Doxycycline as Potential Anti-Cancer Agent

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Review

Doxycycline as Potential Anti-Cancer Agent

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Contents

Abstract:

Cancer cells do create hostile microenvironment (deprivation of nutrients, accumulation of acidity, anoxic habitat). Those cells are not only adapted to this sanctuary environment, blunting of immunity but also, grow, migrate to the distal area (metastasis) and communicate with each other in a unique population structure and organization too (clonal expansion). The adaptation requirements push those types of adaptable cells (cancer cells) to be primitive cells. The prevailing pharmacological approach in treating cancer is developing a chemotherapeutic agent that acts on rapidly proliferating cells that stuck with normally growing epithelium and bone marrow too. The latter approach has been drafted to work on cellular target under the term of "targeted therapy" believing that each target represents Achilles Heels of cancer. In this article, we try to introduce new a concept of cancer pharmacology, by offering new off-label use of Doxycycline, that characterized by selective toxicity, as potential anticancer agents. This notion is relying on the absence of taxonomic barriers.

Keywords: Doxycycline, Cancer

Introduction:

Cancer is a complex genetic disease when the possible causation of it was correlated primarily with environmental factors which affect the key regulatory proteins of the cells. The products of these alterations appear in unusual cellular behavior including growth, metabolism and motility (tumor dissemination) [1]. To scope with a newly way of adaptation requirements, cancer exploits the evolutionary scale to become slightly primitive cells. Therefore, carcinogenesis is a process of convergent and /or reverse evolution [2].

The treatment of cancer includes surgery, chemotherapy, and radiation therapy. The term of Chemotherapy means treatment by chemical. However, pharmacologically, indicates anti-infectious (antibacterial, antifungal, antiviral and antiprotozoal drugs), anti-infestation (anti-worms) and anticancer agents.

Cancer chemotherapy, cytotoxic drugs, is directed to induce cellular death by investment on apoptosis rather than necrosis [3,4], and it action rapidly proliferating

types of cells which have very limited activity on slowly growing tumors. They have serious side effects that affect (i) the quality of life of the patients, and (ii) the patient compliance too. Some of these side effects include hematological toxicity (neutropenia, anemia), dermatological toxicity (alopecia), gastrointestinal toxicity (nausea, vomiting), renal toxicity and many other kinds of toxicity [5]. Also, the vast majority of the chemotherapeutic agents are responsible for recurrence [6], while others are well known as carcinogens and teratogens too[7,8].

1. Doxycycline and its mechanism of action:

Doxycycline is semi-synthetic tetracycline where Tetracycline is a naturally produced by Streptomyces genus that belongs to dominant types of bacteria, Actinobacteria (see Fig. 1) [9]. Doxycycline is an antibiotic, synthesized by a microorganism to inhibit the growth of another microorganism, having broad spectrum bacteriostatic effect. The possible mode of action via a mediating strong inhibitory effect on of protein synthesis. Precisely, Doxycycline inhibits the synthesis of proteins via preventing aminoacyl-tRNA attachment to the ribosome in bacterial cell [10]. In addition to that, Doxycycline has inhibitory effect to the activity of matrix metalloproteinases (MMPs); according to this, it has the potentiality for using as anti-neoplastic and also as anti-inflammatory [9]. Also, it inhibits mitochondrial protein synthesis. It acts as ionophore, binds to some divalent metal cations mainly Zn^{2+}, Ca^{2+} and Mg^{2+}. It result in formation of lipid-soluble complexes which are easily transport crossing the hydrophobic membranes [11]. Doxycycline is a well-tolerated drug, is virtually completely absorbed after oral administration and has good tissue penetration [9]. It has selective toxicity, not like cancer chemotherapeutic agents which show poor selectivity. Doxycycline is cheap and available worldwide antibiotic, its administration may be orally or parentally. These make it is convenient for use in the community [9].

