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Microtubules and Tubulins as Target for Some Natural Anticancer Agents Extracted from Marines,Bacteruim, and Fungus

Newshan Behrangi[1], Mehrdad Hashemi[1]*, Hojat Borna[1] and Alireza Akbarzadeh[1]

[1] Department of Genetics,Islamic Azad University
Tehran Medical Branch, Tehran, Iran

*Correspondence: Mehrdad Hashemi,Department of Genetics
Islamic Azad University ,Tehran Medical Branch, Tehran, Iran
mhashemi@iautmu.ac.ir

Abstract. Microtubules are key components of cytoskeleton and they play role in maintaining cell structure ,providing platforms for intracellular transportation,forming the spindle during mitosis ,signaling cells , in cell division and mitosis.Their dynamic structures and their role in mitosis candidate them an efficient target for anticancer development. Plants ,marine organisms and microorganisms are consisted of substances which have anticancer properties.In present study ,we evaluated natural anticancer agents which are extracted from marines,fungus,and bacterium in order to define their main targets by PASS software.According to our results, *Epothilone, Okadaic acid* , *Ixabepilone, Dictyostatin, Bryostatin, Peloruside A,*and *Saliniketal* are as microtubule stabilizer agents and it is demonstrated *Peloruside A, Epothilone* ,and *Bryostatin* with PASS scores 0.973, 0.948,and 0.913 respectively are as the strongest microtubule stabilizer molecules. In addition , *Pironetin, Discodermolide,*and *Rhizoxin* are as tubulin antagonists agents.Therefore, *Pironetin* with score 0.806 releaved the highest anticancer effects via binding to tubulins among other three molecules.It is intereseting that *Bryostatin, Peloruside A,*and *Rhizoxin* exhibited high cytotoxic activity.

Keywords:Microtubules,anticancer,PASS software,Cytotoxic

INTRODUCTION

Cancer is a crucial public health problem in both developed and developing countries and it represents the largest cause of mortlity in the world .Cancer is a condition in which cells divide without control and results in impairing organisms.Cancer chemotharpy is a vital alternative method for treating successfully some types of solid tumors, lymphomas ,and leukemia.Anticancer drugs ,which are applied to chemotherapy, can be divided into synthetic and natural drugs .Plants ,marine organisms and microorganisms are consisted of substances which have anticancer properties ;therefore, they are used widely in cancer chemotherapy.Chemotherapeutic drugs based on their mode of action can be subdivided into distinct groups: (i)drugs that interfere with DNA synthesis , (ii) Drugs that introduce DNA damage and (iii) drugs that inhibit the function of the mitotic spindle. Several anticancer agents act through the microtubules, they exert their anticancer influence by causing disorganized stabilization of microtubules in area away from the centriol , or causing destabilization of mitiotic spindle in order to interfere with mitosis and subsequently results in inducing tumor cell death.Microtubules are a component of the cytoskeleton and they play role in maintaining cell structure , providing platforms for intracellular transportation, forming the spindle during mitosis as well as other cellular processes.

In this study ,we consider 10 natural anticancer molecules which are extracted from various fungus ,bacteria ,marine sponge and target microtubles ,including: *Epothilone* (metabolites produced by the *myxobacterium Sorangium cellulosum)*, *Okadiac acid* (isolated from the marine sponge *Halichondria okadai*),*Ixabepilone* (produced by *Sorangium cellulosum)*, *Dictyostatin*(isolated from the *marine sponge Spongin)*, *Bryostatin* (isolated from extracts of a species of *bryozoan, Bugula neritina)* , *Peloruside A* (isolated from the *marine sponge Mycale hentschel)i*,and *Saliniketal* (isolated from *marine actinomycete Salinispora arenicola)*.Some of them exert their effects through targeting tubulins ,such as: *Pironetin*(isolated from *Streptomyces Discodermolide*),and *Rhizoxin* (biosynthesised by *Burkholderia rhizoxinica)*.We evaluated their anticancer properties by bioinformatic software ,then ranked them on basis of their anticancer strength .

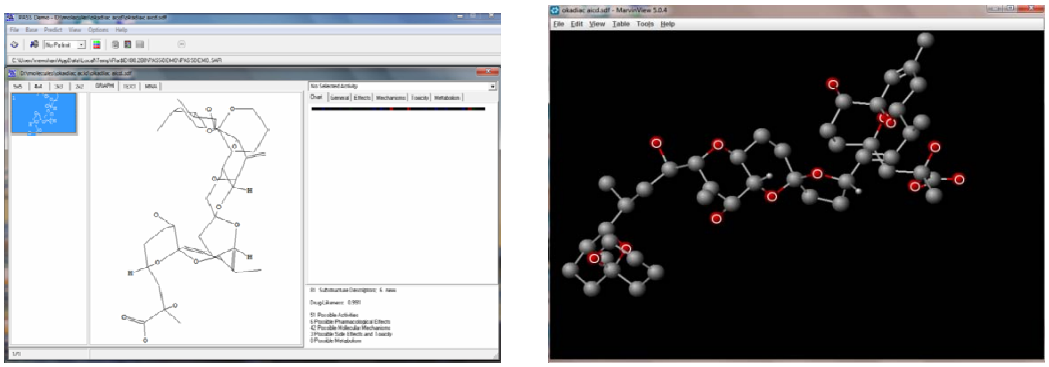
MATERIAL AND METHOD

Data.A paractical database is the main step in bioinformatics projects.Collection of data from Pubmed database were accomplished with general keyword “anticancer”.Most data were gathered from 2010 papers;therefore,anticancer

