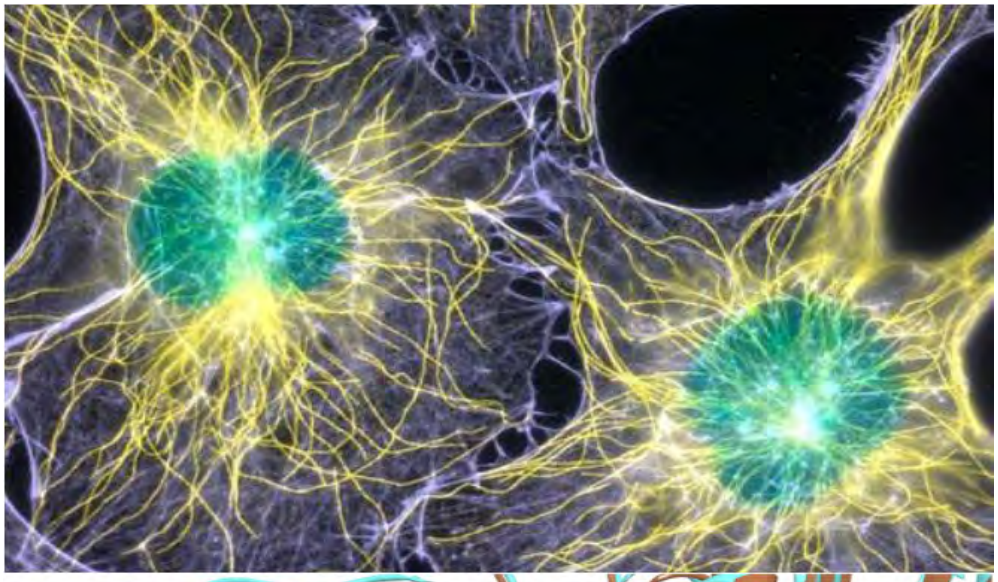
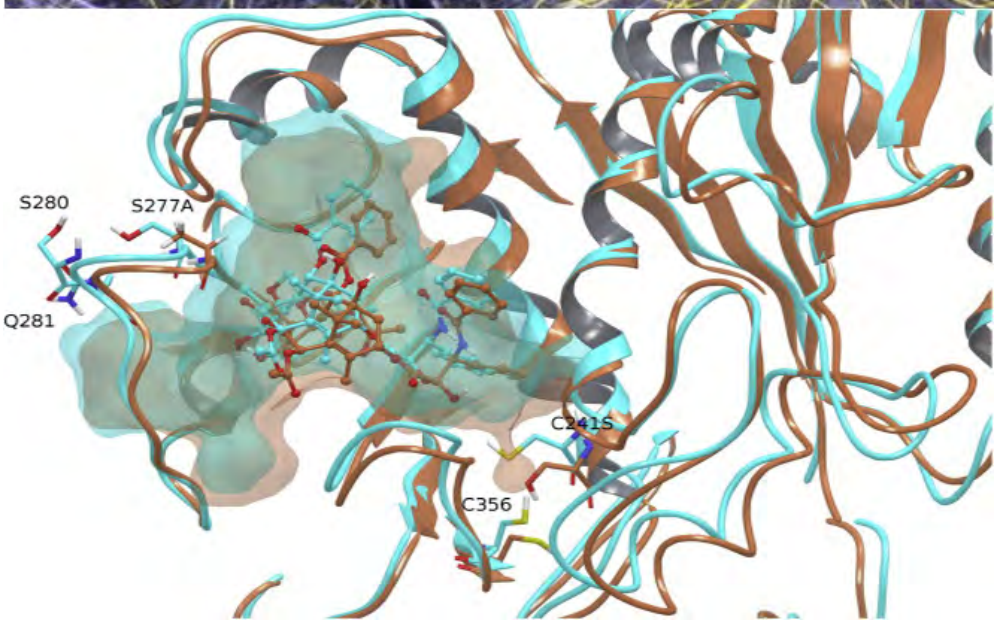
Institute of Materia Medica
Chinese Academy of Medical Sciences

The 4th Workshop on Chemistry and Biology of Microtubules and Microtubule-interacting Agents





Sept. 18~19, 2018
Beijing, China

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Invited Speech

José Fernando Díaz Pereira

Education

1993	PhD. in Biochemistry, Universidad Complutense de Madrid
1988	Ms Sc. in Chemistry, Universidad Complutense de Madrid

Professional Experience

Since 2008	Senior Scientist and Group Leader
1999-2008	Junior Scientist, Centro de Investigaciones Biológicas CSIC
1994-1999	Postdoc, Katholieke Universiteit Leuven

Research Interest

Tubulin pharmacology, microtubule and tubulin structure.

Major Hit

Description of the first covalent tubulin targeted agent, Discovery of three new binding sites in tubulin (Laulimalide, Maytansine and Triazolopirimidines).

Tubulin activity modulation by small molecules. A historical overview

José Fernando Díaz Pereira
(Centro de Investigaciones Biológicas, CSIC, Madrid, Spain)

Abstract Tubulin is an essential and constitutive GTPase of eukaryotic cells that function in a wide range of biological processes including chromosome segregation, cell structural support, intracellular transport, cell motility and angiogenesis. Due to this multiple functions many of known MT functions are crucial on tumour growth and vascularization, which makes tubulin a reference target for antitumoral drugs [1] able to modulate tubulin dynamics engaging the spindle checkpoint, arresting the cell cycle progression at mitosis and leading to cell death [2].

Antitubulin drugs were the second kind of antitumoural agents employed in clinics after antimetabolites Eli Lilly found that alkaloids of the Madagascar periwinkle (Vinca rosea), (which had a popular reputation in indigenous medicine in various parts of the world) originally discovered in a screen for anti-diabetic drugs, blocked proliferation of tumour cells [3]. The antitumour effect of the vinca alkaloids (e.g. vincristine) was later shown to be due to their interaction with microtubules, and therefore cell division[4]. Once tubulin was stablished as a bona fide antimumoural target other successful tubulin binding drugs has reached clinical use and some are in development, either as drugs (paclitaxel) or as drug-antibody conjugate (Maitansine, auristatin).

Keywords: Tubulin, antitumoural, drug-antibody conjugate

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Alexander Dömling

Education

Dr. Alexander Dömling was trained as an organic chemist in the world-famous laboratories of Prof Ivar Ugi (Technische Universität München, TUM) and Prof Barry Sharpless (Nobel Laureate, The Scripps Research Institute). Besides chemistry he also studies biology at the TUM.

