Chapter 4

Neocarzinostatin - New Promises in Anticancer Activities

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4.1 Introduction

Medically cancer is a malignance, a broad cluster of assorted disease. The malignancy may spread to more expelled parts of the body through the lymphatic structure or circulatory framework. While trying to battle uncontrolled development cells, researchers are making an endeavor to seek out molecules to arrest formation of the malignant tumors. These molecules are referred to as anticancer antibiotics. Chemotherapy is typically the primary selection for the treatment of the many cancer sorts. Enediynes antibiotics potential natural toxins that possess potent medicinal drug, malignant tumor activities due to their distinctive molecular structure mode of action, currently the foremost promising leaders within the malignant tumor medical aid and tried clinical affectivity [1]. Among 20 distinct enediynes, the foremost necessary Neocarzinostatin (NCS) in cancer treatment and a potent tumour, drug actions [2] and exerted by DNA cleavage. DNA harming movement essentially in single-strand DNA cuts related yield through an O_2subordinate reaction [3], Thiols [4] and UV radiation [5] enormously upgrade DNA-severing properties of NCS. This Chapter offers a high level read of NCS, synthesis, mode of action and efforts undertaken to vogue artificial enediyne-related DNA cleaving agents.

4.2 Neocarzinostatin Chromophore

NCS is the first member of enediyne antibiotic class [6], isolated from *Streptomycescarzinostaticus* Var. F-41reported by Ishida *et al* in 1965 [7]. In 1966, the complementary chromophore binding protein (apo-NCS) isolated [8] and following Maeda‘s studies primary sequence of protein was published [9], later revised [10] and confirmed by NMR studies [11]. NCS is an acidic protein, MW 10,700, 1:1 non-covalently associated mixture of a protein component (NCS apoprotein) and a chromophoric molecule (NCS chromophore, Figure 1).

It is the in freestanding state is highly unstable upon exposure to heat, high pH or UV-light irradiation.

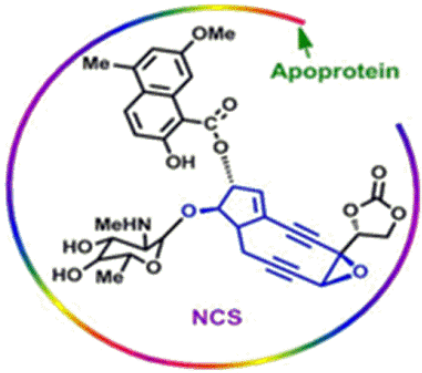
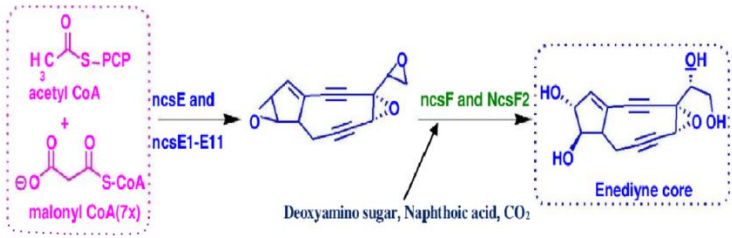


Figure 1. *Neocarzinostatin chromophore (peptide loop composed of 113 amino acids).*

4.3 Enediyne Core of NCS Biosynthesis

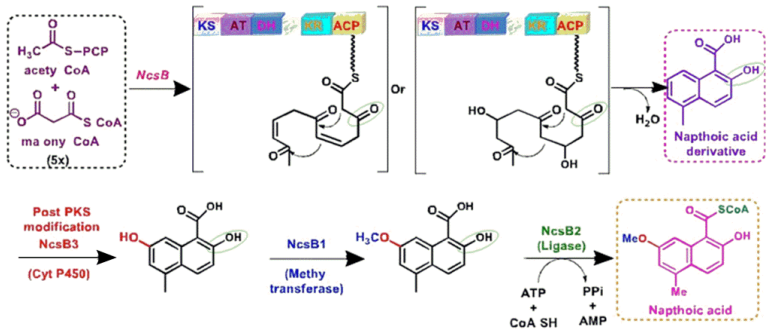
14 genes identified within*ncs* gene (*ncsE*to*ncsE11*and*ncsF1*to*ncsF2*) cluster, important role in the NCS core biosynthesis [12]. The enediyne core previously predicted to be synthesized by an iterative type Ipolyketide synthase (PKS) with five domains, of which keto-synthase (KS), acyltransferase (AT), ketoreductase (KR), and dehydratase (DH) are characteristic of known type I PKSs [13, 14, 15, 16, 17]. NcsE shows head-to-tail sequence homology to the SgcE [13] and CalE8 [14] enediyne PKSs. Consequently, it proposed that NcsE, in a mechanistic analogy to other enediyne PKSs, catalyzes the formation of the nascent linear polyunsaturated intermediate from one acetyl CoA and seven malonyl CoAs in an iterative manner, which processed to form the enediyne core by several gene products, including NcsE1-E11 and epoxide hydrolases F1 and F2 (Scheme 1).