molecules were extracted from this papers ,and defined their targets in apoptotic pathway.In this case molecules were classified based on their origins ,as a result we had 7 groups of anticancer molecule such as molecules in Drug Bank, plants, fruits,microorganisms,semisythetic agents,synthetic agents and finaly ungrouped anticancer agents which their origins were unknown.

Structure. Structural formula of these molecules were investigated from Chemspider,Pubcheme and Wikipedia ,respectively , and the orginal molecular structure of all compounds were found,their skeletal structures drawn with Chemschetch , Chemaxon,version 5.4 software in order to reach 3D structures of molecules within MDL SD file , the same software is used with molecular mechanics algorithm for structural optimization .ChemAxon is a leader in providing Java based chemical software development platform for biotechnology and pharmaceutical industries.Protein Data Bank (PDB), Tripos MOL2, MDL MOL and SD file formats were saved, too (Fig.1b)

Docking. All molecules were predicted for their possible bioactivities with PASS, V.poroikov et al, version 1.917, software.PASS (Predicition of Activity Spectra for Substance) is a simple computational tool that can predict more than 1500 pharmacological effects,molecular mechanisms of action,and toxicities on basis of structural descriptors of compounds.The top molecules with more than 0.6 score anticancer activity were selected and categorized on basis of their targets .



a) b)

Figure .1 : a) Platform and analysis of a molecule for its anticancer activity with PASS software ,b) 3D structure of *Okadaic acid* with ChemAxon within MDL SD file

RESULTS

In this study about 90 anticancer molecules originated from marine sponge, bacteria, and fungus were distilled from Pubmed papers, and their molecular structures were evaluated by PASS software in order to highlight their main targets in cancer pathway.Our results releaved that 10 molecules with PASS score more than 0.6 can target microtubules in the cell efficiently.Therefore ,these molecules by stabilizing microtubules,and inhibiting microtubule formation act as promising anticancer drugs.

Table .1 illustares anticancer molecules which target microtubules and tubulins in order to exert their anticancer exert in the cell.As can be seen from Figure. 2 *Epothilone, Okadaic acid , Ixabepilone , Dictyostatin, Bryostatin , Peloruside A,* and *Saliniketal* act through stabilizing microtubules.Therefore, *Peloruside A* , *Epothilone* ,and *Bryostatin* with scores 0.973, 0.948,and 0.913 resepectively are as potent microtubule stabilizer among other ones.It is nothiceworthy that *Epothilone* is also as an antimitotic and microtubule formation inhibitor agent, so it exerts potent anticancer effect on the cancerous cells .Morever, *Discodermolide* ,*Pironetin* ,and *Rhizoxin* bind to tubulins directely ,and *Pironetin* with score 0.806 is the strongest agent among tubulin antagonists

TABLE .1

PASS Prediction of anticancer molecules

	MOLECULES	PROPERTIES	DRUG LIKENESS
1	Epothilone	Microtubule stabilizator 0.948 Antimitotic 0.706 Microtubule formation inhibitor 0.611	0.991
2	Okadaic acid	Microtubule stabilizator 0.661	0.991
3	Ixabepilone	Microtubule stabilizator 0.765	0.991
4	Dictyostatin	Microtubule stabilizator 0.784	0.994
5	Bryostatin	Microtubule stabilizator 0.913 Cytotoxic 0.665	0.994

6	Peloruside A	Microtubule stabilizator 0.973 Cytotoxic 0.713	0.995
7	Saliniketal	Microtubule stabilizator 0.746	0.995
8	Discodermolide	Beta tubulin antagonist 0.698	0.995
9	Pironetin	Tubulin antagonist 0.806	0.993
10	Rhizoxin	Tubulin antagonist 0.608 Cytotoxic 0.737	0.994