Professional Experience

In his further professional stations in different biotech companies (he cofounded) he could apply and further extend his knowledge in early drug discovery. From research in his biotech companies emerged Almorexant (Phase III) and MCB3837 for the Potential Treatment of Clostridium Difficile Infections (CDI). Currently ongoing biotech companies work on his portfolio of small molecule PD1-PDL1 antagonists (SMIO BV) and arginase inhibitors for the treatment of asthma, COPD and allergic rhinitis (Carmolex Inc). Full professor at the University of Pittburgh (2007-2011) Full professor and chair of department of drug design at the University of Groningen (2011-2018).

Research Interest

Dr. Alexander Dömling technical interests include miniaturization and acceleration of chemistry and preclinical drug discovery by developing a game changing (semi) autonomous technology platform blending Instant Chemistry, nL dispensing, acoustic-MS, uHTS and artificial intelligence.

All his projects are very close to drug discovery and novel exciting molecular drug targets and he is feeling competent to deliver through his drug discovery approach novel advanced leads even for complicated targets such as transcription factors or protein protein interactions or targets form next generation sequencing. His dream is to succeed to bring novel therapeutics for unmet medical needs to the patients.

Tubulysin Totalsynthesis by MCR

Alexander Domling*, Thimmalapura M. Vishwanatha
(University Groningen, Department of Drug Design, Antonius Deusinglaan1, Postbus196, 9700ADGroningen, the Netherlands)

Abstract Tubulysins are very potent antimitotic natural products isolated by Höfle in 2000 (J. Antibiotics, 53: 879 (2000); G. Höfle et al.). To increase their therapeutic window antibody drug conjugates have been designed and evaluated. Several ADCs and other Tubulysin-based medicines are now under development. The natural products is produced by several myxobacterial species. However, the fermentation yields are currently insufficient for production of large amounts in API quantities and qualities. Therefore totalsynthesis is a workable alternative towards production of Tubulysin derivatives. Tubulysin is a ribosomally produced secondary metabolite which consist of a complex peptidic structure involving synthetically demanding chiral building blocks such as -amino acids and thiazole derivatives. Multiple total syntheses have been published recently which all suffer from lengthy, low yielding multiple sequential steps. Here we will first time disclose a very short high yielding total synthesis of a Tubulysin derivative using multicomponent reaction chemistry.

Keywords: Tubulysin, antimitotic, total synthesis, multicomponent reaction

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Susan Lynn Mooberry

Education

The Mooberry Laboratory has expertise in the discovery and mechanisms of action of diverse microtubule targeting agents. Dr. Mooberry received her B.S. degree in Biology from St. Lawrence University and then trained in pharmacology at the Medical University of South Carolina.

Professional Experience

Dr. Susan Lynn Mooberry conducted postdoctoral studies at the University of Hawaii Cancer Center where she moved into a faculty position. In Hawaii she collaborated with natural products chemists and identified several new microtubule targeting compounds, including the cryptophycins, laulimalide and the taccalonolides. She continues her drug discovery efforts for breast and pediatric cancers. She is currently Professor of Pharmacology at UT Health San Antonio. She is currently President of the American Society of Pharmacognosy.

Microtubule targeting drugs rapidly and differentially inhibit oncogenic signaling and this likely contributes to their anticancer efficacies

Susan L. Mooberry *, Nicholas F. Dybdal-Hargreaves, Roma Kaul, April L. Risinger
(Department of Pharmacology and Mays Cancer Center, UT Health San Antonio, 7703 Floyd Curl Drive, San Antonio, TX, 78229, USA.)

Abstract Microtubule targeting agents (MTAs), including the taxanes, vinca alkaloids, ixabepilone, and eribulin represent some of the most valuable anticancer drugs used to treat breast cancer. For decades, the clinical anticancer efficacy of these drugs was attributed strictly to their antimitotic activities and they were commonly referred to as "antimitotics." Building evidence of the non-mitotic effects of MTAs on oncogenic signaling pathways has prompted a reevaluation of their mechanisms of action.[1-5] Additionally, the fact that individual breast cancer patients can respond differently to individual MTAs prompted further evaluations into the distinct effects of these drugs on oncogenic pathways.

A hallmark of aggressive breast cancers is their ability to undergo epithelial-to-mesenchymal-transition (EMT), and this is thought to be a central event in oncogenesis. The loss of cortical E-cadherin is a central defining characteristic of EMT. We were interested in evaluating the initial, early effects of clinically relevant concentrations of MTAs on Src-dependent E-cadherin internalization in triple negative breast cancer cells. In HCC1937 cells, the MTAs rapidly promoted the cortical localization of E-cadherin and yet differences were noted among the MTAs [6].The most robust effects were noted with the microtubule destabilizing drugs, eribulin and vinorelbine. Further mechanistic studies showed that these same effects on cortical localization of E-cadherin were initiated by inhibition of Src kinase with dasatinib or by siRNA-mediated knockdown of the p130Cas (BCAR1) scaffold. Our studies showed that eribulin inhibits the interaction between Src and p130Cas resulting in decreased Src phosphorylation that precedes cortical E-cadherin accumulation [6]. Collectively, these results suggest that the microtubule network facilitates the internalization of E cadherin, a key event in EMT. This provides a direct connection between rapid microtubule disruption and EMT reversal initiated by eribulin. This is consistent with the ability of eribulin to reverse EMT in preclinical models of triple negative breast cancer within 7 days [7].

Another major pathway in EMT is the TGF-β pathway [8]. Ligand-stimulated TGF-β receptor engagement leads to the activation of downstream pathways that induce the expression of the Snail and Slug. These two proteins are transcriptional-repressors that promote EMT. We evaluated whether the disruption of microtubule dynamics and structure by MTAs could rapidly affect TGF-β-dependent induction of Snail and Slug; and if there are differences among the various drugs. The effects of TGF-β stimulation were first evaluated in BT-549 triple negative breast cancer cells that have an activated TGF-β pathway and have undergone EMT. The TGF-β – mediated induction of Snail and Slug were assessed and the results show that the microtubule destabilizers, eribulin and vinorelbine, as well as ixabepilone inhibit TGF-β induced expression of both Snail and Slug at the message and protein levels. Similar results were obtained with other TNBC cells lines. Transient siRNA-mediated knockdown studies demonstrated that the effects of MTAs on Snail expression are mediated by Smad2/3.

These results demonstrate the ability of diverse MTAs to rapidly and differentially alter interphase oncogenic

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signaling related to EMT. Identification of differences among MTAs might facilitate the rational selection of specific drugs depending on tumor characteristics. These studies highlight non-mitotic mechanisms of action of compounds and interesting differences among MTAs. These studies were funded by Eisai Inc.

