

Borrelidin:

A promising anticancer agent from *Streptomyces* species.

Vino S* and Lokesh K.R

Abstract

Borrelidin, a novel nitrile containing macrolide antibiotic obtained from the *Streptomyces* species particularly inhibits threonyl-tRNA synthetase (ThrRS). Also borrelidin was found to possess antiangiogenic activity- a key process in the spread of malignant tumors by inducing the collapse of formed capillary tubes. Biologically active analogues of borrelidin can be produced by incubating the strains with alternative starting materials. Borrelidin analogues were also found to be cytotoxic against some cancer cell lines. This review provides the analysis of borrelidin and its analogues from bacteria.

Keywords: borrelidin, threonyl-tRNA synthetase, polyketide synthase, angiogenesis.

Introduction

Borrelidin, 2-(7-cyano-8, 16 dihydroxy-9, 11, 13, 15-tetramethyl-18-oxooxa cyclo octadeca 4, 6-dien-2yl) cyclo pentanecarboxylic acid is first isolated from *Streptomyces rochei* in 1949. It is crystalline white solid in appearance with molecular weight of 489.6 (molecular formula - $C_{28}H_{43}NO_6$), which is soluble in organic solvents like Dimethyl Sulfoxide (DMSO) and Ethanol.

Borrelidin, 18 membered polyketide macrolide antibiotic, is structurally unique and rarely occurs in nature.¹ The planar structure of borrelidin was elucidated by Keller-Schierlein in 1967² and subsequently refined by the Nuclear Magnetic Resonance (NMR) analysis and X-ray diffraction methods^{3,4}. The absolute configuration of its nine stereogenic centers has been determined by the X-ray diffraction of its crystal in chiral solvent.⁴ The unique structural features of borrelidin includes (i) a deoxy propionate subunit consisting of four 1,3 alternating C-methyl groups with a distinctive syn/syn/anti

relationship at C₄ - C₁₀ (ii) a conjugated diene nitrile chromophore unit at C₁₂ - C₁₅ and (iii) a cyclo pentane carboxylic acid subunit at C₁₇. (Figure 1) The nitrile, lactone and probably the hydroxyl functions are essential for the borrelidin molecule to show antimicrobial

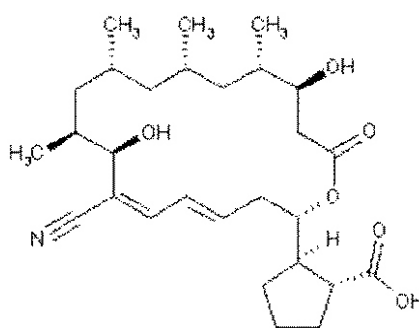


Figure 1 - Chemical structure of Borrelidin

activity. The structural features of borrelidin were similar to the antibiotic, Treponemycin.^{5,6,7}

Biosynthesis of borrelidin

Although, Borrelidin first isolated from a soil sample of *Streptomyces rochei* subsequently

identified from other *Streptomyces* species such as *S. parvulus*, *S. albovinaceous*, *S. Griseus* and an unidentified *Streptomyces* species C2989.⁸⁻¹² Apart from microbial sources, borrelidin also can be synthesized chemically which has similar structure and functions. Novel analogues of borrelidin also can be synthesized by precursor directed biosynthesis.^{5,13}

The biosynthetic pathway of borrelidin falls into four categories based on the genes involved. They are (i) Biosynthesis of polyketide backbone (ii) starter acid biosynthesis (iii) formation of nitrile group (iv) regulation and resistance.¹⁴ the biosynthesis of polyketide backbone occurs by repeated condensation of simple carboxylic acids (3 units of malonyl CoA and 4 units of methyl malonyl CoA). The trans cyclo pentane 1,2 dicarboxylic acid acts as starter molecule and the backbone extended by the addition of carboxylic acid which occurs in seven cycles catalyzed by poly functional type - Poly Ketide Synthase (PKS), also known as modular PKS.¹⁴ Modular PKS are organized into repeating modules, each consisting of a catalytic domain required to perform one round of chain assembly.

