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E7010: INVESTIGATIONAL ANTICANCER AGENTS TARGETING THE MICROTUBULES

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ABSTRACT: E7010 (*N*-[2-[(4-hydroxyphenyl)amino] – 3 pyridinyl] – 4 - methoxybenzenesulfonamide), an orally active sulfonamide antitumor agent that is currently in a Phase I clinical trial, showed rather consistent growth-inhibitory activities against a panel of 26 human tumor cell lines (IC_{50} = 0.06–0.8 µg/ml), in contrast to vincristine (VCR; IC_{50} = 0.0002–0.04 µg/ml), 5-fluorouracil (IC_{50} = 0.2–30 µg/ml), Adriamycin (IC_{50} = 0.002–0.7 µg/ml), 1-β-D-arabinofuranoxylcytosine (IC_{50} = 0.005 to >30 µg/ml), camptothecin (IC_{50} = 0.002–0.4 µg/ml), and cisplatin (IC_{50} = 0.5–20 µg/ml). It caused a dose-dependent increase in the percentage of mitotic cells in parallel with a decrease in cell proliferation, like VCR. It also showed a dose-dependent inhibition of tubulin polymerization, which correlated well with the cell growth-inhibitory activity. ^{14}C-labeled E7010 bound to purified tubulin, and this binding was inhibited by colchicine but not by VCR. However, its binding properties were different from those of colchicine, as well as those of VCR. E7010 was active against two kinds of VCR-resistant P388 cell lines, one of which showed multidrug resistance due to the over expression of P-glycoprotein (resistant to Taxol), and the other did not show multidrug resistance (sensitive to Taxol). Furthermore, four E7010-resistant P388 cell lines showed no cross-resistance to VCR, a different pattern of resistance to podophyllotoxin, and collateral sensitivity to Taxol. Therefore, E7010 is a novel tubulin-binding agent that has a wider antitumor spectrum than VCR and has different properties from those of VCR or Taxol

INTRODUCTION: [1] Cancer is a class of diseases or disorders characterized by uncontrolled/ abnormal division of cells and the ability of these to spread, either by direct growth into adjacent tissue, or by implantation into distant sites by metastasis, in which cancer cells are transported through the bloodstream or lymphatic system. The abnormal cellular division of this is not subject to normal growth controls. It is one of the most dreadful diseases due to incurable affliction that insidiously attacks people of all cultures and ages.

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While, cancer is an ancient disease, the inability to cure cancer has persisted despite impressive advances in scientific knowledge and medical techniques.

Treatment of Cancer: [1]

Cancer can be treated by surgery, chemotherapy radiation, immunotherapy, monoclonal antibody therapy or other methods. The choice of therapy depends upon the location and grade of the tumor and the stage of the disease, as well as the general state of the patient. A number of experimental cancer treatments are also under development. Complete removal of the cancer without damage to the rest of the body is the goal of treatment.

Surgery:

In theory, cancers can be cured if entirely removed by surgery, but this is not always possible. When the cancer has metastasized to other parts in the

body prior to surgery, complete surgical excision is usually impossible. Examples of surgical procedures for cancer include mastectomy for breast cancer and prostatectomy for prostate cancer.

Monoclonal Antibody Therapy:
Immunotherapy is the use of immune mechanisms against tumors. These are used in various forms of cancer, such as breast cancer (trastuzumab/Herceptin) and leukemia (gemtuzumab ozogamicin/ Mylotarg). When a monoclonal antibody attaches to a cancer cell, it can make the cancer cell more visible to the immune system. The immune system attacks foreign invaders in your body, but it doesn't always recognize cancer cells as enemies. A monoclonal antibody can be directed to attach to certain parts of a cancer cell. In this way, the antibody marks the cancer cell and makes it easier for the immune system to find.

Immunotherapy:
Other, more contemporary methods for generating non-specific immune response against tumors include intravesical Bacille Calmette-Guerin (BCG) immunotherapy for superficial bladder cancer, and use of interferon and interleukin. Vaccines to generate non-specific immune responses are the subject of intensive research for a number of tumors, notably malignant melanoma and renal cell carcinoma.

Radiation Therapy:
Radiation therapy also called as radiotherapy, X-ray therapy, or irradiation is the use of ionizing radiation to kill cancer cells and shrink tumors. Radiation therapy can be administered externally via external beam radiotherapy (EBRT) or internally via brachytherapy. Radiation therapy injures or destroys cells in the area being treated by damaging their genetic material, and enables to grow and divide.

Hormonal Suppression:
The growth of some cancers can be inhibited by providing or blocking certain hormones. Common examples of hormone-sensitive tumors include certain types of breast and prostate cancers. Removing or blocking estrogen or testosterone is often an important additional treatment.

Chemotherapy:
Chemotherapy is the treatment of cancer with drugs that can destroy cancer cells by impeding their growth and reproduction. The first drug used for cancer chemotherapy was not originally intended for that purpose. Mustard gas was used as a chemical warfare agent during World War I and was studied further during World War II. During a military operation in World War II, a group of people were accidentally exposed to mustard gas and were later found to have very low white blood cell (WBC) counts. It was reasoned that an agent that damaged the rapidly growing WBC might have a similar effect on cancer.

Therefore, in the 1940s, several patients with advanced lymphomas were given the drug by vein, rather than by breathing the irritating gas. Their improvement, although temporary, was remarkable. That experience led researchers to look for other substances that might have similar effects against cancer. As a result, many other drugs have been developed to treat cancer, and drug development since then has exploded into a multibillion dollar industry. The targeted-therapy revolution has arrived, but the principles and limitations of chemotherapy discovered by the early researchers still apply.

Cancer treatment will be entirely based on person’s unique situation. Certain types of cancer respond very differently to a various types of treatment, so determining the type of cancer is a vital step toward knowing which treatments will be the most effective. The cancer's stage will also determine the best course of treatment, since early-stage cancers respond to different therapies than later-stage ones. Person’s overall health, lifestyle, and personal preferences will also play a part in deciding which treatment options will be best. Although chemotherapeutic drugs attack reproducing cells, they cannot differentiate between reproducing cells of normal tissues and cancer cells. The damage to normal cells can result in side effects.