Scheme 1. *Biosynthetic hypothesis of the enediyne core of NCS.*

4.4 Naphthoic Acid Biosynthesis

Naphthoic acid moiety synthesis from polyketidechain of sixhead-to-tail acetate units are disclosed by isotopic labeling experiments. In NCS gene cluster, major enzymesare (a) an iterative PKS (NcsB), (b) a CoA ligase and (c) several ancillary enzymes. Naphthoic acid synthesis starts with NcsB, an iterative PKS that contain domains (a) the keto-acyl synthase (KS), (b) acyltransferase (AT), (c) keto-reductase (KR), (d) dehydratase and (e) acyl carrier protein domain (ACP) and a core domain with unknown function. NcsB uses acetyl coenzyme A (CoA) as stating material and malonyl-CoA as extender to assemble a nascent hexaketide with reduction and dehydration of the keto groups at C5 and C9 (Scheme 2).

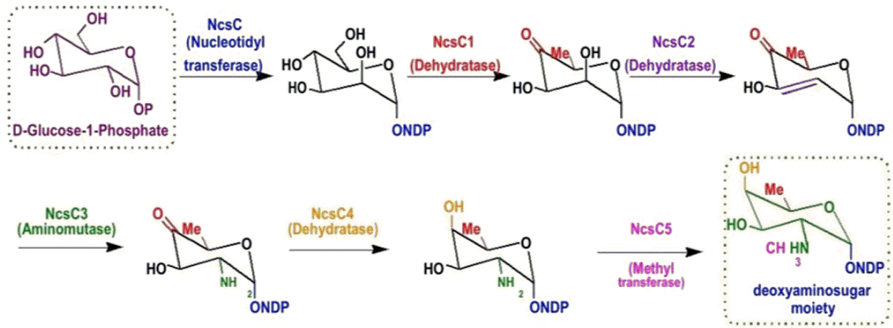


Scheme 2. *Possible biosynthesis mechanism for the naphthoic acid moiety of neocarzinostatin.*

The hexaketide intermediate then undergoes aromatization by intramolecular aldol condensation to furnish the naphthoic acid moiety. The post-PKS modification of the naphthoic acid starts with the incorporation of a OH group at C8 carbon which catalyzed by the cytochrome P450 hydroxylase NcsB3. Ultimatelymethylation of OH group catalyzed by an S-adenosylmethionine (SAM) dependent O-methyltransferase(NcsB1). Then NcsB2 ligase catalyzes the adenylation of 2-hydroxy-7-methoxy-5-methyl-1-naphthoic acid to form its CoA derivative. Finally, putative acyl transferase (NcsB4) responsible for transfer of naphthoic group onto enediyne core.