The genes encoding for polyketide synthase (PKS) are organized in six segments namely *borA1* to *borA6*. These six genes are responsible for the synthesis of 31 individual domains organized into loading modules and six extender modules of PKS. The loading modules are coded by *borA1* and the extension modules are coded by *borA2* (one module), *borA3* (two modules), *borA4* (one

module), *borA5* (one module), *borA6* (one module). All of them contain Keto Synthase and Acyl Carrier Protein domains, modules 2&3 include additional Dehydratase domain,

module 5 contains Dehydratase & Enoyl Reductase domain and all extension module contains Keto Reductase domain. The final *borA6* encodes for Thioesterase domain.¹⁴

synthesis of derivatives and for biological evaluations.

Gene cluster organisation

The genes encoding the enzymes responsible for borrelidin biosynthesis are organized in three clusters. (Figure 2) The six genes (*borA1-A6*) coding for PKS located in the

Domain	Function
keto synthase (KS)	Addition of carboxylic acid into growing polyketide chain
Acyl transferase (AT)	Selects particular carboxylic acid substrate
Acyl carrier protein (ACP)	Retention of growing chain and its transfer on the PKS
Keto reductase (KR), Dehydratase (DH), Enoyl reductase (ER)	Responsible for the reduction of keto groups during chain extension
Thio esterase (TE)	Release of the final poly ketide chain and also the cyclisation of the final product.

The functions of each domain are discussed in Table 1.

The trans-cyclo pentane 1,2 dicarboxylic acid acts as starter molecule for biosynthesis of borrelidin. While feeding exogenous trans-cyclo pentane 1, 2 dicarboxylic acid to wild type and mutants (containing disrupted genes for trans-cyclo pentane 1,2 dicarboxylic acid biosynthesis) it shows increase in borrelidin production by 3.6 ± 0.6 fold and 15.3 ± 1.3 fold respectively. This provides the information that the starter molecule is trans-cyclo pentane 1,2 dicarboxylic acid. Similar effect failed when other dicarboxylic acids are used as starter molecules. The biosynthesis of trans-cyclo pentane 1,2 dicarboxylic acid are encoded by genes namely *borC*, *borD*, *borE*, *borF*, *borK*, *borL*, *borM*, *borG*, *borH* and *borN*.¹⁴

The presence of nitrile group at C₁₂ position is a rare and unusual chemical architecture in borrelidin.¹⁵ The carbon atom of nitrile compound arises from methyl malonyl CoA which is added at third round of chain extension. This methyl group is oxidised and transaminated by the products of two genes (*borI* and *borJ*) which helps in the nitrile formation. Products of *borI* resembles as Cytochrome P450 hydroxylase that catalyse oxidation of C₁₂ methyl group and *borJ* gene product resembles as Pyridoxal-Phosphate (PLP) dependent aminotransferase which

catalyse the incorporation of nitrogen atom for formation of nitrile group in borrelidin. The primary target for borrelidin is threonyl tRNA synthase (ThrRS). As a self-resistance mechanism the *Streptomyces parvulus* produce a borrelidin-resistant ThrRS, which is encoded in *borO* gene. Similarly the products of *borO* also found in *Streptomyces coelicolor A3* that resist borrelidin activity.¹⁶

The structural and biological uniqueness of borrelidin promotes the total synthesis through chemical process. Though borrelidin first isolated more than 50 years ago the synthetic studies towards total synthesis of borrelidin appeared recently.^{5,17-19} the syntheses of precursor fragments of borrelidin were also reported.^{15,20,21} these synthetic studies will contribute to the