These cells usually repair themselves after chemotherapy. Several exciting uses of chemotherapy hold more promise for curing or controlling cancer. New drugs, new combinations of chemotherapy drugs and new delivery

techniques are the expected advances in the coming years for curing or controlling cancer and improving the quality of life for people with cancer. Chemotherapeutic drugs are divided into several categories based on how they affect specific chemical substances within the cancer cells, which cellular activities or processes the drug interferes with, and which specific phases of the cell cycle the drug affects. These include DNA intercalating agents, DNA topoisomerase I and II inhibitors, carbonic anhydrase (CA) inhibitors, CDK inhibitors, tubulin polymerization inhibitors, antimitotic agents, antimetabolites, and miscellaneous agents.

Types of Chemotherapy Drugs: [2]

Chemotherapy drugs are divided into several groups based on how they affect specific chemical substances within cancer cells, which cellular activities or processes the drug interferes with, and which specific phases of the cell cycle the drug affects.

Alkylating Agents:

These agents directly damage DNA to prevent the cancer cell from reproducing. As a class of drugs, these agents are not phase-specific and in other words, they work in all phases of the cell cycle.

Nitrosoureas:

Nitrosoureas act in a similar way to alkylating agents. They interfere with enzymes that help copy and repair DNA. They too, are not phase specific. Unlike many other drugs, these agents are able to travel from the blood to the brain, so they are often used to treat brain tumors.

Antimetabolites:

These are a class of drugs that interfere with DNA and RNA growth. These agents damage cells during the S phase and are commonly used to treat leukemias, tumors of the breast, ovary, and the gastrointestinal tract, as well as other cancers.

Anthracyclines and Related Drugs:

Anthracyclines are antitumor antibiotics that interfere with enzymes involved in DNA replication. These agents work in all phases of the cell cycle. Thus, they are widely used for a variety of cancers.

Topoisomerase Inhibtors:

These drugs interfere with enzymes called topoisomerases, which are important in accurate DNA replication. They are used to treat certain leukemias, as well as lung, ovarian, gastrointestinal, and other cancers.

Mitotic Inhibitors:

Mitotic inhibitors are plant alkaloids and other compounds derived from natural products. They can stop mitosis or inhibit enzymes from making proteins needed for reproduction of the cell. These work primarily during the M phase of the cell cycle but can cause cellular damage in all phases.

Corticosteroid Hormones:

Steroids are natural hormones and hormone-like drugs that are useful in treating some types of cancer such as lymphoma, leukemias, and multiple myeloma as well as other illnesses. When these drugs are used to kill cancer cells or slow their growth, they are considered as chemotherapy drugs.

Sulfonamides as Anticancer Agents:

Sulfonamides constitute an important class of compounds that exhibit a broad spectrum of biological activities like antibacterial, antitumor, diuretic, hypoglycemic, etc. Also, some structurally novel sulfonamide derivatives have recently been reported to show substantial antitumor activity *in vitro* or *in vivo*. E7010 I, ER-34410 II and E7070 (Indisulam) III are examples for antitumor sulfonamides in advanced clinical trials.

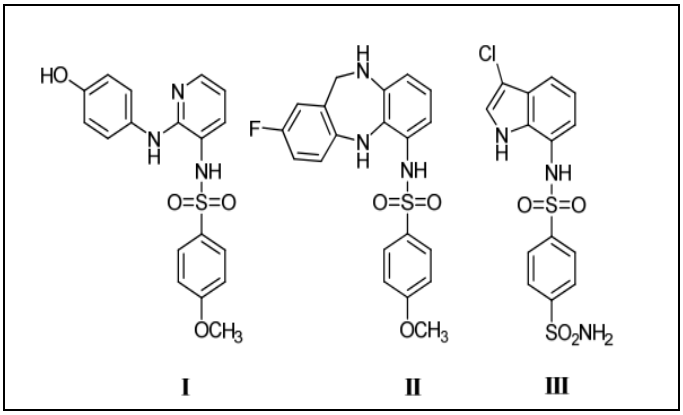

FIG 1: STRUCTURES OF NOVEL SULFONAMIDE DERIVATIVES

There are a variety of mechanisms describing the antitumor action of sulfonamides, such as carbonic

anhydrase (CA) inhibition, cell cycle arrest in the G1 phase, disruption of microtubules and angiogenesis (matrix metalloproteinase, MMP) inhibition. The most prominent mechanism was the inhibition of carbonic anhydrase isozymes (CAs) where as E7010 inhibit microtubule polymerization.

Sulfonamides as Carbonic Anhydrase Inhibitors: [3]

Carbonic Anhydrases:

All the CAs is able to catalyze the hydration of CO_2 to bicarbonate at physiological pH. This chemical interconversion is crucial since bicarbonate is the substrate for several carboxylation steps in a number of fundamental metabolic pathways such as gluconeogenesis, biosynthesis of several amino acids, lipogenesis, ureagenesis and pyrimidine synthesis. Apart from these biosynthetic reactions, some of the CAs are involved in many physiological processes related to respiration and transport of CO_2 /bicarbonate between metabolizing tissues and the lungs, pH homeostasis and electrolyte secretion in a variety of tissues/organs.

Role of Carbonic Anhydrases in Cancer:

The importance of this family of enzymes for the uptake of bicarbonate by many organisms, and the presence of a large number of isoforms (which can be distinguished from each other in activity and are located in different areas inside the cell) make the CAs undoubtedly involved in cell growth. Furthermore, at least three CA isozymes (CA IX, CA XII and CA XIV) have close connections with tumors. The role of CAs in cancer can be explained in light of the metabolic processes required by growing cancer cells that develop with a higher rate of replication than normal cells. Such a circumstance requires a high flux of bicarbonate into the cell in order to provide substrate for the synthesis of either nutritionally essential components (nucleotides) or cell structural components (membrane lipids).

