Advances in Cancer Drug Targets

Volume 3

Editor:

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Bentham e **Books**

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PREFACE

The 3rd volume of the book Series "*Advances in Cancer Drug Targets*" comprises eight chapters written by the leading experts in this field. It is an outstanding collection of well written chapters on cancer drug targets in the field of pharmacology, molecular biology and biochemistry.

Human neutrophil elastase (HNE) plays an important role in the development of chronic obstructive pulmonary diseases. In chapter 1, Alix *et al.*, explain its involvement in non-small cell lung cancer progression. Natural compounds and/or synthesized agents which antagonize HNE activity have been comprehensively reviewed in this chapter. They also focus on substances (*i.e.* lipids and derivatives, phenolics) that exhibit an inhibitory bifunctionality towards HNE and matrix metalloproteinases (MMPs), particularly MMP-2.

The efficacy of cancer-immunotherapy with complement-activating monoclonal antibodies is restricted by over-expression of one or more membrane-bound complement regulatory proteins (mCRPs: CD46, CD55, CD59) that are present on the surface of neoplastic cells. Kirschfink *et al.*, in chapter 2 discuss small interfering RNAs (siRNAs) for post-transcriptional gene knock down of CD46, CD55 and CD59 aiming to sensitize tumor cells.

Hepatocellular carcinoma (HCC) is the third most common cause of deaths from cancer worldwide. There is growing evidence that the deregulation of Wnt/β-catenin signaling pathway plays a critical role in hepatic oncogenesis and mainly occurs at the early stage of hepatocarcinogenesis. In chapter 3, Kim and Wands have summarized the potential molecular targets related to the Wnt/β-catenin signaling pathway along with their therapeutic applications.

A major challenge in treating ovarian cancer is to overcome intrinsic and acquired. Chapter 4 by Ahmed *et al.* presents the recent advances in our understanding of the cellular origin and the molecular mechanisms defining the basis of cancer initiation and malignant transformation with respect to epithelial-mesenchymal transition (EMT) of ovarian cancer cells.

Due to the high expression of Survivin in various carcinomas, it is one of the key anti-apoptotic proteins. It is also associated with their biologically aggressive characteristics and drug resistance. Bisen *et al.*, in chapter 5 elaborate the efficacy of combination of oxaliplatin and paclitaxel as a potential strategy in controlling HNSCC cell proliferation. This review highlights the fact that the co-treatment of cells with paclitaxel and oxaliplatin results in a significantly higher cytotoxicity as compared to individual single drug treatment.

Melatonin has oncostatic effects on different neoplasias, particularly on estrogen-dependent breast cancer. The compound acts by interacting with estrogen-responsive pathways, thus behaving as an antiestrogenic hormone. In chapter 6 by Cos *et al.*, evidence is presented that that melatonin could exert its antitumoral effects on hormone-dependent mammary tumors by down-regulating the sulfatase pathway of the tumoral tissue.

Recent studies have thrown light on the role of mammalian target of rapamycin (mTOR) in the regulation of tumor cell motility, invasion and cancer metastasis. Zhou and Huang in chapter 7 discuss the mTOR complexes and the role of mTOR signaling in tumor cell migration and invasion. The chapter also highlights the findings about the mechanism by which rapamycin inhibits cell migration, invasion and cancer metastasis.

It has been hypothesised that a phenyl hydroxylamine group linked to a second aromatic moiety generates a pharmacophore which can interact with Ras and inhibit its activation In chapter 8, Peri *et al.*, present reports on the synthesis of a library of small molecules with arylamides and arylsulfonamides groups. They also explain their biological activity to inhibit nucleotide exchange on human Ras.

I hope that the current book volume, which provides insights into the development of new approaches to anti-cancer therapy for interested researchers and pharmaceutical scientists, will be received with the same enthusiasm as the previous volumes of this Series. I am grateful to the valuable contributions made by the authors. I greatly appreciate the assistance from the editorial staff, particularly Mr. Mahmood Alam (Director Publications) and Mr. Shehzad Naqvi (Senior Manager) for their hard work and determined efforts.

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Neutrophil Elastase as a Target in Lung Cancer: the State of the Art

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Abstract: Human neutrophil elastase (HNE), a main factor in the development of chronic obstructive pulmonary diseases, has been recently involved in non-small cell lung cancer progression. It can act at several levels (i) intracellularly, cleaving for instance the adaptor molecule insulin receptor substrate-1 (IRS-1) (ii) at the cell surface, hydrolyzing receptors as CD40 (iii) in the extracellular space, generating elastin fragments *i.e.* morphoelastokines which potently stimulate cancer cell invasiveness and angiogenesis.

Since decades, researchers identified natural compounds and/or synthesized agents which antagonize HNE activity that will be described in this review article. Some of these compounds might be of value as therapeutic agents in lung cancer.