Genes	Amino acids	Function in borrelidin biosynthesis
<i>borB</i>	264	Type II thioesterase
<i>borC</i>	265	Starter unit biosynthesis
<i>borD</i>	250	Starter unit biosynthesis
<i>borE</i>	390	Starter unit biosynthesis
<i>borF</i>	272	Starter unit biosynthesis
<i>borG</i>	539	Starter unit biosynthesis
<i>borH</i>	675	Starter unit biosynthesis
<i>borA1</i>	876	PKS loading domain (AT-ACP)
<i>borA2</i>	1571	PKS module 1 (KS-ATa-KR-ACP)
<i>borA3</i>	3500	PKS module 2&3 (KS-Ata-DH-KR-ACP-KS-ATp-DH-KR-ACP)
<i>borA4</i>	1620	PKS module 4 (KS-ATp-KR-ACP)
<i>borA5</i>	2156	PKS module 5 (KS-ATp-DH-ER-KR-ACP)
<i>borA6</i>	1742	PKS module 8 (KS-ATa-KR-ACP-TE)
<i>borI</i>	426	Nitrile biosynthesis
<i>borJ</i>	454	Nitrile biosynthesis
<i>borK</i>	330	Unknown
<i>borL</i>	446	Starter unit biosynthesis
<i>borM</i>	305	Starter unit biosynthesis
<i>borN</i>	248	Starter unit biosynthesis
<i>borO</i>	675	Borrelidin resistance

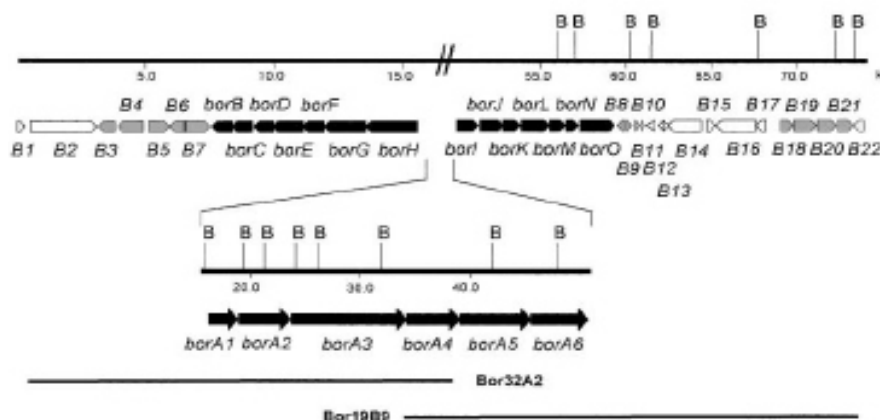


Figure 2 - Schematic representation of Borrelidin gene cluster. Adapted from Chemistry & Biology, Vol. 11, 8797, January, 2004. (Salas, J.A., et al)

center and seven genes (*borB, C, D, E, F, G*, and *H*) coding for borrelidin biosynthesis are present to the left in opposite transcriptional direction. To the right side of PKS in same transcriptional direction are six genes (*borI, J, K, L, M, N* and *O*). Some genes are transcribed in polycistronic nature.¹⁴ The corresponding genes and their functions are discussed in Table 2.

Biosynthesis of borrelidin analogues

The mutants of *Streptomyces parvulus* didn't produce borrelidin or its analogues till the precursor trans cyclo pentane 1,2 dicarboxylic acid was added.¹⁴ The analogues of borrelidin were synthesized by changing the precursor used for borrelidin biosynthesis in *Streptomyces parvulus* mutants. By using these mutants the analogues of borrelidin were synthesized by providing different analogues of trans cyclo pentane 1,2 dicarboxylic acid. This approach has proved a great success in producing analogues of several therapeutic polyketides.²² The analogues of borrelidin containing cyclobutane-trans-1,2 dicarboxylic acid, (2-mercaptoacetyl)-succinic acid, 2,3-dimethyl succinic acid, 2-methyl succinic acid replacing trans cyclo pentane 1,2 dicarboxylic acid were synthesized successfully.¹³