The Action of Sulfonamides as Anticancer Agents through CA Inhibition:

Sulfonamides CA inhibitors reduce the provision of bicarbonate for the synthesis of nucleotides (mediated by carbamoyl phosphate synthetase II) and other cell components such as membrane lipids (mediated by pyruvate carboxylase). Such mechanism would likely involve CA II and CA V. An alternative, or additional mechanism, may involve the acidification of intracellular milieu as a consequence of CA inhibition by these potent CA inhibitors. It is also possible that the sulfonamides interfere with the activity of the CA isozymes known to be present predominantly in tumor cells, CA IX, XII and XIV. A combination of these mechanisms proposed above is also possible.

Sulfonamides Targeting G1 Phase of Cell Cycle:

G1 phase of the cell cycle is an important period where various complex signals interact to decide a cell's fate: proliferation, quiescence, differentiation, or apoptosis. It is now well-recognized that malfunctioning of cell cycle control in G1 phase is among the most critical molecular bases for tumorigenesis and tumor progression. Thus, there is a growing possibility that a small molecule targeting the control machinery in G1 phase can be a new type of drug efficacious against refractory clinical cancers. E7070 III was found to block the entry of human NSCLC A549 cells into the S phase, leading to the accumulation of cells in the late G1 phase. In addition, treatment of A549 cells with E7070 resulted in the inhibition of pRb phosphorylation, a crucial step in the G1/S transition. Down regulation of CDK2 and cyclin A expressions as well as suppression of CDK2 catalytic activity with the induction of p53 and p21, may account for the inhibition of pRb phosphorylation by E7070.

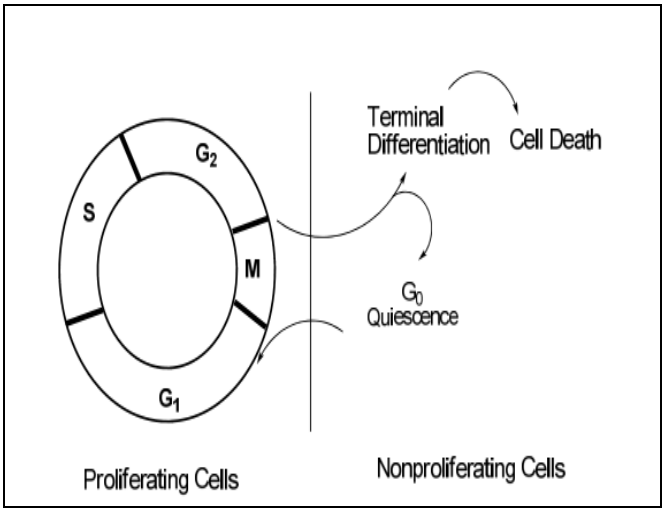

FIG 2: CELL CYCLE SULFONAMIDES CAUSING DISRUPTION OF MICROTUBULES: [4]

In addition, E7070 was shown to inhibit the phosphorylation of CDK2 itself, leading to the inhibition of CDK2 catalytic activity. These results suggest that E7070 may target the late G1 phase of the cell cycle and restore the pRb- dependent growth-inhibitory pathway disrupted in human NSCLC cells. This is accomplished by inhibiting CDK2 catalytic activity.

The effects of E7010 on microtubule structure in colon 38 cells were examined, and E7010 was shown to cause the disappearance of cytoplasmic microtubules and mitotic spindles. The experiments clearly demonstrated that the growth-inhibitory activity of E7010 is caused by the inhibition of microtubule assembly. A novel series of 7-aroylaminoindoline-1-benzenesulfonamides have been identified by Chang et al. as a novel class of highly potent antitubulin agents. The SAR information of the 7-aminoindoline-substitution pattern revealed that the 7-amide bond formation in the indoline-1-sulfonamides contributed to a significant extent for maximal activity rather than the carbamate, carbonate, urea, alkyl, and sulfonamide linkers.

"E7010"- A Novel Sulfonamide:
Formula: $C_{18}H_{17}N_3O_4S$

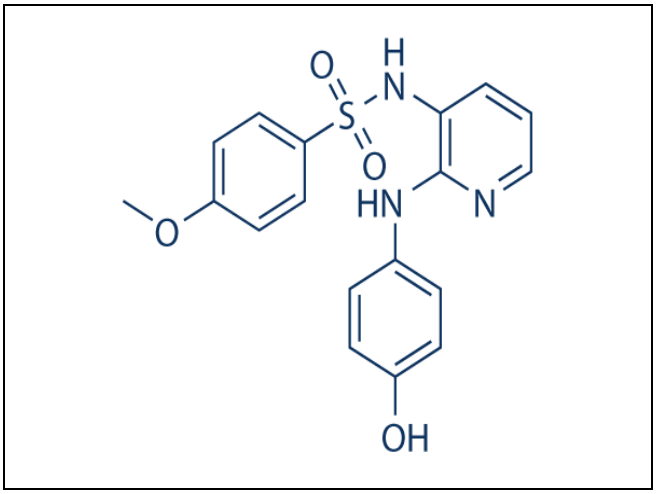

FIG 3: STRUCTURE OF E7010 (ABT-571)

Tubulin inhibitors are drugs that interfere directly with the tubulin system, which is in contrast to those drugs acting on DNA for cancer chemotherapy. Microtubules play an important role in eukaryotic cells. The addition of tubulin ligands can affect microtubule stability and function, including mitosis, cell motion and intracellular organelle transport. Tubulin binding molecules have generated significant interest after the introduction of the taxanes into clinical oncology and the general use of the vinca alkaloids. These compounds inhibit cell mitosis by binding to the protein tubulin in the mitotic spindle and preventing polymerization or depolymerization into the microtubules. This mode of action is also shared with another natural agent called colchicine. Microtubules are the key components of the cytoskeleton of eukaryotic cells and has an important role in various cellular functions such as intracellular migration and transport, cell shape maintenance, polarity, cell signaling and mitosis.[3] It plays a critical role in cell division by involving in the movement and attachment of the chromosomes during various stages of mitosis. Therefore microtubule dynamics is an important target for the developing anti-cancer drugs.[1]

Structure:
Microtubules are composed of two globular protein subunits, α- and β – tubulin. These two subunits combine to form an α, β-heterodimer which then assembles in a filamentous tube-shaped structure. The tubulin hetero dimers arrange themselves in a head to tail manner with the α-subunit of one dimer come in contact with the β- subunit of the other. This arrangement results in the formation of long protein fibres called protofilaments.