Keywords: Microtubules, epithelial-to mesenchymal transition, TGF-β, eribulin, paclitaxel

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Carlo Ballatore

Education

1997-2000	PhD. in Medicinal Chemistry, Cardiff University, University of Wales,
1990-1995	Laurea. in Chemistry and Pharmaceutical Technologies, University of Rome

Professional Experience

Since 2016	Associate Professor, University of California San Diego
2013-2016	Research Associate Professor, University of Pennsylvania
2007-2013	Research Assistant Professor, University of Pennsylvania
2005-2007	Senior Research Investigator, University of Pennsylvania
2003- 2005	Scientist III, Acidophil, LLC/Dihedron Corp., San Diego, CA
2002- 2003	Research Scientist I, NewBiotics, Inc., San Diego, CA
2001-2002	Postdoc, Anderson Cancer Center, University of Texas

Research Interest

Alzheimer's disease and related neurodegenerative tauopathies with specific collaborative programs directed towards the discovery and development of tau aggregation inhibitors; microtubule-stabilizing agents; thromboxane A_2 receptor antagonists; and multi-targeted inhibitors of eicosanoid biosynthesis. In addition, the Ballatore laboratory is also actively involved in the investigation of basic, fundamental principles in medicinal chemistry, such as in the area of isosteric replacements.

Microtubule Stabilizing Agents as Possible Treatments for Alzheimer's Disease and Related Tauopathies

Carlo Ballatore [a,*], John Q. Trojanowski, [b] Virginia M.-Y. Lee, [b] Amos B. Smith, III, [c] Kurt R. Brunden [b]
([a] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0756; [b] Center for Neurodegenerative Disease Research, University of Pennsylvania, 3600 Spruce St, Philadelphia PA 19104; [c] Department of Chemistry, University of Pennsylvania, 231 S. 34th St., Philadelphia PA 19104)

Abstract A number of neurodegenerative tauopathies, which include Alzheimer's disease (AD) Pick's disease and frontotemporal dementia (FTD) with Parkinsonism linked to chromosome 17 (FTDP-17), are characterized by the presence of insoluble proteinaceous deposits comprised of hyperphosphorylated tau proteins. Tau is a microtubule (MT)-associated protein that under physiological conditions promotes MT-stabilization. In neurons, the MT-stabilizing function of tau is believed to play an important role in enabling and maintaining the complex intracellular transport machinery that allows proteins, trophic factors and other cellular constituents, to travel along the axons (axonal transport). Under pathological conditions typical of neurodegenerative tauopathies, tau becomes hyperphosphorylated, disengaged from MTs and ultimately sequestered into intraneuronal inclusions, commonly referred to as neurofibrillary tangles (NFTs). This abnormal disengagement of tau from MTs is in turn believed to trigger increased MT dynamicity, altered axonal transport, and axonal dystrophy that collectively contribute to neuronal dysfunction and death.

MT-stabilizing drugs may compensate for the loss of MT-stabilizing tau function in tauopathy neurons and, therefore, these molecules have been suggested as potential candidates to treat tau-mediated neurodegeneration. Several examples of MT-stabilizing natural products and derivatives thereof have been approved for cancer treatment; however, the use of these compounds for central nervous system (CNS) diseases may be challenging due to limited brain penetration and oral bioavailability, as well as potential systemic side-effects. Nonetheless, selected brain-penetrant MT-stabilizing natural products, including epothilone D (Figure 1), have been identified and found effective in animal models of neurodegenerative tauopathies. Moreover, among non-naturally occurring MT-stabilizing compounds, selected triazolopyrimidines (*e.g.*, 51657, Figure 1) have been identified that are characterized by generally favorable drug-like properties, including brain-penetration and oral bioavailability. Evaluation of 51657, in aged (9-month-old) PS19 tau transgenic mice that harbor NFT-like tau pathology in the CNS revealed that low doses (3 or 10 mg/Kg) twice-weekly for 3 months produce improvements in neuronal outcomes that resemble those previously observed upon treatment with MT-stabilizing natural products. In addition, mice treated with either dose of 51657 did not show signs of compound intolerance during treatment. Taken together, these results suggest that stabilization of axonal MTs may be a promising strategy to treat neurodegenerative tauopathies and that triazolopyrimidines hold promise as CNS-active MT-stabilizing candidate therapeutics.

Keywords: microtubules, axonal transport, Alzheimer's disease, tauopathies, microtubule-stabilizing compounds

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Karl-Heinz Altmann

Education

1986	PhD in organic chemistry, University of Basel
1983	Diploma in chemistry, Johannes-Gutenberg Universität Mainz

Professional Experience

From 1990 to 1996 he was a research scientist and group leader at Ciba-Geigy Central Research in Basel. In 1997 he moved to Oncology Research of Novartis Pharma, until in 2000 he was appointed the Novartis Senior Chemistry Expert. From January to July 2003 he was the acting Global Head of Chemistry of the Novartis Institutes for BioMedical Research. Research in the Altmann group is centered on the chemical synthesis of pharmaceutically relevant natural products natural product analogs and their biological evaluation, with a particular focus on leads for anticancer and antituberculosis drug discovery. In 2014 Professor Altmann was awarded the Paul Ehrlich Prize of the Société de Chimie Thérapeutique, France.

The chemisty and biology of zampanolide and dactylolide

Karl-Heinz Altmann*
(ETH Zürich, Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, HCI H405, Vladimir-Prelog-Weg 4, 8093 Zürich, Switzerland)

Abstract Zampanolide (1) is a marine NP that was first reported in 1996 by Tanaka and Higa[1] and shown to be a potent inhibitor of tumor cell proliferation *in vitro*. In 2009, it was re-extracted from the Togan sponge *Cacospongia mycofijiensis* by Northcote, Miller, and co-workers, who confirmed its antiproliferative activity and uncovered its microtubule-stabilizing and tubulin-polymerizing activity.[2] In 2001, the group of Riccio reported a macrolactone structurally related to zampanolide (1) that had been isolated from the sponge *Dactylospongia sp*.[3]

In contrast to 1, dactylolide (2) is only a moderately potent antiproliferative agent, with IC_{50}'s in the low μM range and the absolute configuration of 2 is opposite to the configuration of the macrolactone core in 1;[4] in fact, *ent*-2, whose configuration corresponds with that of the macrolactone core in zampanolide (1), has not been isolated from natural sources so far.[5]

Before this background, we have developed convergent total syntheses of 1 and 2, which have enabled a range of biochemical and structural studies. At the same time, the total synthesis work has established a platform for the synthesis of analogs for SAR studies. After a brief summary of the total synthesis work and the biological and structural studies with 1 and 2, this contribution will discuss the synthesis and biological activity of a series of zampanolide and dactylolide analogs that we have investigated over the last few year. In addition, a new method will be presented that has allowed to establish the C20 stereocenter in zampanolide and its analogs with high stereoselectivity.