2. Doxycycline inhibits cellular Proliferation:

2.1.1 Effect of Doxycycline in Non-Hodgkin Lymphoma (NHL):

The most type of NHL is diffuse large B-cell lymphomas (DLBCL). It has three subtypes: 1. Activated B-cell (ABC-DLBCL), 2. Germinal center B-cell (GCB-DLBCL), and 3. Mediastinal large B-cell (PMBL-DLBCL). Mainly ABC-DLBCL cells

depend on constitutive NF-κB signaling for proliferation and survival [12–14]. Targeting pathways required for NF-κB activation thus has been proposed as a novel treatment strategy for DLBCL [15,16]. In a study, when doxycycline is used in vitro and in vivo models of DLBCL cells, it has been found to inhibit their growth. It refers to its accumlation in high concenteration in DLBCL cells. Also it affects different signaling pathways that play a central role in lymphomagenesis [17].

2.1.2 Doxycycline appears to affect HSP90 function via an indirect mechanism in DLBCL cells:

The heat shock protein 90 (HSP90) "a chaperone proteins" assists a vast number of proteins in their folding and function of normal proteins or that mutated in cancerous cells too. Dysregulation of HSP90 expression and activity was observed in different types of cancer such as DLBCL [18,19]. HSP90 inhibitors can induce proliferation arrest and apoptosis in DLBCL cells [20,21]. Upon the treatment of DLBCL by doxycycline, the action results from using as an inhibitor of HSP90 activity in cancer cells, it results in degradation of client proteins, such as TYK2 and RIPK1, and reduction in NF-κB signaling, STAT3 phosphorylation and ERK activation [22–27]. It was found that the doxycycline treatment decreased the levels of several known HSP90 client proteins in DLBCL cells but not on mRNA levels. It also reduces the HSP90 client proteins in other types of NHL cells.

2.1.3 Doxycycline impacts on neddylation and radiation:

In recent studies of lymphoma patients, doxycycline shows a positive therapeutic effect [28]. This is occurred by inhibition the activity of CSN5 and by reducing the levels and function of HSP90 [17]. CSN5 belongs to COP-9 "constitutive photo-morphogenesis 9" signalosome. It inhibits the neddylation. A similar process to neddylation is ubiquitination, in which the ubiqutine "a regulatory protein" is added to substrate proteins, this is result in enhancing their degradation via proteasome, or alter their function or location [29–31]. Neddylation involves NEDD8 instead of ubiqutin. Doxycycline inhibits CSN5 function in deneddylation. This leads to impair the cell survival in DLBCL, in which doxycycline mainly induces the degradation of proteins via increasing

the nddylation and ubiquitination processes. Another effects of doxycycline treatment, it leads to cell cycle arrest and inhibition of NF-kB and STAT3 signaling [32]. It has been concluded that the synergism of doxycycline with chemotherapeutic agents in the treatment of diffuse large B-cell lymphoma, doxycycline is very useful in treatment of chemoresistant CSCs. However, it has been shown that inhibition of neddylation does sensitize cancer cells to raditation and chemotherapy although the mechanism behinds this synergy is a conundrum[32,33].

2.2 Cytotoxic Effects of Doxycycline in Hematological Malignancies:

In vitro, doxycycline has been noticed to have a cytotoxic effect, it induces cell death in many cancerous cell lines including mainly leukemia and other types such as breast and prostate cancer [34–37]. Activation of cell death, apoptosis, by cytotoxic drugs can eliminate cancer cells. It can occur via FAS ligand binding the FAS receptor (extrinsic), or by releasing the cytochrome c "a potent catalyst of apoptosis" (intrinsic). The two activate the caspases as a main effectors in apoptosis [38,39]. Members of Bcl-2 family of proteins, they have either proapoptotic or anti-apoptotic effect. The proapoptotic (Bax, Bak, Bid, Bim), BH-3 proteins, activation causes the release of cytochrome c from the mitochondria. It binds to Apaf-1 that result in the activation of caspase-9. PI3K/AKT pathways including mTOR is the most survival signaling in cancer due to multiple genetic aberrations [40]. In acute myelogenous leukemia (AML), this pathway is activated in 50–80% of patients[41] and is frequently associated with the mutations of Ras and c-KIT [41], amplification of PI3K "delta isoform" [42], or survival conveyed by IGF-1 signaling induction [43]. Activation of PI3K leads to AKT activation, which signals to various downstream substrates including GSK-3, FOXO, mTOR, Bad, MDM2 and NF-κB, and modulates diverse cell processes including survival, proliferation, apoptosis, and autophagy, among others [44]. Overexpression of other members of Bcl-2 family, anti-apoptotic, (Bcl-2, Bcl-xL, Bcl-w and Mcl-1), occurs particularly in hematological malignancies (e.g. AML). It result in cell survival and also drug resistance [45]. Several agents have been developed to target these proteins directly, e.g., ABT-737, it antagonizes Bcl-2 and Bcl-xL functions but not Mcl-1.