These protofilaments form the backbone of the hollow, cylindrical microtubule which is about 25 nanometers in diameter and varies from 200 nanometers to 25 micrometers in length. About 12-13 protofilaments arrange themselves in parallel to form a C-shaped protein sheet, which then curls around to give a pipe-like structure called the microtubule. The α-tubulin end has negative (-) charges while the β-tubulin end has positive (+) charges.[3] The microtubule grows from discrete assembly sites in the cells called Microtubule organizing centers (MTOCs), which are a network of microtubule associated proteins (MAP)[4 5].

Two molecules of energy rich guanosine triphosphate (GTP) are also important components

of the microtubule structure. One molecule of GTP is tightly bound to the α-tubulin and is non-exchangeable whereas the other GTP molecule is bound to β-tubulin and can be easily exchanged with guanosine diphosphate (GDP). The stability of the microtubule will depend on whether the β-end is occupied by GTP or GDP. A microtubule having a GTP molecule at the β-end will be stable and continue to grow whereas a microtubule having a GDP molecule at the β-end will be unstable and will depolymerise rapidly.[4 5]

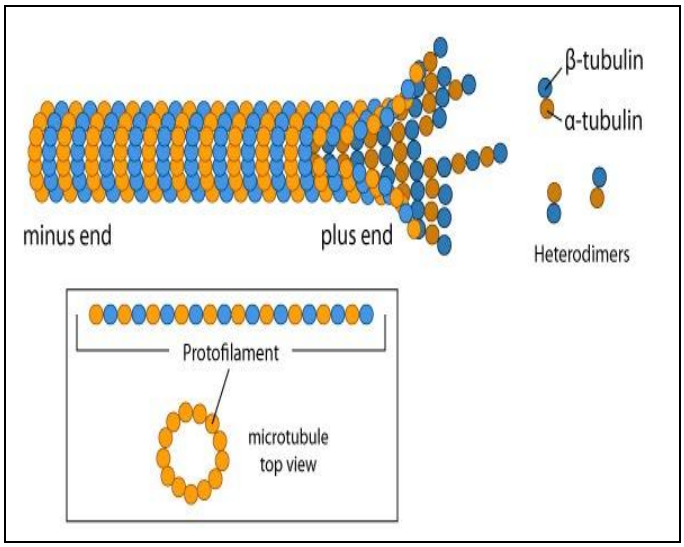

FIG 4: FORMATION OF MICROTUBULE

Limitation:

Although all of the anti microtubule agents effectively inhibit microtubule dynamics *in vitro*, their effects against different types of cancers vary *in vivo*.

Microtubule Dynamics:

Microtubules are not static but they are highly dynamic polymers and exhibit two kinds of dynamic behaviours: 'dynamic instability' and 'treadmilling'. Dynamic instability is a process in which the microtubule ends switches between periods of growth and shortening. The two ends are not equal, the α-tubulin ringed (-)end is less dynamic while the more dynamic β-tubulin ringed (+) end grows and shortens more rapidly. Microtubule undergoes long periods of slow lengthening, brief periods of rapid shortening and also a pause in which there is neither growth nor shortening. The other dynamic behaviour called tread milling is the net growth of the microtubule at one end and the net shortening at the other end. It involves the intrinsic flow of tubulin sub-units from the plus end to the minus end. Both the dynamic behaviors are important and a particular microtubule may exhibit primarily dynamic instability, treadmilling or a mixture of both.[6, 7]

Mechanism of Action:

Agents which act as inhibitors of tubulin, also act as inhibitors of cell division. Microtubule exists in a continuous dynamic state of growing and shortening by reversible association and dissociation of α/β-tubulin heterodimers at both the ends. This dynamic behavior and resulting control over the length of the microtubule is vital to the proper functioning of the mitotic spindle in mitosis i.e., cell division.

Tubulin inhibitors thus act by interfering with the dynamics of the microtubule, i.e., growing (polymerization) and shortening (depolymerization). One class of inhibitors operates by inhibiting polymerization of tubulin to form microtubules and are called polymerization inhibitors like the colchicine analogues and the vinca alkaloids. They decrease the microtubule polymer mass in the cells at high concentration and act as microtubule-destabilizing agents. The other class of inhibitors operates by inhibiting the depolymerization of polymerized tubulin and increases the microtubule polymer mass in the cells. They act as microtubule-stabilizing agents and are called depolymerization inhibitors like the paclitaxel analogues.[3] These two classes of drugs seem to operate by slightly different mechanism.

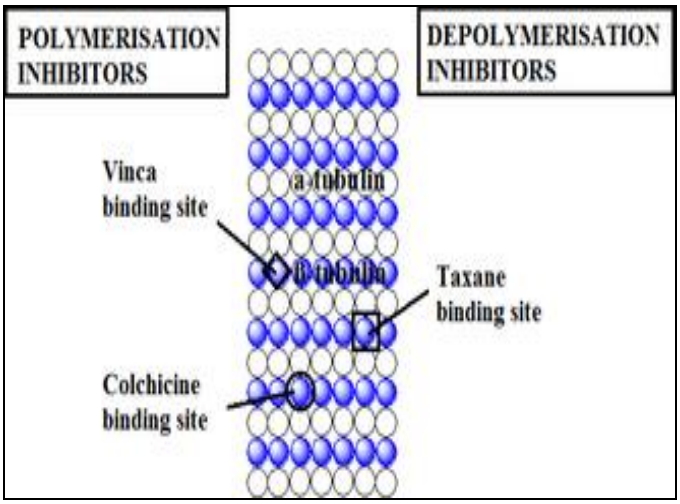

FIG 5: TUBULIN INHIBITORS BINDING SITES

Colchicine analogues blocks cell division by disrupting the microtubule. It has been reported that the β-subunit of tubulin is involved in colchicine binding. It binds to the soluble tubulin to form colchicine-tubulin complex. This complex along with the normal tubulins then undergoes polymerization to form the microtubule. However the presence of this T-C complex prevents further polymerization of the microtubule. This complex brings about a conformational change which blocks the tubulin dimers from further addition and thereby prevents the growth of the microtubule. As the T-C complex slows down the addition of new dimers, the microtubule disassembles due to structural imbalance or instability during the metaphase of mitosis.[11]