Keywords: Dactylolide, microtubule stabilizer, natural product, zampanolide, SAR

References

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Jack Adam Tuszynski

Education

2013-2015	D.Sc. (habilitation) Faculty of Automatic Control, Electronics & Computer Science, Silesian University of Technology
1980-1983	Ph.D. Physics Department, The University of Calgary, Calgary, Canada.
1975-1980	M.Sc. with distinction，The A. Mickiewicz University of Poznan

Professional Experience

Since 2005	Allard Endowed Research Chair, Department of Experimental Oncology, Cross Cancer Institute.
Since 1993	Full Professor, Department of Physics, University of Alberta
1990-1993	Associate Professor, Department of Physics, University of Alberta
1988-1990	Associate Professor, Department of Physics, University of Alberta Field: theoretical condensed matter physics.
1983-1988	Department of Physics, Memorial University of Newfoundland Field: theoretical condensed matter physics.
1983-1983	Post-doctoral Fellow, Chemistry Department, The University of Calgary Field: Molecular biophysics.

Research Interest

His research interests are strongly linked to the protein tubulin and the microtubules assembled from it.

Computational Drug Design, Synthesis and Preclinical Testing of Colchicine Derivatives for Metastatic Bladder Cancer Using Tubulin as a Target

Jack Tuszynski
Allard Chair
Division of Experimental Oncology
Cross Cancer Institute and University of Alberta
Edmonton, Canada

Abstract The significance of microtubules as a molecular target for chemotherapeutic treatments has been known for decades. Tubulin, which makes up microtubules binds numerous small molecule ligands, which result in the alteration of microtubule dynamics leading to cell cycle arrest and cell death. Some of these ligands are currently used clinically for the treatment of several types of cancer and include the drugs paclitaxel and vinblastine. These drugs bind to several distinct binding sites within beta tubulin, which have been identified through electron crystallography. The drawback of these drugs is their indiscriminate binding to all cells leading to the death of both cancerous and healthy cells. Hence despite the overall success of the vinca alkaloid and taxane drug families side effects such as neurodegradation seriously impair the prognosis for many cancer patients treated with them. Moreover, in many cases drug resistance develops in the course of chemotherapy. We have focused on computational searches, optimization and testing new and re-purposing old molecules that interfere with the formation of mitotic spindles during cell division in tumors. To build the molecular models of our target tubulin, we used the program Modeller that uses alignment of the sequences with known related structures to obtain spatial restraints that the output structure must satisfy. Missing regions are predicted by simulated annealing of a molecular mechanics model. The existence and distribution of various tubulin isoforms is the basis for novel chemotherapeutic drug design that can differentiate between different cell types to reduce side effects. The quality of the resulting models for tubulin isoforms was investigated by an analysis of ten human beta tubulin isoforms regarding their differences within ligand binding sites. New promising colchicine derivatives have been designed and computationally tested for isoform specificity. The stabilities of these derivatives have been computationally evaluated using quantum mechanical methods. They have been synthesized and tested in vitro and in vivo. Testing of these compounds on a panel of tumour cell cultures has produced promising results for their ability to selectively target specific cancer cells. Mitotic abnormalities, such as an impaired spindle were also observed in the treated cells and almost all the cells were blocked in prometaphase. The cytotoxicity of the colchicine derivatives was further quantitated by utilizing clonogenic assays. We have also determined that the colchicine derivatives control the migration of vascular endothelial cells for additional therapeutic benefits. We have shown that a class of novel colchicine derivatives: (a) can inhibit migration in primary endothelial cells, (b) can selectively induce cytotoxicity in rapidly dividing cells, (c) in mouse models can cause anti-angiogenic effects. We will also report the results of in vivo studies of our lead compound in patient-derived xenograft mouse tests for efficacy in metastatic bladder cancer and in healthy rats for toxicity. The lead compound, CR42-24, is currently completing pre-clinical studies and is expected to be submitted for IND approval by FDA in a year.

Susan Bane Tuttle

Education

Susan Bane is a Professor in the Department of Chemistry and Director of the Biochemistry Program at Binghamton University, where she has been a faculty member since 1985. She received her BS degree in Chemistry at Davidson College and her Ph. D. in Biochemistry at Vanderbilt University Medical School. After postdoctoral studies in organic chemistry, she joined the faculty of Binghamton University as a bioorganic chemist. She received the Chancellor's Award for Excellence in Scholarship and Creative Activities in from the SUNY system in 2012.

Professional Experience

Susan has studied various aspects of tubulin biochemistry and antimicrotubule drugs since graduate school. Her research lies at the intersection of chemistry and biomedical science, and her recent interests include using organic chemistry to understand and to manipulate biological systems.

Tyrosination of tubulin with tyrosine derivatives

Susan Bane Tuttle *
(State University of New York, Department of Chemistry, 25 Murray Hill Road, Binghamton, USA)

Abstract modification of tyrosination/detyrosination. The genetically encoded C-terminal tyrosine is removed by a recently identified carboxypeptidase, and is reintroduced by another specific enzyme, tubulin tyrosine ligase (TTL). Disruption of this cycle has been implicated in pathologies such as cancer aggressiveness. Enzymatic tyrosination of α-tubulin is a unique posttranslational modification; thus, tubulin tyrosination is a potential tool for precise modification of the protein and a potential target for drug development. It has long been known that some structural variation in the amino acid substrate is tolerated by TTL. Our group demonstrated a substrate that can be used for *in vitro* and intracellular bioorthogonal labeling of tubulin that relies upon the functional versatility of TTL. The adaptability versatility and applications of this approach for site-specific labeling of tubulin will be presented. The advantages, limitations and future avenues of this work will be discussed.

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Carlos M. Galmarini

Education

Department at PharmaMar since 2008. Dr Galmarini received his MD from the University of Buenos Aires (Argentina). He completed an internship in Oncology at the "Hospital Municipal de Oncología María Curie" in Buenos Aires, and also different fellowships in hematology/oncology at the City of Hope National Cancer Center in Los Angeles and the "Centre León Bérard" and "Hôpital Lyon Sud" in Lyon (France).

Professional Experience

Dr Galmarini received as well his PhD and HDR from the Université Claude Bernard Lyon 1 (France) where he was appointed as an Associate Professor. Dr Galmarini is the author of more than 100 papers published in peer reviewed international journals, and of different chapters in books on cancer chemotherapy.