4.5 Deoxyamino Sugar Biosynthesis

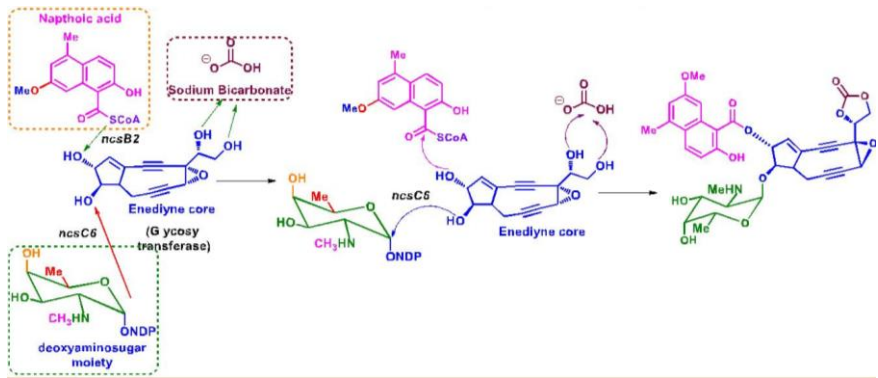
The deoxy amino sugar moiety biosynthesis start with activation of monosaccharide as its nucleotide diphospho (NDP) derivative by nucleotidyltransferase NcsC (Scheme 3). Several gene products have been proposed for the activation of sugar ring via formation of a 4-keto intermediate, deoxygenation at C6 and installation of amino group at C2. The isotopic labeling experiments with [methyl-3H] methionine revealed that the N-methyl of deoxy amino sugar originates from methionine of SAM. The methylation catalyzed by methyltransferase NcsC5, whereas glycosyltransferase NcsC6 may transfer sugar moiety onto enediyne core.



Scheme 3. *Possible mechanism of biosynthetic hypothesis of deoxyaminosugar moiety.*

4.6 Biosynthesis of NCS by Joining Together Peripheral Moieties to Enediyne Core

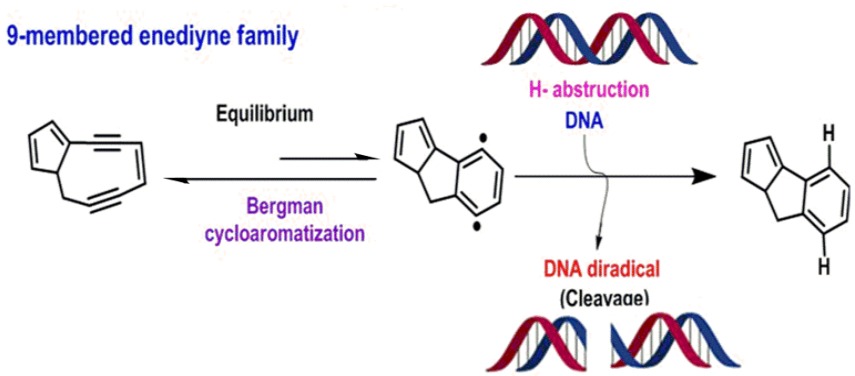
A convergent strategy could be envisaged for the assembly of the NCS chromophore from three individual building blocks of deoxy amino sugar, naphthoic acid, and enediyne core (Scheme 4). The coupling between dNDP-sugar and enediyne core catalyzed by NcsC6 glycosyltransferase while the other coupling between naphthoyl-S-NcsB and enediyne core catalyzed by NcsB2 CoA ligase. Although the cyclic carbonyl carbon of NCS previously shown to originate from carbonate, no obvious candidate catalyzing the attachment of carbonate could be identified within the gene cluster.



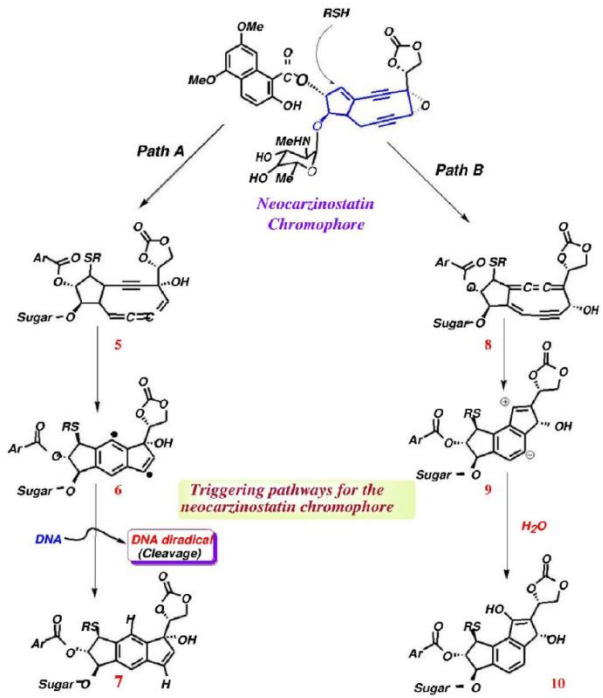
Scheme 4. *Biosynthesis of enediyne core of NCS with peripheral moieties.*