The Vinca alkaloids bind to the β-subunit of tubulin dimers at a distinct region called the Vinca-binding domain. They bind to tubulin rapidly, and this binding is reversible and independent of temperature (between 0 °C and 37 °C). In contrast to colchicine, vinca alkaloids bind to the microtubule directly. They do not first form a complex with the soluble tubulin or do they copolymerize to form the microtubule, however they are capable of bringing about a conformational change in tubulin in connection with tubulin self-association.[6] Vinca alkaloids bind to the tubulin with high affinity at the microtubule ends but with low affinity at the tubulin sites present along the sides of the microtubule cylinder. The binding of these drugs at the high affinity sites results in strong kinetic suppression of tubulin exchange even at low drug concentration while their binding to the low affinity sites in relatively high drug concentration depolymerizes microtubules.[1]

In contrast to colchine and vinca alkaloids, paclitaxel enhances microtubule polymerization promoting both the nucleation and elongation phases of the polymerization reaction, and it reduces the critical tubulin sub-unit concentration (i.e., soluble tubulin concentration at steady- state). Microtubules polymerized in presence of paclitaxel are extremely stable.[1] The binding mechanism of the paclitaxel mimics that of the GTP nucleotide along with some important differences. GTP binds at one end of the tubulin dimer keeping contact with the next dimer along each of the protofilament while the paclitaxel binds to one side of β-tubulin keeping contact with the next protofilament. GTP binds to unassembled tubulin dimers whereas paclitaxel binding sites are located only in assembled tubulin. The hydrolysis of GTP permits the disassembly and the regulation of the microtubule system; however, the activation of tubulin by paclitaxel results in permanent stabilization of the microtubule. Thus the suppression of microtubule dynamics was described to be the main cause of the inhibition of cell division and of tumor cell death in paclitaxel treated cells.[12]

A number of sulfonamides have also been screened particularly for their antitumor activity, which led to the discovery of a novel sulfonamide N-[2-[(4-hydroxyphenyl)amino]-3-pyridinyl] – 4 - methoxy benzenesulfonamide (E7010), which inhibits tubulin polymerization. This compound causes cell cycle arrest and apoptosis in M phase and is shown to exhibit microtubule assembly owing to its reversible binding to the colchicine binding site on tubulin. It is quite soluble in water as an acid salt. This compound exhibits good *In vivo* antitumour activity against several rodent tumor and human tumor xenografts12 and presently undergoing the stages of clinical trials. N-Phenyl nicotinamides are known as apoptosis inducers which blocks the cell cycle in G2/M phase and structure–activity relationship (SAR) studies indicate that the 3-pyridyl group is crucial for their activity.

Methoxybenzene-sulfonamide showed good results against a wide range of tumor cells including vinca alkaloid resistant solid tumors. Results from animal studies indicated activity against colorectal, breast and lung cancer tissues. In recent years, several molecules structurally distinct from colchicines have been crystallized in the colchicine binding site. E7010 is a sulfonamide that binds to colchicine binding site on tubulin subunit leading to cell cycle arrest in G2/M phase. It is an orally bioavailable tubulin binding agent presently under phase II clinical trials. [14]

Structure Activity Relationship (SAR):

Colchicine is one of the oldest known antimitotic drugs and in the past years, much research has been done in order to isolate or develop compounds

having similar structure but high activity and less toxicity. This resulted in the discovery of a number of colchicine analogues. The structure of colchicine is made up of three rings, a trimethoxy benzene ring (ring A), a methoxy tropone ring (ring C) and a seven-membered ring (ring B) with an acetamido group located at its C-7 position.

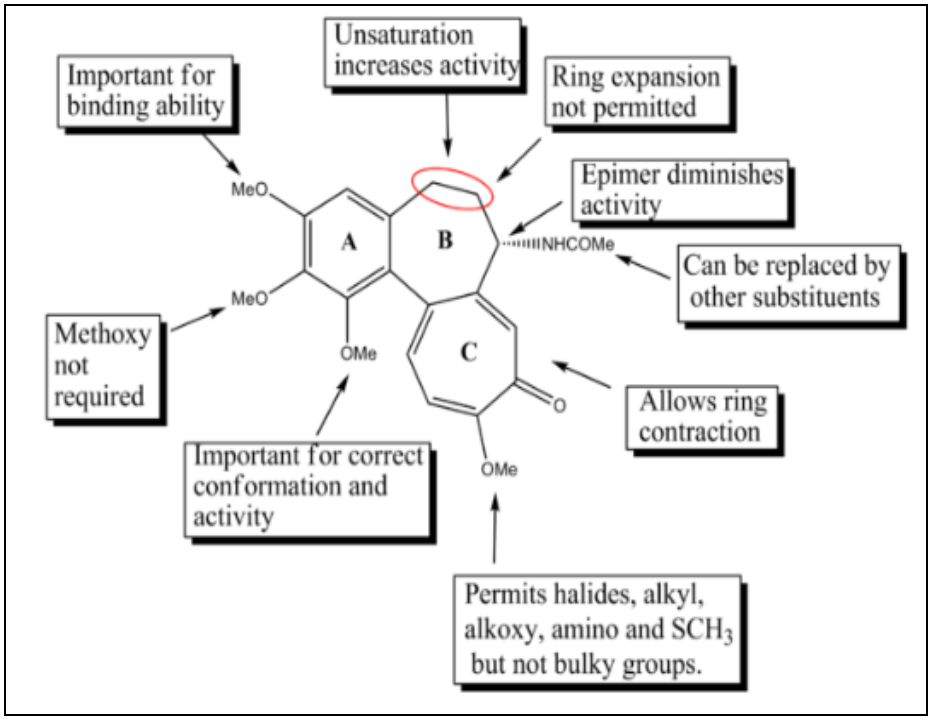

FIG. 6: SAR OF COLCHICINE ANALOGUES

The trimethoxy phenyl group of colchicine not only helps in stabilizing the tubulin- colchicine complex but is also important for antitubulin activity in conjunction with the ring C. The 3-methoxy group increased the binding ability whereas the 1-methoxy group helped in attaining the correct conformation of the molecule. The stability of the tropone ring and the position of the methoxy and carbonyl group are crucial for the binding ability of the compound. The 10-methoxy group can be replaced with halogen, alkyl, alkoxy or amino groups without affecting tubulin binding affinity, while bulky substituents reduce the activity. Ring B when expanded showed reduced activity, however the ring and its C-7 side chain is thought to affect the conformation of the colchine analogues rather than their tubulin binding ability. Substitution at C-5 resulted in loss of activity whereas attachment of annulated heterocyclic ring systems to ring B resulted in highly potent compound.[11]

