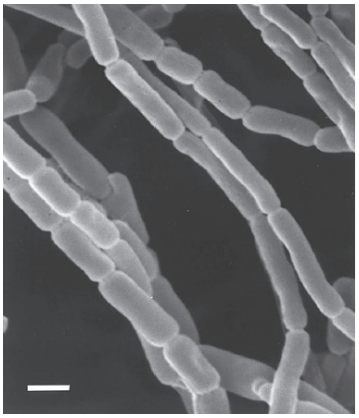
Staurosporine ©

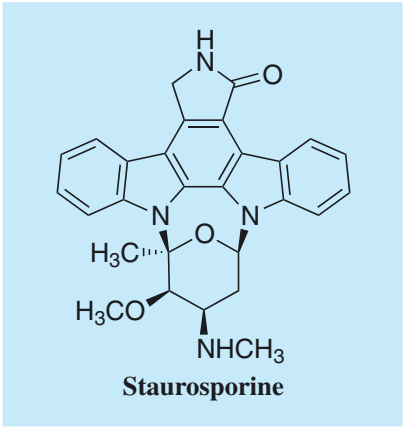
1. Discovery, producing organism[23)]

Staurosporine was originally discovered in 1977 from culture of *Saccharothrix aerocolonigenes* starin AM-2282^T during a screening program for microbial alkaloid using a chemical detection method.[1,2,3)] During the past 30 years, staurosporine and related indolocarbazole natural compounds have been isolated from not only actinomycetes but also *myxomycetes* (slime molds), cyanobacteria and marine invertebrates such as sponges.

In 1986, 10 years after the discovery, staurosporine was found to be a nano molar inhibitor of protein kinases.[4,5)] Staurosporine is commercially available for biochemical research.



Saccharothrix aerocolonigenes subsp. *staurosporeus* AM-2282^T
(*Lentzea albida* AM-2282)



Staurosporine

2. Physicochemical data and structure

Pale yellow crystals. $C_{28}H_{26}N_4O_3$; mol wt 466.20; Sol. in DMSO, DMF. Slightly sol. in $CHCl_3$, MeOH.

The structure and absolute configuration of staurosporine was revealed by X-ray crystallographic analysis [6,7)] and confirmed by total synthesis by Danishefsky *et al*.[8)] and an another group.

3. Biosynthesis of Staurosporine

Biosynthesis of staurosporine and rebeccamycin has been revealed by genetic and biochemical studies (Sanchez *et al*. [9,10)] , Onaka *et al*. [11,12)] and Groom *et al*.[24)] see figure).

In staurosporine biosynthsis, StaO initiates the synthesis by catalyzing L-trypthophan to imine form of indole-3-pyruvic acid (IPA imine) and StatD then catalyzes the coupling of two IPA imines to yield chromopyrrrolic acid(CPA). The formation of indolocarbazole core of staurosporine is catalyzed by StaP. StaP converts CPA into three indolocarbazole compounds; staurosporine aglycone (K252c), 7-hydroxy-K252-c and acrylflavin A by intramolecular C-C bond formation and oxidative decarboxylation. The presence of StaC directs formation of K252c predominantly. StaG catalyzes a glycosidic bond formation between N-13 and C-6' and then StaN, a P-450 homolog, catalyzes an additional C-N bond formation between N-12 and C-2'. These two enzymes convert Staurosporine aglycone (K252c) to 3' *O*-demethyl, 4'-*N*-demethyl-staurosporine via Holyrine A and Holyrine B. StaMA catalyzes *N*-metylation of 3' *O*-demethyl, 4'-*N*-demethyl- staurosporine and StaMB catalyzes *O*-methylation which result in formation of staurosporine.

4 Biological activities

In mid-1980s , staurosporine and related indolocarbazole K-252 were shown to be nano molar inhibitors of protein kinases Kyowa Hakko Co. The reports led many laboratories and pharmaceutical companies to find selective protein kinase inhibitors by chemical synthesis or screening of new natural products. In 1996, a bcr-abl tysorine kinase inhibitor by chemical synthesis, Gleevec, entered human clinical trial of chronic myelogenous leukaemia and was approved in 2001 in USA.[10)]