MI130004: A novel antibody-drug conjugate combining trastuzumab with PM050489, a marine derived tubulin-binding agent

Carlos M. Galmarini
(Cell biology and pharmacogenomics department Pharma Mar S.A. Avda. de los Reyes 1 28770)

Abstract In the search for novel payloads to design new antibody–drug conjugates (ADC), marine compounds represent an interesting opportunity given their unique chemical features. PM050489 is a marine compound that binds b-tubulin at a new site and disrupts the microtubule network, hence leading to mitotic aberrations and cell death. PM050489 has been conjugated to trastuzumab via Cys residues through a noncleavable linker, and the resulting ADC, named MI130004, has been studied. Analysis of MI130004 delivered data consistent with the presence of two molecules of PM050489 per antibody molecule, likely bound to both sides of the intermolecular disulfide bond connecting the antibody light and heavy chains. The antitumor activity of MI130004 was analyzed in vitro and in vivo in different cell lines of diverse tumor origin (breast, ovary, and gastric cancer) expressing different levels of HER2. MI130004 showed very high in vitro potency and good selectivity for tumor cells that overexpressed HER2. At the cellular level, MI130004 impaired tubulin polymerization, causing disorganization and disintegration of the microtubule network, which ultimately led to mitotic failure, mirroring the effect of its payload. Treatment withMI130004 in mice carrying histologically diverse tumors expressing HER2 induced a longlasting antitumor effect with statistically significant inhibition of tumor growth coupled with increases in median survival time compared with vehicle or trastuzumab. These results strongly suggest that MI130004 is endowed with remarkable anticancer activity and confirm the extraordinary potential of marine compounds for the design of new ADCs.

Norbert SEWALD

Education

1998	Habilitation, Organic Chemistry, Leipzig University
1991	Dr. rer. nat., Organic Chemistry, TU München
1988	Studies of Chemistry, TU München

Professional Experience

Since 1999	Full Professor for Organic and Bioorganic Chemistry, Bielefeld University
1991-1992	Postdoctoral fellow (funded by EU), Prof. J.E. Baldwin, Oxford University

Research Interest

Amino acid and peptide chemistry, Isolation of natural products, Total synthesis of peptide-based natural products and peptidomimetics, Biocatalytic halogenation, Molecular tools for the life sciences, Peptide-drug conjugates

Cytotoxic Peptide-Drug Conjugates Based on Cryptophycins

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Abstract Many anticancer chemotherapeutics interfere with microtubule dynamics and prevent microtubules from forming correct mitotic spindles, which causes cell-cycle arrest and apoptosis. Cryptophycins are a class of 16-membered highly cytotoxic macrocyclic depsipeptides isolated from cyanobacteria [1]. The biological activity is based on their ability to interact with tubulin. Strong antiproliferative activities with 100- to 1000-fold increased potency compared to paclitaxel and vinblastine have been observed. Cryptophycins are highly interesting drug candidates, since their biological activity is not negatively affected by P-glycoprotein, a drug efflux system commonly found in multidrug resistant cancer cell lines and solid tumors [2]. These characteristics made the synthetic analog cryptophycin-52 (LY355703) a promising candidate for cancer treatment. However, the clinical trials had to be discontinued because of neurotoxic side effects and lacking efficacy *in vivo* [3].

Experimental evidence suggests that cryptophycin non-covalently binds to tubulin. A conformational change of free tubulin dimers occurs upon binding of cryptophycin preventing the polymerization to microtubules. This even occurs when substoichiometric amounts of cryptophycin-1 are administered. The exact binding site of cryptophycins and the orientation of the drug inside the binding pocket are still unknown since no X-ray structure of a cryptophycin-tubulin complex has been published yet. The structurally diverse (depsi)peptides cryptophycin, dolastatin-10, hemiasterlin, and phomopsin A are supposed to have a common binding site [2].

We have developed efficient strategies for the synthesis of cryptophycins and their analogues [2] taking specific emphasis on the synthetically most challenging unit A [4]. In addition, new interesting functionalities have been introduced in different positions for SAR studies [2,4,5].

We additionally synthesized an analogue of cryptophycin-52 where the trans-amide bond between units B and C is replaced by a 1,4-disubstituted 1*H*-1,2,3-triazole to probe the quasi-isosterism of 1,4-disubstituted 1*H*-1,2,3-triazoles and trans-amide bonds. The cytotoxic activity is largely retained for this "clicktophycin", generated by a [3+2] "click" cycloaddition reaction. This proves the bio-equivalence of 1,4-disubstituted 1*H*-1,2,3-triazoles and trans-amide bonds even in complex compounds [6].

Selectivity issues may be tackled with in a directed therapy approach. The lack of an addressable functional group in cryptophycin-52 hampers the conjugation to a homing device. Structure-activity relationship (SAR) studies have been done aiming at the introduction of a new functional group for bioconjugation while maintaining the high biological activity of the parent compound [7,8].

Cryptophycin conjugates with peptides and antibodies have been developed for targeted delivery in tumor therapy. The conjugation of cryptophycin derivatives to monoclonal antibodies have provided antibody-drug conjugates (ADC) with high potency and remarkable selectivity [2,9].

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The cyclic RGD peptide Cilengitide, c-(-Arg-Gly-Asp-D-Phe-NMe-Val-), addresses integrins that are highly expressed on tumor cells. Cryptophycin-55 glycinate was conjugated to monomeric or tetrameric RGD peptides, c-(-Arg-Gly-Asp-D-Phe-Lys-), as homing devices targeting integrin $\alpha_v\beta_3$. The protease cleavable cleavage dipeptide site Val-Cit and the PABC self-immolative spacer were inserted between the drug and the tumor targeting ligand. The cytotoxicity of the conjugates was evaluated *in vitro* on M21, and M21-L human melanoma cells, showing increased antitumor activity and selectivity of the tetrameric conjugate against the $\alpha_v\beta_3$ positive M21 cell line. The metabolic plasma stability of the monomer conjugate and the lysosomal drug release have been investigated *in vitro*.

Keywords: tubulin binder, small molecule drug conjugate, SAR studies

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Andrea E. Prota

Education

Andrea E. Prota obtained his degree in pharmaceutical sciences and his Ph.D. in natural sciences at the ETH in Zürich in 1995 and 1999, respectively.

Professional Experience

In 2000 Dr. Prota joined the groups of Joyce Fingeroth and Thilo Stehle at the Beth Israel Deaconess Medical Center and the Massachusetts General Hospital of Harvard Medical School in Boston (USA). During this time, his work focused on the structural analysis of viral receptors and attachment proteins by X-ray crystallography. This research led to the structure determination of the reovirus attachment protein sigma1, the Epstein Barr virus receptor CD21 and the reovirus receptor hJAM1. In June 2002, he moved to the Paul Scherrer Institute in Switzerland, where he first worked as a postdoctoral fellow with Fritz Winkler on the structural analysis of eukaryotic DNA mismatch repair proteins. In 2004 he then was appointed senior scientist and established his own research group studying key cell surface receptors and ligands in tumor angiogenesis, with particular focus on VEGF-receptors, neuropilins and VEGFs. Since early 2010 he is a tenured principal investigator in the laboratory of Michel Steinmetz at the Paul Scherrer Institute where he focuses on the use of X-ray crystallography in combination with biochemical and biophysical methods to provide the molecular mechanism of protein interactions implied in the regulation of microtubule cytoskeleton.