The advent of high-throughput screening systems has allowed evaluating a large number of small molecules in parallel and automated fashions. In response to this screening innovation, one of the greatest concerns in recent drug discovery programs has been directed toward how to design and prepare compound libraries for getting "hits" in various biological assays. In this regard, historical reviews of drug discovery often give practical lessons.

One highly informative example is represented by the sequential development of sulfonamide therapeutics such as antibiotic sulfa drugs, insulin-releasing hypoglycemic agents, carbonic anhydrase-inhibitory diuretics, high-ceiling diuretics, and antihypertensive drugs.

These diverse pharmacological effects were serendipitously found through the serial derivatization of a single chemical structure of sulfanilamide, indicating that the sulfonamide moiety is a crucial functionality capable of interacting with multiple cellular targets. Therefore, it has seriously considered that novel anticancer chemotherapeutics might be discovered from the sulfonamide class. In fact, the drug discovery

efforts using sulfonamide-focused libraries have resulted in the finding of E7010 and E7070 as anticancer drug candidates.

E7010 was shown to reversibly bind to the colchicine site of β-tubulin, thereby halting mitosis. The compound exhibited good *in vivo* efficacy against rodent tumors and human tumor xenografts. As an active antimitotic agent, E7010 demonstrated objective responses in 2 of 16 patients in the single-dose study of Phase I trials; spinal cord metastasis was reduced by 74% in a patient with uterine sarcoma, and a minor response was observed in a pulmonary adenocarcinoma patient. In contrast to E7010, E7070 was found to block cell cycle progression of P388 murine leukemia cells in the G1 phase, accompanied by a decrease in the S-phase fraction.

Although its precise mode of action has not yet been determined, E7070 appears to be considerably different from conventional anticancer drugs in clinical use with respect to its cell cycle effect and its tumor type selectivity. Furthermore, preclinical animal tests established the promising efficacy of E7070 against human tumor xenografts. Thus, the compound has progressed to clinical evaluation in collaboration with the European Organization for Research and Treatment of Cancer.

In the Phase I setting, one patient with a uterine adenocarcinoma experienced a partial response with a >50% shrinkage of measurable tumors after i.v administration on the weekly schedule. Another partial response was reported in a patient with breast cancer on the daily × 5 schedule. Phase II studies of E7070 are currently ongoing in Europe and the United States.

On the basis of the significant observations described above, decided to further examine E7010, E7070, and some other antitumor sulfonamides from these two classes of cell cycle inhibitors with special interest in their effects on gene expression. Array-based hybridization technology has enabled monitoring of the mRNA levels of thousands of genes simultaneously, displaying the patterns of transcriptional changes under various conditions. In particular, global gene expression analysis with a biologically active small molecule represents the chemical genomic approach to understanding complex cellular processes relevant to drug activity.

Discovery Pathway of E7010:

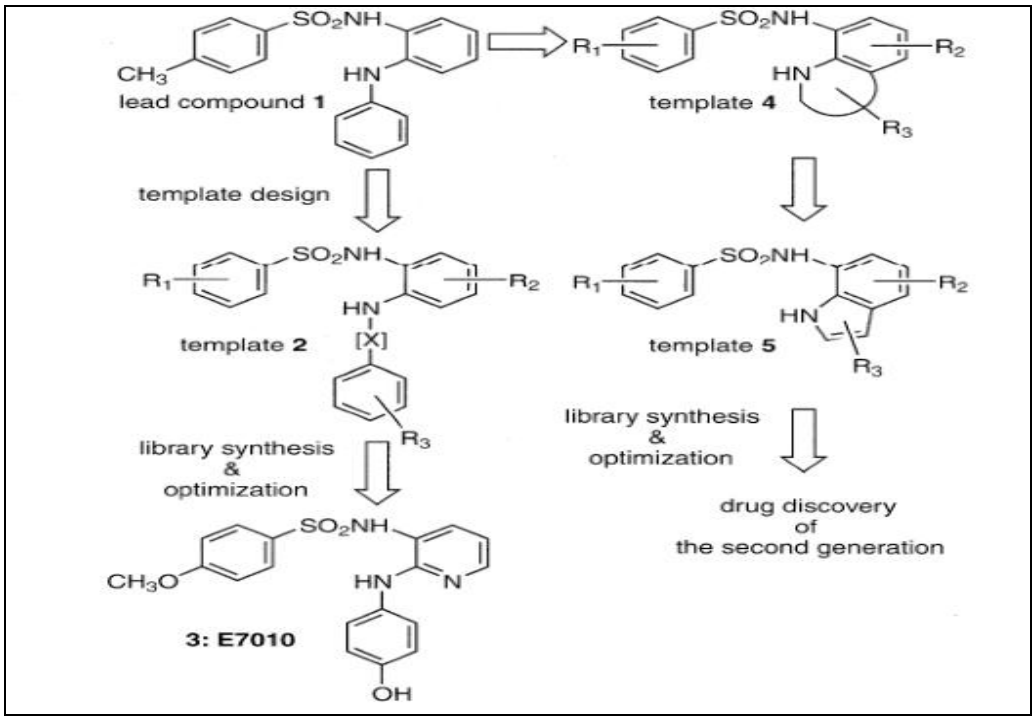

FIG 7: FLOW CHART OF DRUG DISCOVERY OF E7010

Current Clinical Aspects of E7010:

Clinical Trial 1:

In vivo tumor growth inhibition produced by a novel sulfonamide, E7010 against rodent and human tumors, American association for cancer research, nozomu koyanagi, takeshi nagasu, fumiko fujita, et.al., april 1, 1994, (54), pg.no:1702. When administered orally, E7010 showed good antitumor activity against various rodent tumors and human tumor xenografts. In tests on mouse tumor, E7010, administered in doses of 25–100 mg/kg daily for 8 days, inhibited the growth of colon 38 carcinoma inoculated s.c. in mice by 60–99%. E7010 was active against s.c. inoculated M5076 fibrosarcoma (75% tumor growth inhibition), s.c. inoculated Lewis lung carcinoma (84% increase in life span), and i.p. inoculated P388 leukemia (118% increase in life span).