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However, it is now widely accepted that lung tumor invasion and metastasis involve proteolytic cascades. Accordingly, we will here mainly focus our attention to natural substances able to display a dual inhibitory capacity (*i.e.* lipids and derivatives, phenolics) towards HNE and matrix metalloproteinases (MMPs), particularly MMP-2. To that purpose, we had synthesized substances named "LipoGalardin" exhibiting such inhibitory bifunctionality. At last, we will propose an original synthetic scheme for designing a potent biheaded HNE/MMP-2 inhibitor.

Keywords: Bifunctionality, Caffeic acid phenethyl ester (CAPE), (Dual) inhibitors, Elafin, Elastokines, (-)-Epigallocatechin-3-gallate (EGCG), Flexible docking, Lipogalardin, Lung cancer, Molecular modelling, Neutrophil Elastase, (Potent) angiogenic molecules, Potent biheaded lnhibitor, (Potent) chemiotactic activity.

1. INTRODUCTION: NEUTROPHIL ELASTASE/A-1ANTITRYPSIN IMBALANCE AS A LINK BETWEEN CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND LUNG CANCER

Cigarette smoke is widely considered as the causative agent in chronic obstructive pulmonary disease (COPD) and lung cancer, two leading causes of death worldwide [1, 2]. This common initiating agent is able to generate in the lung reactive oxygen species resulting in NF-kappa B activation and inflammation [3] but consequences appear distinct in the two diseases. COPD is characterized mainly by matrix degradation, incomplete tissue repair with excessive apoptosis and impaired neovessel formation, while excessive DNA damage and its incomplete repair are hallmarks of lung cancer [2]. However, recent investigations pinpointed that smokers who suffer from COPD appear to be at increased risk for developing adenocarcinoma of the lung, suggesting that it might exist a link between these pathologies [4 - 6]. Such link between COPD and lung cancer has been clearly evidenced in population based study [7].

Alpha-1 antitrypsin (α-1AT) deficiency caused by the homozygous ZZ allele is responsible for liver disease in children and emphysema in young adults [8, 9]. These individuals have a shorter life span than the general population and lung cancer, an age-related disease, was not detected in patients. However, a 14 fold increased risk of lung cancer was evidenced in non-smokers α-1AT deficiency

gene-carriers younger than 60 years of age [10]. Particularly the frequency of M1 allele and PiM1 α-1AT homozygotes in lung cancer patients was found to be significantly elevated as compared to healthy individuals [11, 12]. Alpha-1 Antitrypsin belongs to the serpin family, and it displays the highest affinity among serine proteinases for neutrophil elastase (NE) [13]. To that respect, since decades, the elastase/α-1 antitrypsin imbalance was considered as one pivotal mechanism in the formation of emphysematous lesions characterized by intense elastin fragmentation and airways enlargement [14]. Imbalance between enzyme and inhibitor might originate from genetic deficiency of α-1AT; alternatively, the oxidation of Met 358 in the α-1AT active site markedly impairs its interactions with NE. Also, Matrix Metalloproteinases (MMP), mainly matrilysin, as overexpressed in COPD and lung cancer, can further inactivate native or oxidized α-1AT by proteolysis [15]. Altogether, these mechanisms led to the generation of excess NE in pathological lung tissues. In the search of pathological biomarkers a recent *in vivo* study pointed out that Aα-Val360, an HNE specific fibrinogen degradation product may represent an ideal indicator for COPD disease severity and progression. This report is considered as the first *in vivo* data supporting physiopathological role of HNE in COPD [16]. Human NE (HNE: EC 3.4.21.37) gene encodes a 267 amino acid residues preprotein and its transcription is restricted to the promyelocytic stage of granulocyte development. Mature HNE contains 218 amino acid residues and two sites of *N*-glycosylation have been identified, but isoenzymes display similar kinetic constants with substrate and inhibitors. Enzyme at high concentration: 3 pg/cell is sequestered within azurophilic granules of polymorphonuclear neutrophils (PMN) and its activity is mainly controlled by compartmentalization [17, 18]. The main physiological function of NE, together with reactive oxygen species and other PMN proteases, consists in fighting against microbial action [13, 17]. Activation of PMN with bacterial products and cytokines only minimally influences NE release. On the contrary, frustrated phagocytosis or PMN necrosis as it occurred in COPD, leads to intense release of elastase from cells [17].

A recent mass spectrometric proteome analysis has evidenced that a histone H2A specific protease (H2Asp), discovered more than 35 years ago and implicated in the truncation of the histone H2A C-tail at V114 in myeloid cells is finally

Inhibition of Membrane Complement Inhibitor Expression (CD46, CD55, CD59) by siRNA Sensitizes Tumor Cells to Complement Attack

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Abstract: The efficacy of cancer-immunotherapy with complement-activating monoclonal antibodies is limited by over-expression of one or more membrane-bound complement regulatory proteins (mCRPs: CD46, CD55, CD59) on the surface of neoplastic cells.