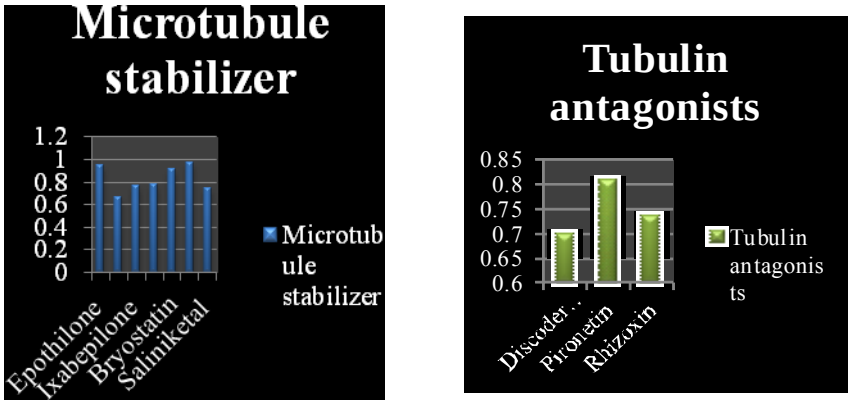


Figure .2: Microtubule Stabilizer and Tubulin antagonists activity of natural anticancer molecules

Epothilon is a potent anticancer agent that acts through stabilizing microtules, inhibiting the formation of microtubules,and is as a antimitotic drug.
According to Table .1 *Bryostatin* , *Peloruside A*,and *Rhizoxin* act as tubulin antagonists as well as they are as cytotoxic agents.Therefore, *Peloruside A* with PASS score 0.973 is as the strongest tubulin antagonist and *Rhizoxin* with score

0.737 categorized as the anticancer agent which has the highest cytotoxic activity among other mentioned molecules.

DISCUSSION

Microtubules are key component of cytoskeleton which have cylindrical structure. They play role in maintaining cell structure ,providing platforms for intracellular transportation,forming the spindle during mitosis ,signaling cells , in cell division and mitosis .They are polymeres of α- and β-tubulin dimmers and are packed around a central core. An important feature of microtubules is polarity that during the end to end polymerization process, alpha subunit of one tubulin is attached to beta subunits of the others . Therefore, this leads to the formation of protofilament that one end will have the α subunit exposed while the other end will have the β subunit exposed. These ends are designated the (−) and (+) ends, respectively.The minus end is capped ,elongation occurs from the plus end .Polymerization and de-polymerization are complex and interesting process are regulated both spatially and temporally.
Microtubules play vital role in mitosis because they array in the cell through mitotic spindles.Mitotic spindles are formed by attachemt of GTP-tubulin to the growing end of the protofilaments.Highly dynamic microtubules in the spindles are required for all stages of mitosis .Firstly,for timely and precise attachment of chromosomes at their kinetochores to spindle during prometaphase after nuclear – envelope breakdown.Second,for complex movement of the chromosomes that bring them to their properly aligned positions at the metaphase .Last,for synchronous separation of the chromosomes in anaphase and telophase after the metaphase-anaphase checkpoint is complete.Stabilizing microtubules dynamic is as a efficacious sterategy that can block mitosis and results in killing tumor cells .

In our study we elucidate some natural microtubule stabilizer agents which are extracted from various sources such as marine,bacterium,fungus. *Epothilone* is a natural anticancer molecule which is produced by the *myxobacterium Sorangium cellulosum* ,and [12]reported that it has antifungal and cytotoxic activity as well as [11] calimed that *Epothilone* and its associated analoges are efficient in treatment of breast cancer. Our results illustrate these anticancer agent is as Microtubule stabilizator ,Antimitotic ,and Microtubule formation inhibitor .Therefore, it exerts strong effect on cancerous cells through three modes. As can be seen from Table 1, *Epothilone* with socre 0.948 is as a potent microtubule stabilizer in campare to its other properties.Other molecules such as *Okadiac acid* ,

Ixabepilone, Dictyostatin, Bryostatin ,Peloruside A, and *Saliniketals* are as microtubule stabilizer agents.Previous research indicated [13] *Peloruside A* binds to a unique site on the tubulin alpha,beta-heterodimer as well as *Bryostatin* [13] is as a Protein Kinase C modulator.It is interesting that our results demonstrated *Peloruside A, Epothilone* ,and *Bryostatin* with PASS scores 0.973, 0.948,and 0.913 respectively are as the strongest microtubule stabilizer molecules.Morever, *Peloruside A* and *Bryostatin* have cytotoxic activity ;thus,these molecules are as promising anticancer agents.Tubulin is as a member of globular protein . α- and β-tubulins are the most common members of tubulin family and they make up microtubules. Some anticancer drugs induce their anticancer effects through targeting tubulins,and they have gained much interest among cytotoxic agents due to its success in clinical oncology .In our study about three molecules are as tubulin antagonists, including: *Discodermolide, Pironetin* ,and *Rhizoxin.*.According to our results , *Pironetin* with score 0.806 releaved the highest anticancer effects via binding to tubulins.It is interesting that *Rhizoxin* represented high cytotoxic activity and it is the most potent cytotoxic agent in compare to *Bryostatin* ,*Peloruside A.* Previous research [15] reported *Rhizoxin* has broad activity against murine tumour models and is also active against vinca alkaloid-resistant cells .It is supposed that by applying in vitro and in vivo experiments ,some unknown properties of these molecules will be appeard and side effects of them will be compared with each other.

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