In a test on rat tumor, E7010 inhibited the growth of SST-2 mammary carcinoma inoculated s.c. in rats by 84%. In tests on s.c. inoculated human tumor xenografts, E7010, when administered orally, showed a broad spectrum of activity. E7010 inhibited the growth of: four kinds of gastric cancer, H-81, H-111, SC-2, and SC-6 by 60–78%; three kinds of colon cancer, H-143, COLO320DM, and WiDr by 58–83%; three kinds of lung cancer, LC-376, LC-6, and LX-1 by 63–82%; and two kinds of breast cancer, H-31 and MX-1 by 79–87%. In studies on drug-resistant P388 leukemia, E7010 was effective against vincristine-resistant P388, cisplatin-resistant P388, and 5-fluorouracil-resistant P388 sublines in mice. Because of its good activity against rodent tumors and human tumor xenografts, E7010 is currently undergoing Phase I clinical trials.

Clinical Trial 2:

Phase I study of E7010, kaichiro yamamoto, kiichiro noda, et. al., cancer chemotherapy and pharmacology, june 1998, (42), 127-134.

E7010 is a novel sulfonamide which was discovered using slow-growing colon 38 carcinoma cells as a screening model. The objective of this phase I study was to determine the maximum allowable dose (MAD), toxicity, and pharmacokinetics of single or 5-day repeated doses of E7010. In the single-dose study, E7010 was administered orally to 16 patients at doses ranging from 80 to 480 mg/m2. The dose-limiting toxicity was peripheral neuropathy at a dose of 480 mg/m2. Hematological and gastrointestinal toxicities were mild. In the 5-day repeated-dose study, 41 patients were given E7010 at doses ranging from 30 to 240 mg/m2 per day. The dose-limiting toxicities were peripheral neuropathy and intestinal paralysis. Gastrointestinal toxicity was dose-dependent but not severe. Hematological toxicity was not dose-dependent. Pharmacokinetic analysis in the single-dose study showed a rapid increase in the plasma levels of the drug after administration, followed by disappearance with a t1/2 of 4.4-16.6 h. The variation in area under the plasma concentration-time curve (AUC) between the patients was small and increased in a dose-dependent manner. Total drug recovery in urine 72 h after administration was 77.8+/-11.4%, indicating that E7010 has favorable absorption and elimination profiles. The changes in the plasma levels of E7010 on day 5 in the 5-day repeated-dose study were almost the same as those on day 1, indicating that the drug did not accumulate.

In the single-dose study, spinal cord metastasis exhibited a 74% reduction in a patient with uterine sarcoma and a minor response (MR) was observed in a pulmonary adenocarcinoma patient. In the 5-day repeated-dose study decreases in the tumor markers carcinoembryonic antigen (CEA) and squamous cell carcinoma antigen (SCC) were observed in a patient with stomach cancer and in a patient with recurrent uterine cervical carcinoma, respectively.

The recommended phase II doses are 320 mg/m2 for a single-dose study and 200 mg/m2 per day for a 5-day repeated-dose study. Since the activity of E7010 is time-dependent, i.e. a certain concentration of E7010 is required for more than 12 h to suppress the growth of P388 leukemia cells, it is recommended that subsequent phase I/II studies be conducted using a divided dose schedule in order to maintain the blood level of E7010.

Clinical Trial 3:

Preferential Binding of E7010 to Murine β β3-Tubulin and Decreased β β3-Tubulin in E7010 resistant Cell Lines, Yasuo Iwamoto, Kazuto

Nishio, Hisao Fukumoto, et.al., Jpn. J. Cancer Res. 89, 954–962, September 1998.

N-[2-[(4-Hydroxyphenyl)amino] - 3 - pyridyl] - 4-methoxybenzenesulfonamide (E7010) is a novel sulfonamide antimitotic agent, which is active against mouse and human tumors. E7010 binds to β-tubulin and inhibits polymerization of microtubules. In order to clarify the mechanisms of E7010 resistance, two murine leukemic P388 subclones resistant to E7010, 0.5r-D and 4.0r-M, were characterized. The two clones showed approximately 10- and 100-fold resistance to E7010-induced growth-inhibitory effects, respectively, compared with the parental cells in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

These cell lines showed no cross-resistance to other anticancer agents such as taxanes, vinca alkaloids, mitomycin C, cisplatin and irinotecan hydro-chloride (CPT-11). Increased α α- and β β-tubulin protein and mRNA levels were observed in 0.5r-D and 4.0r-M cells as compared with the parental cells. We examined the isotype-specific expression of β β-tubulin in these E7010-resistant cells by a competitive reverse transcription-polymerase chain reaction method.

Although a 50% increase in β β5 isotype mRNA levels was observed in 4.0r-M cells, the levels of β β3 isotype message in the two resistant clones were approximately 50% less than the parental cells. To elucidate the binding properties of E7010 with β β-tubulin isotypes, we prepared isotype specific fusion proteins of β-tubulins. Direct photoaffinity labeling of the isotype specific fusion proteins with [14C] E7010 revealed that E7010 preferentially binds to the β β3 isotype rather than β β2, β β4, and β β5 isotype proteins. Therefore, altered expression of β β-tubulin isotypes, especially β β3 isotype, to which E7010 binds with high affinity, may account for the decreased sensitivity of these resistant clones to E7010.

Clinical Trial 4:

Effect of E7010 on liver metastasis and life span of syngeneic C57BL/6 mice bearing orthotopically transplanted murine Colon 38 tumor, Yasuhiro Funahashi, Nozomu Koyanagi, Kyosuke Kitoh, et.al., Cancer Chemotherapy and Pharmacology, 47(2):179-84.