Research Interest

His current research activity is devoted to the structural analysis of tubulin interactions with microtubules targeting agents.

Microtubule-targeting agents: Strategies to hijack the cytoskeleton

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Abstract Microtubule-targeting agents (MTAs) like paclitaxel and the vinca alkaloids belong to the most important medical weapons to combat cancer. MTAs interfere with intracellular transport, inhibit eukaryotic cell proliferation, and promote cell death by suppressing microtubule dynamics. Recent advances in the tubulin structural biology field have enabled the discovery and the extensive characterization of new ligand-binding sites on microtubules and their building block tubulin, and made it possible to investigate the molecular mechanisms of action of a wide range of different MTAs at the atomic level. Notably, until 2013 three distinct MTA-binding sites on tubulin, referred to as the taxane site, the colchicine site, and the vinca site, had been structurally characterized to high resolution. Since then three additional binding sites have been investigated by X-ray crystallography and cryo-electron microscopy (cryo-EM) in great detail; they are referred to as the maytansine site, the laulimalide/peloruside site, and the pironetin site.

My presentation will cover our current knowledge on the six known tubulin-binding modes of MTAs and a review of the molecular mechanisms of action of MTAs on tubulin and microtubules. I will start by highlighting and discussing the different molecular mechanisms by which MSAs may achieve their microtubule-stabilizing effect by binding to the taxane site, a pocket of β-tubulin located on the luminal side of microtubules. The differential occupation of the taxane site has been reported to either lead to the structuring of the otherwise disordered M-loop into a short helix, thereby promoting lateral tubulin contacts in microtubules, or to strengthen longitudinal tubulin contacts via an allosteric mechanism. Further, I will present how both the MSAs laulimalide and peloruside A may inhibit microtubule disassembly by acting as molecular 'clamps' that hold together protofilaments. Both agents target a common pocket on β-tubulin which is positioned near the lateral interface between protofilaments on the outer surface of the microtubule. A description will follow of how all the so far structurally characterized MDAs targeting the colchicine site cluster at the tubulin intradimer interface into two distinct zones, which either face the α-tubulin subunit or are buried deeper in the β-tubulin subunit. Microtubule formation is thereby mainly inhibited by preventing the curved-to-straight conformational change in tubulin. A further mechanism of microtubule destabilization is promoted by ligands targeting the vinca site. These agents bind at the inter-dimer interface between two longitudinally aligned tubulin dimers and either introduce a molecular 'wedge' at the tip of microtubules, which prevents the curved-to-straight transition of tubulin necessary for proper incorporation into microtubules, or sequester tubulin dimers into ring-like oligomers that are incompatible with the straight protofilament structure in microtubules. Two additional mechanisms of microtubule destabilization will be highlighted by presenting the molecular details of ligands binding to both the maytansine site and the pironetin site. Maytansine site binding agents directly block the formation of longitudinal tubulin contacts in microtubules either by inhibiting the addition of further tubulin dimers to the plus ends of growing microtubules, or by forming assembly incompetent tubulin–ligand complexes at high ligand concentrations. A similar effect is promoted by pironetin, which covalently binds to an extended hydrophobic pocket on α-tubulin and causes both a disorder of the T7 loop and a conformational perturbation in the N-terminal section of helix H8 of α-tubulin. These two secondary structural elements are known to establish key longitudinal tubulin contacts along protofilaments in microtubules.

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The final part of my talk will cover the description of dual inhibitors that interact with kinases and microtubules, a property that represents a challenge in developing target-specific drugs with improved safety profiles. Moreover, I will raise some outstanding questions related to MTA structural biology and discuss possible routes for the development of next-generation MTAs for future use in the clinic. I will conclude by presenting preliminary results on one of the possible routes for future investigations of this fascinating class of antimitotic agents.

Keywords: Microtubule-targeting agents; tubulin-ligand binding modes; molecular mechanisms of action

Pedro Alejandro Sánchez Murcia

Education

Pedro Sanchez-Murcia graduated in Chemistry (2005) and Biochemistry (2008) at the Universities of Murcia and Complutense Madrid, respectively, and obtained PhD with honors in Chemistry in 2013.

Professional Experience

During predoctoral period, Dr. Pedro Alejandro Sánchez Murcia recognized twice with the Young Researcher PharmaMar SEQT Award in 2009 and 2011. In addition, he was a visiting student at the Humbold-Universität Berlin (Germany) and University of Aarhus (Denmark). He carried out my first postdoctoral stay at the laboratory of Prof. Federico Gago (University of Alcalá, Spain). After that, he moved to Austria to the laboratory of Prof. Leticia González (University of Vienna) where he is currently senior postdoc.

Research Interest

His scientific interests are framed within the fields of Biological and Medicinal Chemistry. Specifically, in the use of computational methods to study (and hopefully predict) properties of small and macromolecules in the biological context.

Theoretical insights into photoactivatable microtubule-targeting agents

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Abstract Over the last two decades several experimental X-ray crystallographic, NMR and cryo-EM models have contributed to a better understanding of the mechanism of action of microtubule-targeting agents (MTAs). Nowadays, the acquired structural knowledge has allowed the rationalization of the structure−activity relationships of some of these MTAs by means of, among others, computational methods. However, the chemical diversity and complexity of these MTAs hampers a complete understanding of their mode of action and the subtle differences among them.

Photoaffinity labeling has been shown as a versatile technique in the identification of the binding mode of (novel) small molecules to macromolecules. In this method, a molecule/ligand with high affinity for the macromolecule is chemically modified to incorporate a photoreactive group, which labels the target upon illumination. To date, several photoreactive groups have been reported, such as benzophenones, aryl azides, and diazirines. In the case of the MTAs, there are a few examples of photochemical probes based on paclitaxel, discodermolide, and ephothilone. Notably, many of them were designed and synthesized prior to the availability of any experimentally derived structural model.

The main goal of this talk is to provide some guidelines to carry out a rational design of MTA-based photoaffinity probes targeting tubulin. To this end, molecular models of ephothilone A, paclitaxel and discodermolide bound to tubulin will be used to discuss the use of different photoreactive groups. In addition, the photophysics and photochemistry of these reactants will be shortly explained.

Keywords: photoaffinity labeling, MTAs, photoreactivity

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Oral Report

Drug discovery at the speed of sound

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Abstract A blockbuster drug generates > $ 1 billion revenues per year. Each day not on the market corresponds to a loss of > $ 2.7 million. Multiple benchmark reports suggest development costs of drugs are skyrocketing while the introduction of novel drugs is decreasing or at best stagnating. Part of the problems can be attributed to the preclinical drug discovery and development involving expensive high throughput screening (HTS) and hit-to-lead campaigns using mostly traditional technologies.