Preclinical studies demonstrated that ABT-737 induces apoptosis and potentiates the anti-tumor activity of multiple agents in various cancers, including leukemia [46]. Recent studies indicate that PI3K inhibitors efficiently down-regulate Mcl-1, an event that plays a significant role in transformed cell lethality. Tetracycline was found to induce Bcl-2 and Bcl-xL knockdown, that sharply increase the lethality upon PI3K/AKT/mTOR inhibiton. Many of PI3K/mTOR inhibitors can mediate cell death in leukemic cell lines such as "BEZ235, PI-103" [47]. In a study using acute myeloid leukemia (AML) cell lines, which was treated with doxycycline for 24 hrs. This result in inhibition of the viability of cell lines. This inhibitory effect depends on the concentration of doxycycline that was used [37].

2.3 Cell signaling in cancer:

FAK, or focal adhesion kinase, it is encoded by the PTK2 gene "protein tyrosine kinase 2". It is involved in Src gene"a proto-oncogene" in tumor cell invasion and motility. Upon the activation, FAK undergoes auto phosphorylation at Tyr 397, then the SH2 domain of Src gene interacts with Tyr 397 and result in phosphorylation of many residues of tyrosine in FAK enzyme creating docking sites for SH2 domain-containing signaling proteins, such as the adaptor Grb2 [48].

PI3K "Phosphatidylinositol 3-kinase" pathway regulates cell growth, motility, and survival. It is inhibited by the action of PTEN which is a tumor suppressor gene; it encodes a phosphatase that converts phosphatidylinositol-3,4,5 triphosphate to phosphatidylinositol-4,5bisphosphate. PI3K activates protein kinase B (Akt) and phosphoinositide-dependent kinase-1 (PDK1). PDK1 is full activating Akt and initiate a kinase cascade that plays an important role in growth of cancer cells. Activation of Akt results in activation of the mammalian target of rapamycin (mTOR), which enables the translation process. Also activation of Akt pathways enhance the cell survival via activation of mTOR, Iκ-kinase (IKK) and Mdm2, and by inhibition of Bax and Bad "pro-apoptotic Bcl-2 family members" and pro-apoptotic transcription factors such as FKHR [48].

Wnt Signaling directs mainly cell proliferation (normal or neoplastic). It regulates the transcriptional coactivator β-catenin which involves transcription of critical genes. B-catenin in the cytosol is inhibited by the action of GSK3 "Glycogen synthase kinase 3".

It phosphorylates the β-catenin resulting in its ubiquitination by β-Trcp, which is an E3 ubiqutin ligase, it frequently leads to β-catenin degradation [49], (see Fig. 2).

STAT pathway "signal transducer and activator of transcription" is mediated JAK "Janus kinase" by many activators such as IFNα-, IFNγ- and IL-6. There are different members of STAT protein family, the only have a role in cancer cell proliferation or survival are STAT3 and STAT5. STAT3 signaling has another central roles mainly in cancer stem cells (CSCs) and inflammation-mediated cancer. The main activator for it is IL-6 and also LIF "leukemia inhibitory factor" which belongs to IL-6 family. It appears in different types of cancer including glioma stem cells (GSCs). The levels of LIF is controlled by TGF-β "transforming growth factor β" signaling. In GSCs the two signaling pathways are important for tumor development [50].

