]
N23S <1> (<1> k_{cat} is 21.9% of wild-type value [29]) [29]
R127H <1> (<1> k_{cat} is 69.7% of wild-type value [29]) [29]
S142L <1> (<1> k_{cat} is 62.3% of wild-type value [29]) [29]
T80V <1> (<1> k_{cat} is 55.1% of wild-type value [29]) [29]
W27F <64> (<64> the replacement of tryptophan 27 by aliphatic amino acids substantially reduces the affinity of the enzyme for riboflavin and for the substrate, 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [12]) [12]
W27G <8,64> (<8> does not bind riboflavin [28]; <64> the replacement of tryptophan 27 by aliphatic amino acids substantially reduces the affinity of the enzyme for riboflavin and for the substrate, 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [12]) [12,28]
W27H <64> (<64> the replacement of tryptophan 27 by aliphatic amino acids substantially reduces the affinity of the enzyme for riboflavin and for the substrate, 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [12]) [12]
W27I <64> (<64> the replacement of tryptophan 27 by aliphatic amino acids substantially reduces the affinity of the enzyme for riboflavin and for the substrate, 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [12]) [12]
W27S <64> (<64> the replacement of tryptophan 27 by aliphatic amino acids substantially reduces the affinity of the enzyme for riboflavin and for the substrate, 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [12]) [12]
W27Y <8,64> (<64> the replacement of tryptophan 27 by aliphatic amino acids substantially reduces the affinity of the enzyme for riboflavin and for the substrate, 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione [12]; <8> whereas the indole system of W27 forms a coplanar pi-complex with riboflavin, the corresponding phenyl ring in the W27Y mutant establishes only peripheral contact with the heterocyclic ring system of the bound riboflavin [14]) [12,14]
W63Y <8> (<8> does not bind riboflavin [28]) [28]
W63Y/L119F <8> (<8> does not bind riboflavin [28]) [28]

Application

medicine <1,10,11,23> (<11> Brucella lumazine synthase can be used as both an antigen-carrier and as an adjuvant in the design of new oral subunit vac-

cines [38]; <1> certain purinetriones bearing phosphate side chains can inhibit both lumazine synthase as well as riboflavin synthase, and molecular modeling with 3-(1,3,7,9-tetrahydro-9-D-ribityl-2,6,8-trioxopurin-7-yl)propane 1-phosphate suggests possible binding modes to each enzyme. Antibiotics that would inhibit both lumazine synthase and riboflavin synthase would be less likely to suffer from the development of antibiotic resistance by the organisms that they are supposed to treat, since pathogenic microorganisms would have to simultaneously select for mutations in both enzymes in order to escape the cytotoxic effects of the antibiotics [32]; <11> feasibility of using Brucella spp. lumazine synthase as a novel and effective delivery system to induce a protective immune response against rotavirus disease. In particular, previous results showing the plasticity of the Brucella spp. lumazine synthase scaffold for the production of polyvalent chimeras suggest that VP8 from different strains can be coupled to Brucella spp. lumazine synthase in order to elicit wide-protecting neutralizing antibodies against different field strains of rotavirus [41]; <11> lumazine synthase is a potent delivery system for the improvement of subunit vaccines [40]; <10> the fact that the enzymes of the riboflavin biosynthesis pathway are not present in the human or animal host makes them potential targets for anti-infective agents [22]; <23> this protein constitutes an interesting candidate for serological diagnosis and for the design of specific chemotherapeutic agents, and its polymeric characteristics could provide the basis for the development of an acellular vaccine [23]) [22,23,32,38,40,41]

6 Stability

Temperature stability

20 <11> (<11> the higher molecular order of the decameric enzyme increases its stability at 20°C compared with pentameric lumazine synthases [21]) [21]

88 <11> (<11> melting temperature. The loss of secondary structure is not recovered after slow cooling of the samples, indicating that an irreversible unfolding takes place under these conditions [21]) [21]

120 <13> (<13> melting temperature: 119.9°C [27]) [27]

Organic solvent stability

guanidine-HCl <11> (<11> produces a cooperative and reversible change in the tertiary structure reflected by a decrease in tryptophan fluorescence emission. In addition, guanidine-HCl incubation produces a complete loss of secondary structure of BLS as monitored by CD spectra [21]) [21]

urea <11> (<11> 8 M, the absence of structural changes indicates that the quaternary arrangement of the enzyme is very stable [21]) [21]

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