E7010 is an orally active sulfonamide antitumor agent showing good activity against various subcutaneously inoculated rodent tumors and human tumor xenografts. The purpose of this study was to evaluate the effect of E7010 on liver metastasis and life span of mice bearing orthotopically transplanted murine Colon 38 tumor. Orthotopic transplantation of murine Colon 38 tumor as intact tissue yielded hepatic metastasis with a high incidence in about 1 month in C57BL/6 mice, and the mice died in about 2 months with cachexia.

In this model, the maximum tolerated dose of E7010 (100 mg/kg per day) was administered orally on various schedules, including for 14 days or daily until death, starting at 14 days after transplantation, or for 8 days from 21 days after transplantation. E7010 showed tumor growth inhibition (T/C=40%) at the orthotopic site similar to that at the subcutaneous site (T/C = 32%) when administered from 14 days after transplantation. When E7010 was started from 21 days after transplantation, it significantly decreased the number of hepatic metastases (control 17.1+/-20.8, E7010 2.6+/-5.3), although inhibition of tumor growth at the orthotopic site was only moderate (T/C=60%).

The administration of E7010 until death produced a significant increase in life span (control 49.8+/-8.9 days, E7010 62.5+/-6.1 days). Although the tumor weight of the E7010-treated group on the day of death was similar to that of the untreated group (control 1.166+/-0.507 g, E7010 1.211+/-0.632 g), there were significantly fewer liver metastases in the E7010-treated group (control 41.3+/-31.1, E7010 2.0+/-2.0). E7010 suppressed tumor growth at both primary and metastatic sites and increased life span in an orthotopic transplantation model of murine Colon 38 tumor in syngeneic C57BL/6 mice. Hepatic metastasis was inhibited more effectively than the growth of the primary tumor.

Clinical Trial 5:

Microtubule-targeted antitumor drugs: chemistry, mechanisms and nanoparticle formulations, Teni

Boulikas, Ioannis Tsogas, Gene Ther Mol Biol, Vol 12, 343-387, 2008.

ABT-751 is an agent that targets the colchicine site of tubulin. It is the one of the approach is to enhance the activity of currently available agents by targeting detoxification and resistance pathways. ABT-751 is a novel oral antimitotic agent that binds to the colchicine site on tubulin and inhibits polymerization of microtubules. Disruption of new microtubules leads to arrest in the cell division cycle and induction of apoptosis. There is currently no colchicine-site agents approved for cancer chemotherapy. ABT-751 has demonstrated anti-tumor activity against a variety of syngeneic and human xenograft tumor models including colon, lung, stomach, breast, and nasopharyngeal cancer models, and is also active *in vivo* against vincristine-, cisplatin- and 5-fluorouracil-resistant cells. ABT-751 is not a multiple drug resistance (MDR) substrate.

A phase I trial of orally administered ABT-751 given daily x 7 every 3 weeks was instituted to determine the dose limiting toxicity (DLT), maximum tolerated dose (MTD) and pharmacokinetics. A total of 15 patients have been studied to date. Doses of 200 and 250 mg pc qd x 7 were found to be tolerable. DLT was seen in 2/6 patients (neuropathy and ileus) at the qd dose of 300 mg/d. One of 3 patients studied to date at the 125 mg pc bid x 7d dose developed a supraventricular arrhythmia. This occurred 17 days after ABT dosing and is thought unlikely to be drug related. Grade 2 toxicities noted to date include constipation, fatigue, nausea, vomiting, myalgias, and anemia. Myelosuppression has not been noted. Pharmacokinetic studies were performed on day 7 of therapy.

DISCUSSION: E70l0 was found by screening a number of sulfonamides with widely different chemical structures and shows a broad spectrum of activity against mouse and human tumors. In a Phase I study of E70l0 using a single-dose schedule, some responses were observed and as demonstrated that E7010 is an antimitotic agent, and the target of its antiproliferative action is tubulin. Most of the tumor cell lines tested was almost equally sensitive to E7010. This is consistent with the idea that the target of E7010 is tubulin, because microtubules in the mitotic spindle are important for cell proliferation of all cells. The patterns of antiproliferative activity against tumor cell lines except HCT-l5 and DLD-l were similar for VCR and E7010, although the ranges of IC50 values were different.

To examine the mechanism of action of E70l0, first examine the effect on cell cycle progression, because this is related to the mechanism of action. Antimetabolites, such as MTX and Ara-C, cause cells to accumulate in the S phase. Topoisomerase inhibitors, VP16 and CPT, ADM, and MMC inhibit cell cycle progression in the 02 phase. Vinca alkaloids and Taxol arrest cells in the M phase. By flow cytometric analysis, it found that E70l0 accumulated P388 cells in G2-M phase. E70l0 caused dose-dependent increases in the mitotic index in parallel with an inhibition of cell proliferation, like VCR.

The effects of E70l0 on microtubule structure in colon 38 cells were examined, and E7010 was shown to cause the disappearance of cytoplasmic microtubules and mitotic spindles. Therefore, the effects of E7010 and E70l0 derivatives on microtubule assembly in a cell-free system were examined and compared with the growth-inhibitory activities. These experiments clearly demonstrated that the growth-inhibitory activity of E70l0 is caused by the inhibition of rnicrotubule assembly. Later it proved that E7010 binds to purified tubulin and that the binding site is the CLC site, not the Vinca site, although the binding properties of E7010 to tubulin were quite different from those of CLC. Therefore, E7010 is quite distinct in its action from clinically used Vinca alkaloids or Taxol.

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CONCLUSION: E7010 was found to possess a wide spectrum of antitumor activity and is also found to be effective against certain multidrug and vincristine resistant cell lines. A close analysis of the structure of E7010 binding to colchicine binding site reveals that its pyridine and methoxy groups superimpose with A and C rings of the colchicine, respectively, while the sulphonamide bridge overlaps with the B ring. Interestingly, it binds much deeply than colchicine there by we conclude that E7010 is a potential microtubule inhibitor when compared to colchicine.

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