NF-κB signaling "the nuclear factor kappa-light chain" controls the expression of different genes. It is mainly regulates anti-apoptotic genes which enhance the cell survival. Therefore the activation of it is associated with mainly activated B-cell lymphoma (ABC-DLBCL). NF-κB pathway is involved also in the induction of cytotoxic drug-mediated senescence in cancer cells that result in more complexity for NF-κB role in oncogenesis [51]. Table (1), summaries some signaling proteins that have a role in oncogenesis.

3. Doxycycline inhibits Metastasis:

3.1 Effect of doxycycline on matrix metalloproteinases (MMPs):

MMPs are zinc-dependent endopeptidases. Their primary mechanism of action collectively involves degradation the components of extracellular matrix (ECM), also MMPs can cleave growth factors and play a crucial role in cell motility and proliferation [52,53]. In humans, the members of MMPs that have been reported are 28. They are classified according to their substrates. The most important are collagenases (MMP-1, -8 and -13), gelatinases (MMP-2, -9), membrane-bound (MMP-14 to -17, -24 and -25) [54,55]. MMPs are highly upregulated and expressed in cancerous tissues compared to normal [56].

They are involved in cancer proliferation, invasion, metastasis and angiogenesis.

In breast cancers, several studies have shown the role of MMPs which are highly expressed, and these types are (MMP-1, -2, -8 to -13, -15, -19, -23, -24, -27 and -28)[57,58]. A study was done in India; the results show that some MMPs were upregulated and the others were downregulated at the level of mRNA expressions. The MMP-1, -9,-11,-15,-24 and -25 were upregulated and MMP-10 and MMP-19 were downregulated in cancer tissue, and that are grade dependent increase of their mRNA expression are membrane-associated MMPs like MMP-15 and MMP-24 [58]. In Glioblastoma multiform (GBM) "a primary brain tumor", it was found that MMP2 and MMP9 have an important role in enhancing glioma tumor migration, invasion, and angiogenesis [59]. Oral squamous cell carcinoma (OSCC) was associated with high production of MMPs [60]. As in glioma elevated levels of MMP-2 and MMP-9 have been observed in pulmonary adenocarcinoma [61], ovarian carcinoma [62], OSCC, and many other types of cancer [63,64].

One of the action of doxycycline is the inhibition of MMPs synthesis and is approved by Food and Drug Association for dental applications as the only MMP inhibitor [65]. Upon the treatment of breast cancer with doxycycline, it has been observed a reduction of proliferation, bone metastasis and the activity of MMP-2 and MMP-9. It is found that Doxycycline decreases the tumor burden by 70% in bone metastasis in breast cancer [66]. In glioma tumor doxycycline mainly decreases MMP2 activity [59]. In (OSCC) also doxycycline decreases the activity of MMP-2 and MMp-9, it was altered the expression of level of MMP-9 mRNA but not MMP-2 mRNA, indicating the effect of doxycycline on MMP-9 expression at transcriptional level but MMP-2 expression at the post-transcriptional level [60]. Moreover, in prostate cancer doxycycline inhibited its cell proliferation, invasion, and metastasis [67,68].

3.2 Inhibition of cell migration in Hematological Malignancies:

In literature, it was found that the Doxycycline has inhibitory effect on the FAK signaling pathway. It is mainly decreased the total protein expression but not at FAK mRNA level. This effect appears following 12 hours treatment with doxycycline. This result in inhibition of leukemic cell migration. [69].