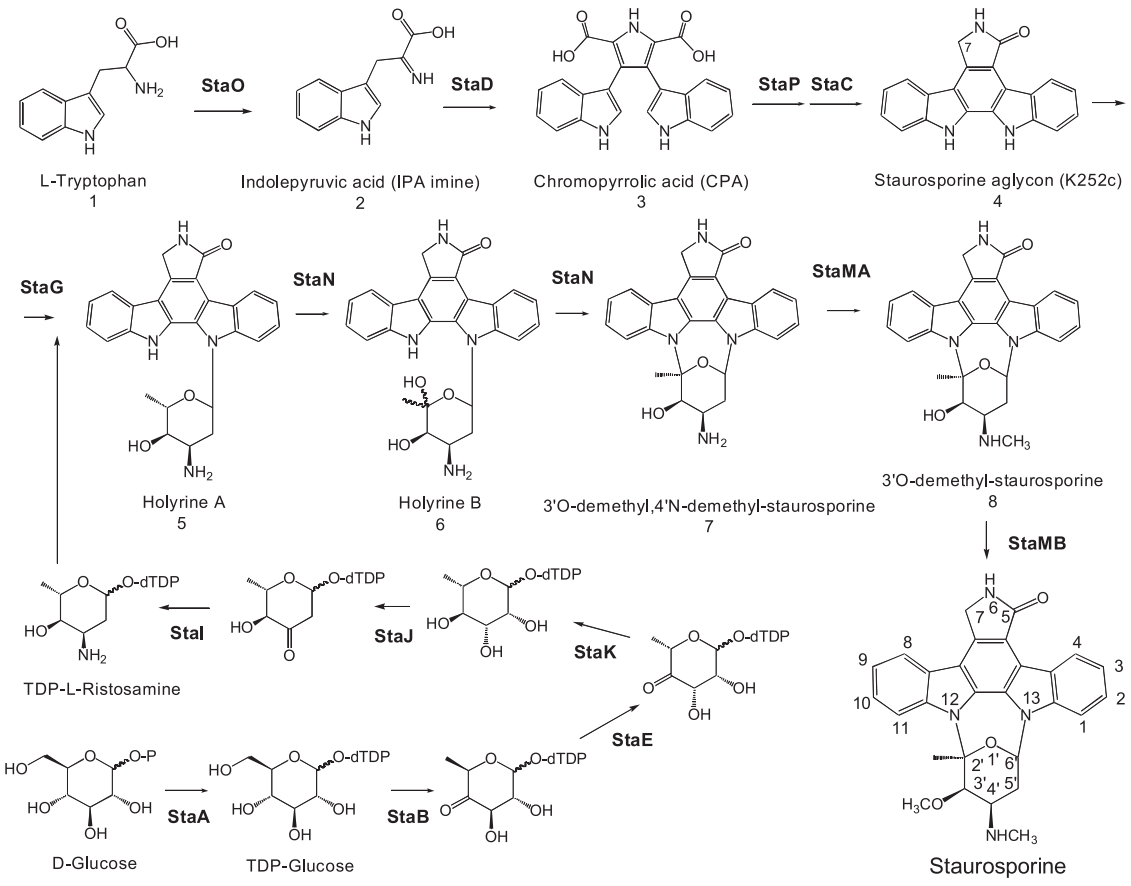
1) Inhibition of protein kinases

Staurosporine has been use as the reference compound of many protein kinase assays. The figure shows IC_{50} against 235 protein kinase assayed by Carna Biosciences Inc.[22)]

Following the discovery that staurosporine was a nanomolar inhibitor of protein serine/threonine kinases such as protein kinase C [4)] and also protein tyrosine kinase v-src [5)], many derivatives and mimics of staurosporine and indole carbazole were made.

7-Hydroxystaurosporine (UCN-01) showed antitumor activity in mouse tumor models and entered clinical studies.[14)] In 2001 Gleevec targeting ABL(Abelson protein tyrosine kinase) was approved for clinical use and protein kinases have became one of the major drug targets of the 21st century.[15)]

The crystal structures of sturosporine and protein kinases, including PKC isozymes, cyclin-dependent kinases and EGF-Receptor tyrosine kinase, revealed that staurosporine overlaps well with the adenosine group of ATP. The lactam ring mimics the hydrogen bonding interactions of adenine, while the glycosyl group mimics those of ribose. Staurosporine related structures such as indolocarbazole aglycon, diindolylmaleimides and dianilinophthalamides have been derivatized and developed into selective inhibitors for pharmacologically interesting targets.[15)]



Biosynthetic pathway of Staurosporine (modified from ref.12)

2) Action on Topisomerases with ATP binding site

DNA topoisomerases have been shown to be an important target of antitumor drugs or antibacterial agents. Both antitumor drugs in eukaryotes and quinolone antibiotics in prokaryotes stabilize DNA-cleavable complex with topoisomerase. It was shown that staurosporine related structures induce DNA cleavable complex with mammalian topoisomerases. Semisynthetic derivatives of K252a were potent inducer of DNA cleavable complex with Topoisomerase I, whereas rebbecamycin was a weak inducer in the same assay.[16)]

3) Anti-microbial and pathogenic activity

Staurosporine was shown to have weak antifungal activity since the discovery by Ōmura *et al*.[1,2)] Later, protein Ser/Thr kinases were identified in many pathogenic organisms including mycobacteria, malaria and viruses.

Staurosporine and K-252 was found to inhibit mycobacterial growth at 5-50 μM.[17)]

In *Plasmodium falciparum*, a cyclin- nuclear division cycle dependent kinase (CDK) PfPK5, was identified as the first such compound not derived from humans. Staurosporine inhibited PfPK5 with IC_{50} at 1 μM.[18)] Antiviral properties of indolocarbazoles have been reported including human immunodeficiency virus, cytomegalovirus and Epstein–Barr virus.

4) Cytotoxic activity/Acute toxicity

Staurosporine showed a wide range of growth inhibition effects against cultured cells and was extremely cytotoxic in some cases (4pM, Hela-S3, 72 hr exposure).