4.7 Mechanism of Action of NCSon Cancer Cell

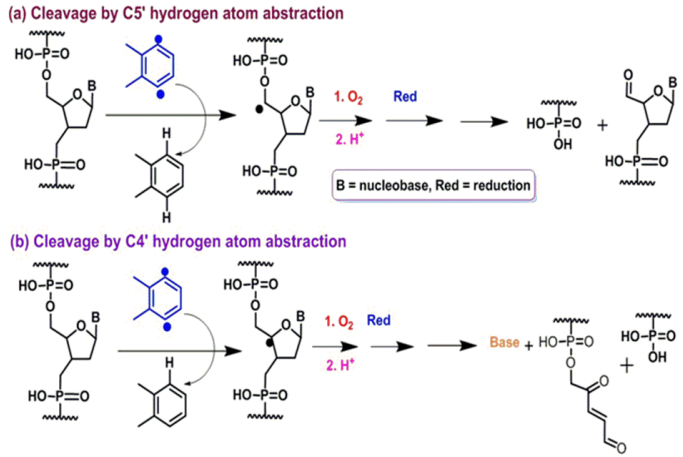
High-resolution X-ray diffraction, NMR techniques, together with thermodynamic studies and molecular modeling disclosed basic principles in DNA-drug interaction [18-20] and NCS-DNA mechanism first reported in 1987 [21]. In pathway A (Schemes 5 & 6), DNA damage initiated by stereospecific nucleophilic attack at C-12. This triggering reaction accompanied by rearrangement of ring skeleton with epoxide opening and formation of cumulene observed by NMR at low temperature [22] in 5 as shown in Scheme 6. Then this reactive intermediate undergoes a rapid cycloaromatization to form diradical in 6, and then which proceeds to attack DNA by removing hydrogen atoms in **7** and this scenario provided by using $HSCH_2CO_2Me$ [21-24], $NaBH_4$ [24] as nucleophiles in *in vitro* experiments. The methyl thioglycolate isolated, fully characterized and the evidence of the basic methyl amino side chain on the sugar residue assists the thiol addition at C-12 through base catalysis provided by Myers [22, 25]. The additional information provided by three-dimensional structure of intact NCS [26] - the amino methyl group of sugar forced into close proximity to C-12(4.3Å) due to a salt bridge with Asp33, suggesting that nucleophilic attack at C-12 assisted by nitrogen, and together with additional steric hindrance at C-12 from the side chains of Ser98, Asp33, Phe52 and the positioning of epoxide in a hydrophobic pocket away from an acid catalyst, this indicates that how apoprotein serves to stabilize the chromophore. In pathway B cycloaromatization, NCS chromophore incubated with 2-mercaptoethanol in the presence of apoprotein in which the zwitterionic intermediate 9 (Scheme 6) is indicated [27, 28], although this mechanism probably does not operate for the free chromophore. Since it is thought that dissociation of NCS chromophore from apoprotein and subsequent DNA binding precedes activation of chromophore, the biological relevance of this second mechanism seems dubious, and this pathway is not responsible for DNA cleavage reported by Chin and Goldberg in 1993 [29].



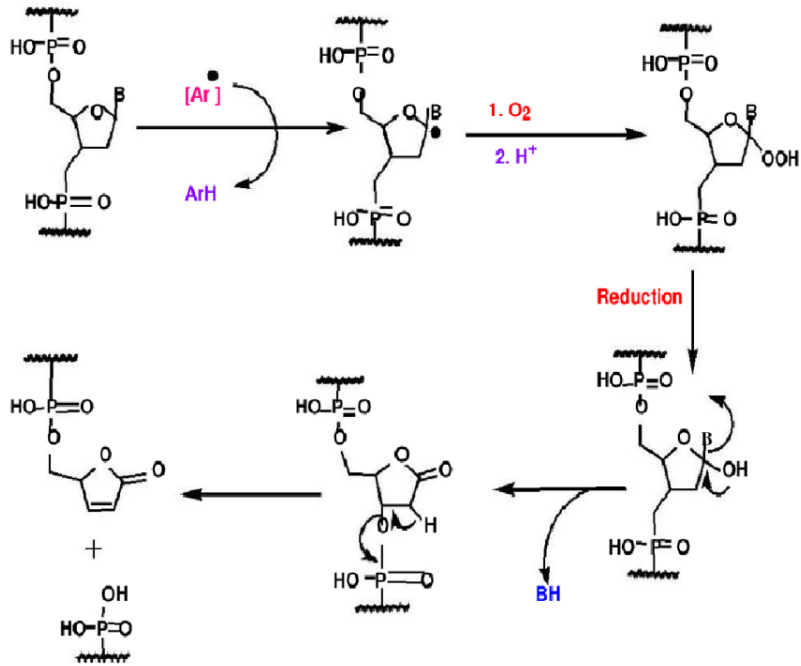
Scheme 5. *General mechanism of action of enediyne anticancer antibiotics: DNA cleavage.*