4. The doxycycline effect on stem cells:

In normal and induced pluripotent stem cells, doxycycline exerts survival and self-renewal effects upon the activation of corresponding signaling pathway such as PI3K/Akt [70]. In breast cancer, it was noticed with the formation of mammospheres which are formed due to the growth of cancer stem cells in the cell population. They are one of the hallmark characteristics of stem cells. Some studies show that the higher level of mitochondrial proteins in breast cancer stem cells "CSC", defined by their capability to formation the mammospheres. When it was treated with doxycycline, it is noticed that the number of mammospheres are reduced, and this refers to the eradication of the "CSCs" by doxycycline. The mechanisms by which the effect of doxycycline occurred; the level of mitochondrial proteins are measured, specifically DNA-PK enzyme, which is involved in DNA repair (NHEJ "non-homologous end-joining"). In the treated cells the level of DNA-PK enzyme was reduced compared to cells not treated which is in high level than in normal cells. It was suggested that DNA-PK enzyme plays a role in breast cancer stem cells, confers the radioresistance in these cells. It is suitable target for cancer treatment with new pharmaceutical development [71]. Doxycycline reduces the expression of DNA-PK proteins. When it was compared with the existing DNA-PK inhibitors it has many advantages beyond the DNA-PK inhibitors such as (NU7441). It has excellent pharmacokinetics, it is nearly 100% absorbed when taken orally (200mg per day) and it has long half-life (18-22) hrs. where is the NU7441 suffer from poor pharmacokinetics, it is not well absorbed, unstable and with a short half-life [71]. A closer look at the chemical structure, of doxycycline and NU7441 , see Fig. 3, reveals that doxycycline is a reduced carba-analogue of NU7441 [32]. It was found that DNA-PK has been shown to interact directly with LEF1, Lymphoid enhancer-binding factor 1, which acts downstream in

Wnt signaling [72]. It was observed that doxycycline blocks signaling pathways normally associated with stem cells such as Wnt signaling [71].

5. Doxycycline as radio-sensitizer:

One of the types of treatment of cancer that are used is radiation, another one is the chemotherapy. Both, the mechanisms are worked by inducing DNA damage, which is lead to the cells death unless it is repaired. In CSCs, they resist the radiation by protecting themselves from DNA damage through enhancing the repairing system of DNA. It was found that this process is active in CSCs [73].

Radiotherapy is the main treatment in glioblastoma, but also it is commonly associated with radioresistance, that because the activation of PI3K pathway which is commonly activated in this type of tumor. Even by PTEN gene mutation or possibly by epidermal growth factor receptor overexpression (EGFR)[74,75]. Ras/PI3K/Akt pathway is correlated with its radio-resistance. In literature it was found that the correlation between the reducing survival times in patients with the activation of PI3K signaling pathway. Also it was found that Akt signaling accelerates DNA repair and consequently, improves post-irradiation cell survival [76]. All of that lead to the radioresistance in glioma tumor. Using of doxycycline to affect PI3K/Akt signaling pathway, it may modulate DNA repair to improve the efficacy of radiation therapy [77].

6. Doxycycline as Quorum Sensing Inhibitor:

Quorum sensing is a proposed system that coordinates the population density [78]. Many species of bacteria use quorum sensing to manipulate gene expression according to the density of their local population [78]. Bacteria use quorum as an anti-predation strategy. Also, Bacteria use quorum sensing to communicate, coordinate withing the same species (intraspecies) and/or inter-species intraspecies or interspecies, and can regulate the host response to maintain the population density and guarantee the evolutionary trajectory of those species [78]. Therefore, developing of quorum sensing inhibitor will work on the whole population (group selection) rather

than individual microorganism (individual selection) [79]. It has been found that targeting group has greater impact compare to targeting an individual because the emergence of resistance to the whole colony occurs at slower rate compare to individual microorganism[80].

Quorum sensing has also been found within the tumor cells (intraspecies) [81,82] and tumor with other types of microorganism e.g. gut microorganism (interspecies) [83]. Doxycycline is a quorum sensing inhibitor [84] which indicates another mode of action of this medicine as promising anti-cancer agent.