Here, we introduce a fundamentally novel approach towards preclinical drug discovery and development by blending Instant Chemistry, nL dispensing, acoustic-MS, uHTS and artificial intelligence.

Acoustic droplet ejection (ADE) technology allows for the fast, contact-less and accurate transfer of very small droplets (nL) from plate to plate of different high density formats. ADE has had a dramatic impact in different technology areas, including drug discovery, cancer research and genomic research and is used in many laboratories world-wide. However, ADE has never been used in miniaturization and acceleration of library synthesis for uHT to dramatically accelerate the preclinical drug discovery cycle. Instant Chemistry (MCR) is suitable to create very large libraries of small molecules and unusual scaffolds, such as macrocycles[1-2]. A prototype instrumentation platform is developed which allows for the parallel synthesis of hundreds of libraries of scaffolds on an unprecedented dense format. The platform is integrated with acoustic-MS and UPLC-MS for quality control and different biophysical and phenotypical screening platforms using the same high density format. Artificial intelligence is developed to ensure never-seen-before fast cycle times for hit-2-lead progression optimizing against multiple parameters at the same time.

We applied speed of sound technology successfully to different protein-protein interactions (microtubule spindle interacting agents) and enzymes (phosphatases, caspases).

Keywords: Nano Scale Synthesis, Automated Chemistry, microtubule spindle interacting agents, Artificial Intelligence, High Throughput Synthesis

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Small molecules promote selective denaturation and degradation of tubulin heterodimers by specific deprotonation of Glu198 of β-tubulin

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Abstract Target protein degradation is a promising strategy for drug design and functional study. Recently, proteolysis targeting chimaeras (PROTACs) have served as a fascinating method to selectively degrade target proteins. Here. we found a small molecule , 3-(3-Phenoxybenzyl)amino-β-carboline (PAC, a β-carboline derivative) , could promote tubulin denaturation and degrade tubulin in an ubiquitin- and proteasome-dependent pathway with high selectivity by binding to the colchicine site of tubulin. Crystallographic data, single amino acid substitution and structure-activity relationship reveal that pyridine nitrogen in PAC and βGlu198 in tubulin are the key elements that mediate the tubulin-binding and tubulin-degradation activities of PAC. Quantum mechanical/molecular mechanical (QM/MM) calculation identified that a low barrier hydrogen bond (LBHB) formed between these two elements. These results reveal a novel protein degradation mechanism: PAC deprotonates and charges βGlu198 through a LBHB ; The negatively charged βGlu198 is incompatible with its surrounding hydrophobic environments and then results in tubulin denaturation. In contrast, normal hydrogen bond between Glu198 and nocodazole or plinabulin showed no degradation effect. To prove this theory, we synthetized a small molecule which could form LBHB with Asp133 of beta2 adrenoceptor, and found this compound could promote beta2 adrenoceptor degradation. Thus, Our results implies that charging of residues in interior hydrophobic region of proteins by small molecules could be an alternative strategy to promote target protein degradation.

Keywords: tubulin denaturation; degradation of tubulin heterodimers

Novel Podophyllotoxin Scaffold-Based Tubulin Inhibitors as Potent Antitumor Agents: Design, Structural Basis and Antitumor Mechanism

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Abstract Tubulin inhibitors exert anti-tumor activity by causing the disorder of normal microtubule assembly and inducing apoptosis of tumor cells. To summarize the molecular interaction mechanism between drug molecules and tubulin from the atomic level is an effective way to discover new high-efficiency tubulin inhibitors. Podophyllotoxin, a main active component of Chinese Medicinal *podophyllum versipelle Hance*, has been used as a model for the study of tubulin colchicine binding site inhibitors and discovery of new antitumor drugs. Since 2005, we explore the theory method of podophyllotoxin molecular structure design and modification [1,2]. A design method for targeting inhibitors based on colchicine binding sites and adjacent alpha subunit GTP domains was established for development a series of nitrogen-containing heterocyclic substituted podophyllotoxin derivatives. The stronger inhibition of tubulin polymerization for Compound 1S was better than that of podophyllotoxin or colchicine. The antitumor activity of Compound 1S on tumor cells was also significantly enhanced by comparing to those of PTOX and colchicine.

The molecular interaction mechanism of 1S-tubulin was studied by structural biology [3]. The results of 1S-tubulin crystal structure (5JCB) analysis showed that Compound 1S could target both alpha and beta subunits of tubulin, mainly acting on two domains of alpha subunit H7-T5 loop-S8 and beta subunit colchicine binding site. The N-containing aromatic heterocycle triazole substituted at C-4 of podophyllotoxin connects with α-T5 of the neighboring α-subunit and forms mainly hydrophilic contacts with several residues of α-tubulin as well as a hydrogen bond. The α-T5 loop is directly bound with compound 1S in the α-subunit. There are mainly hydrogen bonds, including ones between the NH and nitrogen atom of compound 1S and the carbonyl oxygen and OH group, respectively, of Ser178 on the α-T5 loop. In addition, the A, B, C, D, and E rings of compound 1S are deeply buried in the colchicine domain, and they form mainly hydrophobic contacts with several residues of β-tubulin with a hydrogen bond. Hydrophobic interactions are formed between the methoxy group of the E ring and several residues of β-tubulin, whereas hydrophilic interactions are formed between the triazole group of compound 1S and several residues of α-tubulin. Compound 1S stretches the space configuration of alpha T5 loop, deflecting its angle by 33.7°, and causes the migration of beta T7 loop from beta subunit to alpha subunit by 2.0-2.7 Å. Together with the biochemical results from Compound 1S, the structural data highlight the main contributors in the alpha subunits and the colchicine domain beta subunits: the dual-target binding sites in the α-T7 loop and β-H7−T7 loop of tubulin. Compound 1S can synchronously bind to αβ-tubulin. The structures also highlight common features for the design and development of novel potent microtubule destabilizing agents.

Based on the understanding of the above structural basis, the theoretical methods of designing and modifying natural drugs targeting tubulin was further improved. We proposed that the conjugated and electronegative pharmacophores were introduced at the C-4 site of podophyllotoxin could further enhance the affinity on tubulin. In our previous study of crystal structure (PDB 5JCB), a binding site α178Ser on α-tubulin near colchicine binding domain was explored to improve tubulin affinity of podophyllotoxin. A high affinity fragment integration strategy on the basis of the binding domain (i.e., α178Ser, α182Val, and α241Phe) on α-tubulin and

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colchicine binding domain was further developed. A series of affinity fragments as α-tubulin affinity fragment was integrated at C-4 site of podophyllotoxin. The IC_{50} value of compounds were reach to nanomolar-potency on tumor cells (i.e., HepG2, A549, HeLa, and MCF-7), especially candidate drug 1. Candidate drug 1 not only exhibited nanomolar antitumor potency *in vitro* but also significantly destroyed solid tumor growth without lethal toxicity *in vivo*.