Scheme 6. *Mechanism of action of enediyne anticancer antibiotics: DNA cleavage initiated by C4'- or C5'-hydrogen atom abstraction.*



Scheme 7. *Mechanism of action of enediyne anticancer antibiotics: DNA cleavage initiated by (a) C4' or (b) C5' hydrogen atom abstraction.*



Scheme 8. *Mechanism of action of enediyne anticancer antibiotics: DNA cleavage initiated by C1'- hydrogen atom abstraction.*

Several scientists worked out to disclose the details of DNA damage by NCS chromophore diradical 6. Itdemonstrated that at least 80% of DNA cleavage leads to 5'-aldehyde of A and T residues selectively [30]. These cleaves involves hydrogen atom abstraction from C-5' of deoxyribose and reaction with molecular oxygen as showed in scheme 7. Less than 20% of strand breaks result from hydrogen atom abstraction at C-4'[31-35] and C-1' [32] (Schemes 7 & 8). The radical at C-2 of 6 particularly susceptible to both internal and external quenching up to 70% under physiological conditions reported by Goldberg [36]. A convincing explanation that the NCS chromophore effects primarily single-stranded DNA cuts by C-6 radical at C-5' of deoxyribose, whereas those double stranded lesions are involved hydrogen abstraction by C-2 radical from C-1' or C-4' of deoxyribose on complementary strand. Further insight into the interaction of NCS chromophore with DNA, recent observation made clear that a thiol independent cleavage mode is possible with single-stranded DNA bulges, the regions where double-stranded structuresgenerated intra molecularly [37]. These logical consequences indicated that DNA is an active participant in its own destruction, since DNAs containing point mutations which disrupt the bulge are not cleavage substrates.

4.8 Conclusion and Future Prospects

The studies described above indicate that how the mechanistic and synthetic challenges resulting from discovery of the neocarzinostatin antibiotic have been approached. Designed enediynes demonstrated abilities to cleave DNA and exhibited selective cytotoxicity against tumor cells versus normal cells [38, 39]. Enediynes have been implicated in the puzzling but important phenomenon of programmed cell death (apoptosis) [40] and the total synthesis of prominent and complex member of enediyne class has been achieved [41]. The most studied systems relate to neocarzinostatin, perhaps because this available for longest

period of time. This compound shown to possess antitumor activity in patients with liver cancer, bladder cancer, stomach cancer, and leukemia as well as in various animal tumors [42]. Polystyrene-co-maleic acid-NCS shown high antitumor activity in animal models following oral administration [43, 44]. Immuno-conjugates of neocarzinostatin such as A7-NCS [45] showing increased survival times when administered to postoperative cancer patients (both with and without metastases) when compared with other chemotherapies. Therefore these novel natural products with their unprecedented modes of action are clearly more than a scientific curiosity, and it remains to be seen whether enediynes, either natural or designed, will become useful additions to the arsenal of chemotherapies available to clinicians for the treatment of cancer.

The next phase of research in enediyne field will undoubtedly include further synthetic attempts at naturally occurring targets, new designed enediynes with sophisticated mechanisms of *in vitro* and *in vivo* activation, and attachment of these systems to suitable delivery systems. Targeting devices may include antibodies, oligonucleotides, oligosaccharides, peptides and proteins, DNA intercalators, DNA groove binders, hormones, and other ligands. Hybrid molecules between enediyne "molecular warheads" and such delivery systems should provide new insights into biological phenomena and may facilitate drug design and development.

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