Conclusion:

Last but not least, doxycycline is an excellent example of how existing, inexpensive, well-tolerated drug might be extended to use as new cancer therapeutic agent (Off-label). In this regard, it is important to establish accurately the bioequivalent dose required to block CSCs and other kinds of cancer cells. According to the different mechanisms of action of doxycycline, the anti-MMPs effect, its action as DNA-PK inhibitor, its cytotoxic effect on malignant cells and its sensitization of some types of cancer cells for radiation therapy, all of that give us a promising treatment type of cancers such as breast cancer, glioblastoma, leukemia and others.

Declarations

Authors' Contribution:

IA, KOA, LS, SJR and MEI conceived of the study, drafted and designed the manuscript. All authors read and approved the final manuscript.

Conflict of Interest statement:

The authors declare that they have no competing interests.

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Tables:

Table (1): some signaling proteins that have an oncogenic role.

Affected protein	DNA/mRNA alteration	Activators	Cancer type
CREBP(CBP)	Translocation/ missense		Lymphoma/ leukemia
GSK3b	Miss-splicing, in-frame deletion		Leukemia
STAT3		IL 10	DLBCL

Figures and Legends:

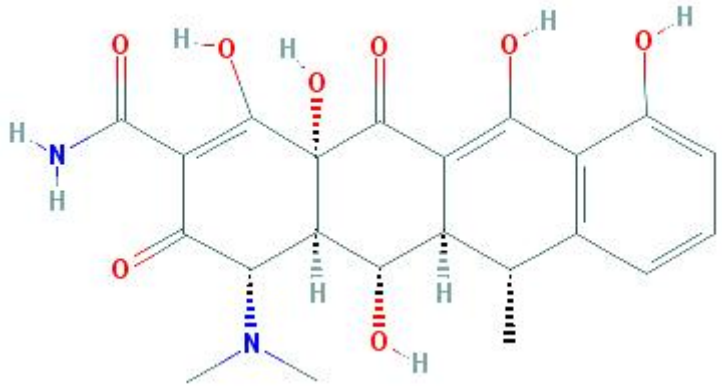

Figure (1): Doxycycline, (6-Deoxy-5-hydroxytetracycline).