Activity against tRNA^{thr} synthetase

The formation of aminoacyl tRNA is the first committed step in protein synthesis. The aminoacyl tRNA synthetase esterifies the 20 different activated amino acids into their corresponding tRNA. These aminoacyl tRNA's plays a vital role in coordinating certain aspects of cellular metabolism and protein synthesis. The macrolide antibiotic borrelidin acts as non-competitive inhibitor to the ThrRS with respect to threonine and inhibits the transfer of activated threonine into tRNA^{thr}. The binding of borrelidin to ThrRS was predicted and it was found to inhibit the first step of threonine activation, thus the overall aminoacylation.²³⁻²⁸

In ThrRS borrelidin acts particularly on cluster A which consists of Thr-307, His-309, Cys-334, Pro-335, Leu-489 and Leu-493. The Cys-334 of cluster A is of particular importance as it directly contacts the zinc ion which is essential for ThrRS activity. Borrelidin binds to cluster A may cause relocation of Cys-334, resulting in distortion of the zinc ion coordination and thereby disruption of ThrRS activity. The amino acids in cluster A are conserved and found to be similar in many species of prokaryotes and eukaryotes which are borrelidin sensitive.²⁸

The novel feature of borrelidin inhibition is that, it does not bind to active site of ThrRS unlike other inhibitors such as Indolmycin (analogue of tryptophan), sulfonamides (mimics activated substrate) and oligonucleotide inhibitors (mimics tRNA features).²⁹⁻³¹ These inhibitors compete with substrates for binding to active site and generally are reversible inhibitors.²⁸ One of the possible effects of borrelidin treatment is inhibition of protein synthesis, in certain case like yeast the borrelidin induces the transcription of amino acid synthetic enzymes. The transcriptional activation occurs through GCN4-transcriptional factor dependent pathway, which controls amino acids under the starvation condition. The possible mechanism is that the inhibition of ThrRS leads to accumulation of uncharged tRNA, this in turn activates *Gcn2p* protein kinase activity. The *Gcn2p* leads to phosphorylation activation of eukaryotic translation initiation factor 2 (eIF-2), which results in increased translation of *Gcn4p*. The increased *Gcn4p* results in transcription of 70 genes involved in amino acid biosynthesis.^{32,33}

Anticancer activity

Cancer cells grow more rapidly than the blood vessels, which nourish them; thus when a solid tumor grows they are unable to obtain oxygen efficiently. In other words, they begin to experience hypoxia. Glycolysis, leading to lactic acid fermentation becomes the primary source of ATP. Under hypoxia conditions the expression of Hypoxia Inducible Transcription Factors (HIF-1) favors expression of glycolytic enzymes and glucose transporters. These adaptations by the cancer

cells help to survive until vascularization occurs. HIF-1 also stimulates the growth of new tumors by increasing the expression of signaling molecule such as Vascular Endothelial Growth Factors (VEGF), that facilitates the growth of new blood vessels. Without vascularisation the tumors would cease to grow and either die or remain harmless. The process of vascularisation of cancer cells is called as angiogenesis.³⁴

Many therapeutic drugs, which inhibit the angiogenesis, are widely used in cancer treatment. Targeting tumor angiogenesis prevents the effects of other anticancer therapeutic modalities due to the diversity of cancer types and drug resistance.³⁵ Borrelidin with the property of inhibiting ThrRS has a huge potential to inhibit angiogenesis. In rat aorta matrix culture model, borrelidin suppresses the formation of capillary tubes and also collapses the newly formed capillary tube. Similar results are observed in culture model of Human Umbilical Vein Endothelial Cells (HUVEC). Borrelidin activates Caspase-3 and -8, which leads to the apoptosis in capillaries. The anti-angiogenic effect of borrelidin is mediated through at least two mechanisms, i.e., by threonine dependent inhibition of new capillary tube formation and other by threonine independent through induction of apoptosis.³⁶