Finally, in order to understand the molecular events, the induced apoptotic mechanisms of podophyllotoxin derivatives were systematically studied and elucidated [4]. A large number of microtubules were depolymerized by heterocyclic-podophyllotoxin derivatives, and then increasing free tubulin bond with voltage-dependent anion-selective channel (VDAC). The activated cAMP-dependent protein kinase A (PKA) not only caused mitochondria depolarization but also activated c-Jun N-terminal kinase (JNK) resulting in the high level of reactive oxygen species. Understanding the molecular events provide a paradigm for a more rational approach to antitumor drug design.

Keywords: tubulin, podophyllotoxin, structure-based drug design, activity and toxicity evaluation, antitumor mechanism

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Abstract

Studies on the Synthesis and Biological Activity Evaluation of C-3'-N-Fluorinated Modified Taxane Analogues

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Abstract A serious of novel C-3'-N-fluorinated modified taxane analogues were synthesized and their cytotoxic activities against MCF-7 and A-549 cell lines were reported. The chlorine group at C-7 enable the behavior of these compounds to be evidently distinct from other similar compounds. The strong cytotoxicity in the two cell lines showed by the newly synthesized taxane analogue F9 indicated it as potential lead compounds for anticancer drug design.

In spite of the clinical application of paclitaxel and docetaxel as anticancer agents[1,2], taxoids remain a subject of research for the design of novel anticancer drugs and a more complete knowledge of their interaction with microtubules. Structure–activity relationship studies (SARs) performed by a number of different groups have demonstrated that the C7-C10 region is quite tolerant to modifications, probably due to the fact that these centers do not interact directly with tublin. And the functionalities on the bottom part of the molecule (including C2, C4, oxetane ring and C-13) are involved in the intimate interaction with the receptor[3]. Structure-activity relationship studies (SARs) also showed that the 1-hydroxyl group is not crucial for the tubulin assembly activities of paclitaxel[4]. Among the compounds that have been prepared are many with modified acyl groups at the C-13 and C-7positions, and one of the compounds in a clinical trial conducted by Bristol-Myers Squibb as a second-generation paclitaxel analogue is a C-7 derivative[5]. However, their metabolic stability, bioavailability and aqueous solubility, as well as their limited targeting specificity and strong side effects during treatment are their major drawbacks for an effective cancer therapy[6]

The fluorine for hydrogen replacement has been extensively employed in drug design, because this strategy can not only modulate the binding and functional properties of the parent compound, but also confer upon the parent compound a stronger resistance to the metabolic degradation due to the greater bond strength of C－F than that of C－H. The pioneering work reported by Ojima that the 3'-trifluoromethyl-10-acetylan analogues of docetaxel exhibited stronger cytotoxicity than paclitaxel and was over one order of magnitude more potent than paclitaxel and docetaxel,when evaluated with a drug resistant human breast cancer cell line[7,8].

As an ongoing part of our research on 1-deoxypaclitaxel analogues[9,10], we have become interested in developing the syntheses and biological activities of a series of 1-deoxypaclitaxel analogues bearing different fluorinated groups at the C-3'-N-Acyl position and the chlorine group at the C-7 position from 1-deoxybaccatin VI. The activities of these newly synthesized compounds against MCF-7 and A-549 cell lines are reported in this paper. These analogues may also be against drug-resistant tumor cell lines, and relevant works are still ongoing.

Cytotoxicity tests showed that the newly synthesized paclitaxel analogue has similar anticancer activity to paclitaxel, especially compound F9 .It shows better cytotoxicity than paclitaxel in breast cancer.

Keywords: Paclitaxel, Tubulin, Semisynthetic, Activity, 1-Deoxybaccatin VI

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Synthesis and Bioactivities Evaluation of Novel 3'-N-sulfonyl Modified Taxane Analogues from 1-deoxybaccatin-VI

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Abstract A series of new 3'-N-sulfonyl 1-deoxypaclitaxel analogs were designed, synthesized and evaluated for their anti-cancer activities in vitro and potency to inhibit the LPS-induced TNFα and NO by murine macrophages. The newly synthesized taxane analougues 8b and 8c exhibited comparable anti-cancer activity against A549 and HepG2 cells than paclitaxel. Besides, the anti-inflammatory assays of terminal compounds indicated that 1-deoxypaclitaxel analogues can competitively inhibit the LPS-induced NO and have lower cytotoxicity. It appears that different sulfonyl substituent group at 3'-N position has a significant effect on activity, and we have identified some very promising taxoids for further optimization.

Paclitaxel is a diterpenoid natural compound originally isolated from the bark of Taxus brevifolia and used in cancer chemotherapy as a mitotic inhibitor. It binds to the β-subunit of tubulin and stabilizes polymerized microtubules [1]. 1-Deoxybaccatin VI 1 that possesses the typical tetracyclic taxoid core while lacking the C-1 hydroxy group is readily available from T. mairei[2]. A large number of structure-activity studies of paclitaxel has proved that removal of the C-l hydroxyl group caused a modest loss of activity, and some of 1-deoxybaccatin-VI analogues exhibit higher activity than paclitaxel in vitro. Thus, the development of a method using 1-deoxybaccatin VI as the starting point for preparation of new active taxoids will be of value. Moreover, SAR studies have revealed that the C-13 side chain is an indispensable part for its antitumor activity [3-5]. The best-known C-13-N-acyl analog of taxol is of course docetaxel, and it has taken its place beside taxol as a clinically used anticancer drug [6]. Although a large number of 3'-N-acyl analogues were investigated, SAR studies of 3'-N-sulfonyl analogues have received little attention. Herein, we report the synthesis and biological evaluation of several novel 3'-N-sulfonyl 1-deoxypaclitaxel analogs.

The synthesis of compounds 8a-h is shown in Scheme 1. The cytotoxicity of paclitaxel and its analogues against hepatic carcinoma were evaluated by CKK-8 method employing HepG2 cell lines. Compounds 8b and 8c indicated more potent cytotoxicity activities than that of paclitaxel in the assay. Especially compound 8b which exhibited excellent cytotoxicity activities. For the A549 cell line, the compounds 8b and 8c exhibited potent cytotoxicity activities. The results also indicated them as potential lead compounds for anticancer drug design. Paclitaxel and its analogs were evaluated for their cytotoxicity and suppressive activity against TNFα and NO production using macrophage cell line, Raw264.7 induced by lipopolysaccharide (LPS) as the first in vitro screen. Compound 8b and 8c showed better anti-inflammatory effect on the LPS-induced TNFα and NO and have lower cytotoxicity. These results highlight these derivatives for further anti-inflammatory drug development and optimization.

Keywords: Paclitaxel; 1-deoxybaccatin VI; Tubulin; MD-2; Activity

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