Staurosporine induced apoptosis as do anticancer drugs, but via partly distinct pathways. Several cell lines are completely resistant towards different anticancer drugs, but remain sensitive towards staurosporine-induced apoptosis. These finding encouraged further clinical trials for the use of staurosporine derivatives in antitumor therapy.

Acute toxicity (mice, i.p.) LD_{50} = 6.6 mg/kg

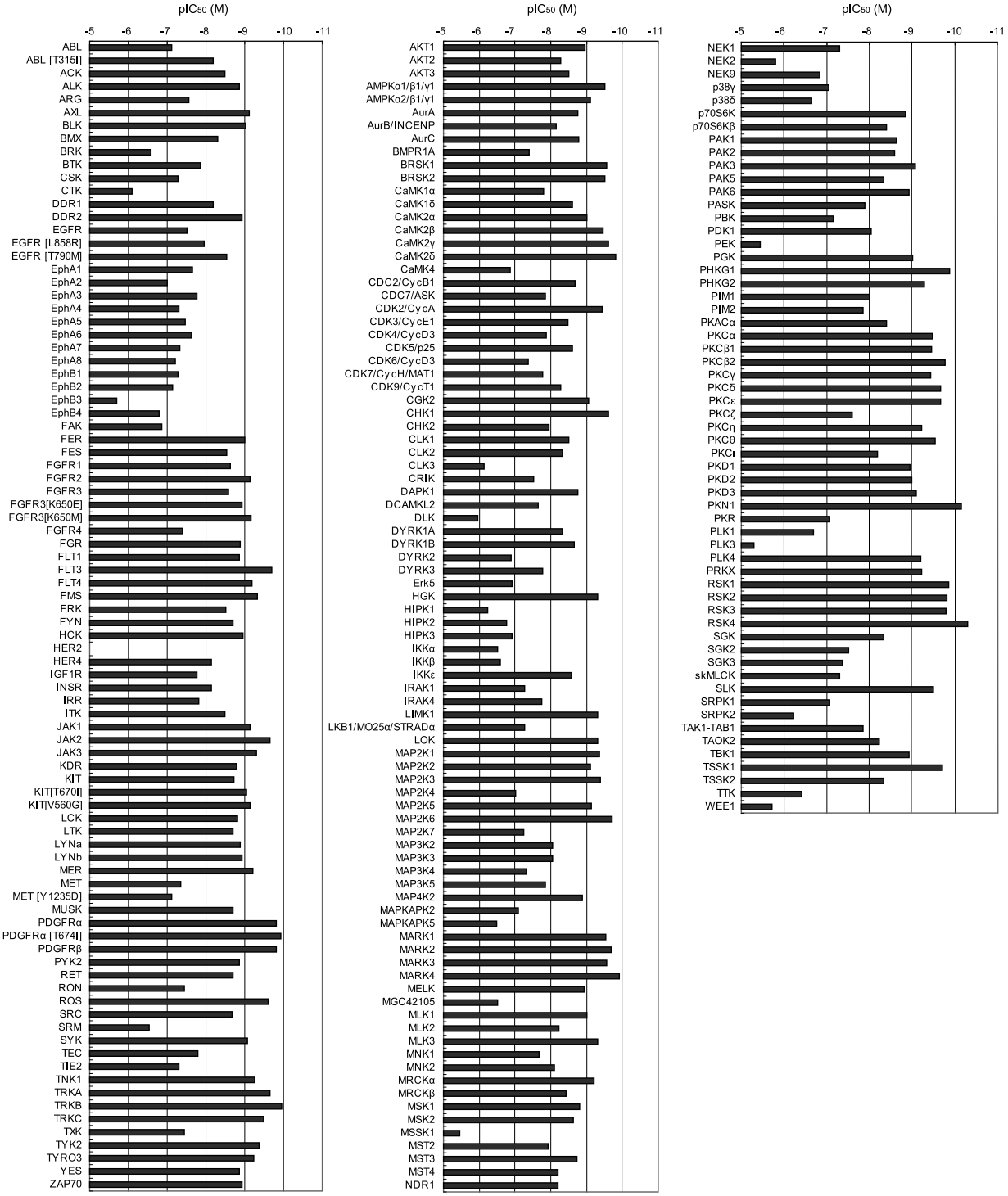
5) Other biological activities

Staurosporine and indolocarbazole derivatives showed many other biological activities.

Anti-angiogenesis activity of staurosporine derivatives with modification in its amino sugar moiety was reported[19)].

Ruboxistaurin (Arxxant, LY333531) is selective inhibitor of Protein kinase C isoforms β and was evaluated in clinical studies Ruboxistaurin has been submitted to FDA for diabetic retinopathy.[20)]

Other reported activities include; inhibition of smooth muscle contraction[21)], insecticidal activity, hypotensive properties, inhibition of platelet aggregation, activation of macrophages, immunosuppression, inhibition of the osteoclast proton pump, neuroprotection.



Inhibitory activity of staurosporine against protein kinases [22)]

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