The Inhibitory Concentration (IC₅₀) of inhibiting capillary tube formation was 0.8 nM which is 50-fold lower than for the inhibition of protein synthesis through ThrRS. The angiogenesis induced by VEGF was inhibited in vivo model of mouse dorsal air sac model. Borrelidin serves as efficient inhibition of lung metastasis of B16-BL6 melanoma cells at the same dosage that inhibits angiogenesis.³⁷

Antimicrobial activity

Borrelidin was found to possess specific antibiotic activity against *Borrelia*, the spirochetes causing relapsing fever and hence it has named after the activity against *Borrelia* as borrelidin, it also inhibits spirochetes of genus *Treponema*.^{6, 8} Borrelidin containing non glycosylated, polyketide lactone ring, does not directly attack any biochemical reactions

other than enzymatic activity of ThrRS.^{38,39} Borrelidin inhibits many species of prokaryotes and eukaryotes which shares the same structural features of ThrRS.²⁸ The organisms which have conserved amino acids in cluster A are susceptible to borrelidin. Examples of highly sensitive organisms are

and chloroquine both in vivo and in vitro. Borrelidin was also found to possess antiviral activity. Borrelidin effectively acts against the viruses such as *Classical fowl plague* and *Pseudo rabies*.^{46,47} Moreover borrelidin also has insecticidal and herbicidal properties to some extent.⁴⁸

Table 3 *In vitro* and *In vivo* antimalarial activities of borrelidin

Compound	<i>In vitro</i> (IC ₅₀ nM)		<i>In vivo</i> (P.berghei)	
	K1 strain	FCR3 strain	ED ₅₀ (mg/kg)	ED ₉₀ (mg/kg)
Borrelidin	1.9	1.8	0.18	2.0
Artemether	7.6	2.2	0.95	3.8
Artesunate	11	2.7	1.7	10.0
Chloroquine	357	29	1.5	2.5

E.Coli and *S. cerevisiae*.^{38, 40} Borrelidin also inhibit the cyclin-dependent kinase Cdc28/Cln2 in *S.cerevisiae*. It arrest both haploid and diploid cells at G1 phase at concentration that do not affect gross protein biosynthesis (IC₅₀ value = 24 μM), which is an anti-mitotic activity.⁴¹ Few species which have different amino acids in cluster A which are resistant to borrelidin are pathogens (like *Helicobacter pylori*, *Mycobacterium*, *Methanococcus maripaludis*, *Archaeoglobus fulgidus*.²⁸

The resistance to borrelidin can be offered by increasing levels of ThrRS and structural modification of ThrRS.⁴² The modification or absence of cluster A results in resistance to borrelidin.

Antimalarial & Antiviral activity

The development of new drugs for malarial disease is important now-a-days as the parasite *Plasmodium falciparum* become resistant to present drugs. Recently, borrelidin shows an effective inhibitory activity against chloroquine resistant *P.falciparum* strain *in vivo* and *in vitro* models.⁴³ The anti-malarial activity of borrelidin isolated from *Actinomyces* strain OM-0060 comparing with standard anti-malarial drugs against the parasite *in vivo* and *in vitro* given in Table 3.^{44,45} Borrelidin shows more potent anti-malarial effect than artemether, artesunate

Conclusion

Borrelidin produced from the *Streptomyces* species especially from *S. parvulus* do possess an excellent anticancer, antiangiogenesis, antiviral, antimalarial and antimicrobial property beyond its apoptosis inducing ability. Very little works had been reported in the areas of strain improvement. With the knowledge of enzymes in the biosynthetic pathway and bioprocessing skills the strains can be altered to make it more productive. Even though it has a wide spectrum effect it is not a successful drug in case of humans due to the levels of toxicity. Research has to be initiated to produce novel derivatives of borrelidin to be more target specific.

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Author for correspondence:**Vino S**

School of Biotechnology,
Chemical and
Biomedical Engineering,
VIT University,
Vellore-632014,
Tamil Nadu, India
Tel: 91-416-2202608;
Fax: 91-416-2243092
E-mail: vinokhanna22@yahoo.com