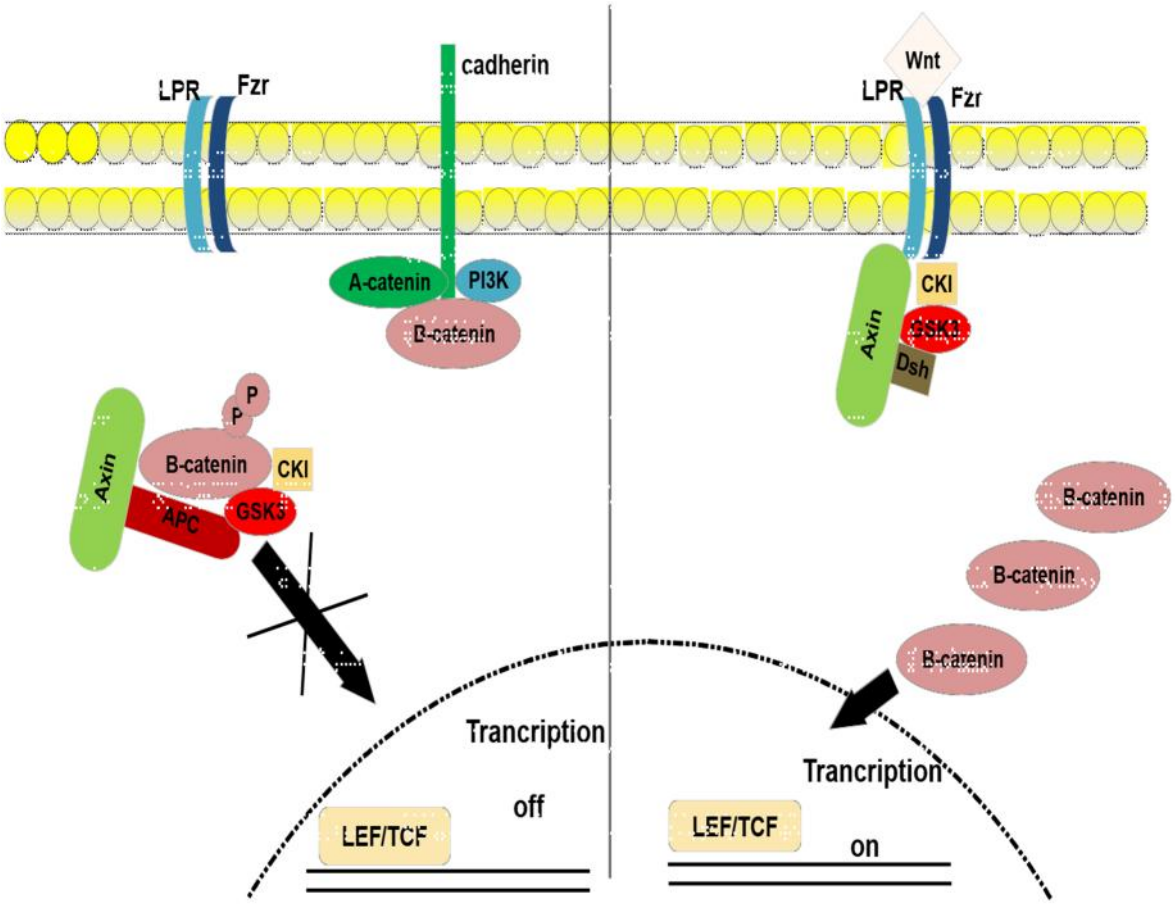

Figure (2): Overview of Wnt/β-catenin signaling: A picture on the left, the absence of Wnt, β-catenin forms a complex in the cytoplasm with Axin, APC, GSK3 and CK1, first it is phosphorylated by CK1 (in orange) and subsequently by GSK3 (in red). Phosphorylated β-catenin is recognized by the E3 ubiquitin ligase β-Trcp, which targets βcatenin for proteasomal degradation. A picture on the right, the presence of Wnt ligand, leads the receptor to form complex. This disrupts Axin-mediated phosphorylation/degradation of β-catenin, allowing β-catenin to accumulate in the nucleus where it serves as a co-activator for TCF transcription factor.

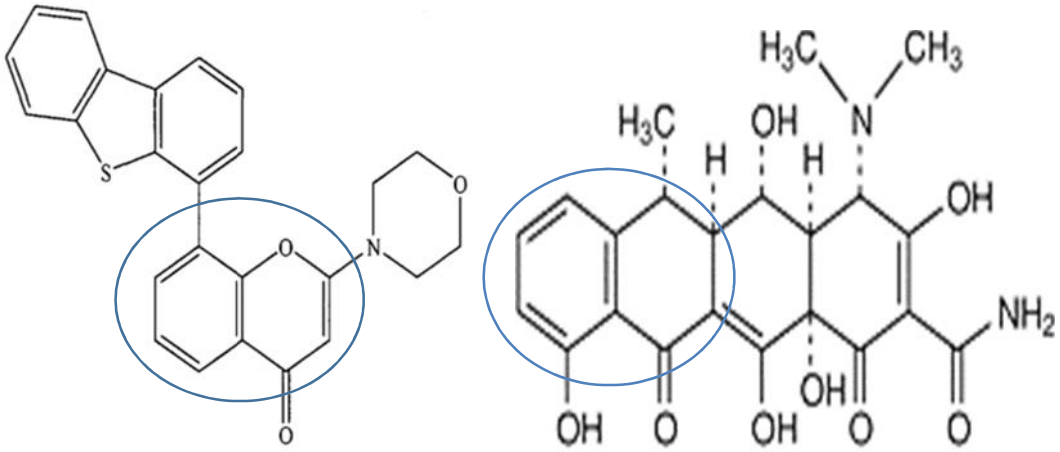

Figure (3): Chemical structure and comparison between doxycycline and a conserved chemical entity within DNA-PK inhibitors such as “Nu7441”. Doxycycline is a reduced carba-analogue of other DNA-PK inhibitors.