In this study we designed small interfering RNAs (siRNAs) for posttranscriptional gene knock down of CD46, CD55 and CD59 aiming to sensitize tumor cells to complement attack and thereby exploiting complement for tumor cell destruction. Tumor cell lines of different origin, such as Du145 (prostate), BT474 (breast) and K562 (erythroleukemia) were selected for the study. FACS-analysis demonstrated that siRNA anti-CD46 (301) reduced CD46 protein expression up to 80%, siRNA anti-CD55 (255) diminished CD55 protein expression up to 49%, and CD59 protein expression was inhibited up to 82% by siRNA anti-CD59 (1339). Time course experiments revealed a long-lasting silencing effect with >50% complement regulator inhibition up to day 13. Upon mCRP knock down, complement-dependent cytotoxicity (CDC) was augmented by 20-30% for CD46, by up to 24% for CD55 and by up to 55% for CD59. The combined inhibition of all three inhibitors further enhanced CDC by up to 66%. Dependent on the cell line, CD46 and CD55 downregulation increased significantly C3 opsonization, which is known to support cell-mediated defense mechanisms. mCRP blocking antibodies were only partly able to further augment the tumor cells' susceptibility to complement lysis.

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Thus, siRNA-induced inhibition of complement regulator expression clearly sensitizes malignant cells to complement attack and, if specifically targeted to the tumor, appears suited as adjuvant to improve antibody-based cancer immunotherapy.

Keywords: Complement resistance, Membrane complement regulatory proteins, siRNA, Tumor immunology.

INTRODUCTION

The potential of complement activation in the control of neoplastic cells is hampered by various protective mechanisms employed by the tumor cell. The same mechanisms, which protect normal cells from accidental complement lysis, are also involved in tumor cell resistance to complement. Among these mechanisms, the expression of the membrane-bound complement regulatory proteins (mCRP) CD46, CD55 and CD59 appears most important and consequently gained most attention [1 - 5].

Compared to the corresponding unaffected tissue, cancer cells often over-express one or more of these mCRPs. As previously suggested this may be due to selective forces caused by repeated complement attacks during neoplastic transformation [1]. Furthermore, an increasing number of studies showed that mCRP over-expression is a result of different mechanisms related to activation of tumor cells. For example, Prostaglandin E_2 can up regulate CD55 expression [6]. CD46 mRNA expression is induced by activation of the signal transducers and activators of transcription 3 (STAT-3) pathway [7], which is - in contrast to normal tissues or cells - persistently activated in most cancer cells and primary tumor tissues [8]. CD59 can be upregulated as a result of increased acetylation of p53, a process that particularly occurs in tumors [9]. It has been demonstrated that enhanced expression of membrane regulators is associated with reduced survival in cancer patients [10, 11].

The inhibitors CD46 [12] and CD55 [13] block the complement cascade at the C3 activation stage, thereby not only preventing direct complement-dependent cytotoxicity, but also limiting the generation of the anaphylatoxins C3a and C5a. Subsequently, an attenuated inflammatory reaction in combination with a reduced amount of C3 opsonins deposited on the cell surface restricts cell-mediated

immune defense mechanisms.

CD59 [14] interferes with the assembly of the membrane attack complex (MAC) of complement *via* binding to C9, thereby preventing the formation of the lytic pore. CD59 is considered to be the most effective surface inhibitor, acting additively or even synergistically with CD46 and CD55 [15].

Neutralization of one or more membrane regulators renders tumor cells more susceptible to complement attack. This has been verified in the past by either blocking antibodies against mCRPs [1, 4, 16], enzymatic removal of the GPI-anchored CD55 and CD59 by PI-phospholipase C [17] or by cytokine-mediated downregulation [18].

We have identified CD46- and CD55-specific phosphorothioate antisense-oligonucleotides (S-ODNs) to immediately interfere with synthesis of both mCRPs in neoplastic cells leading to a significantly reduced complement resistance [19].

RNA interference (RNAi) based on small interfering RNAs (siRNAs) is considered to be more efficient than traditional antisense strategies [20]. SiRNAs are short double-stranded RNA oligomers consisting of 21-23 bp, which induce highly sequence-specific degradation of homologous mRNA, thereby silencing the target gene by posttranscriptional gene knock down [21, 22].

We here demonstrate a significant reduction of tumor cell complement resistance upon the application of siRNAs targeting all three mCRPs (CD46, CD55 and CD59).

MATERIALS AND METHODS

Cell Culture

DU145 prostate carcinoma cells ATCC (American Type Culture Collection, Manassas, VA, USA) number: HTB-81, BT474 breast carcinoma cells (ATCC number: HTB-20) and K562 erythroleukemic cells (ATCC number: CCL 243) were all cultured in RPMI 1640 (Cambrex, Verviers, Belgium), supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS, Invitrogen, Karlsruhe,

Points of Therapeutic Intervention along the Wnt Signaling Pathway in Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is the third most common cause related to cancer mortality worldwide. Due to frequently late diagnosis, overall prognosis of patients with liver cancer is poor. Unfortunately, there is no targeted therapeutics for the treatment of HCC except sorafenib, which has exhibited notable results in certain advanced HCC. Increasing evidences indicate that deregulation of Wnt/β-catenin signaling pathway plays a critical role in hepatic oncogenesis and mainly occurs at the early stage of hepatocarcinogenesis. In addition, aberrant activation of the Wnt/β-catenin signaling pathway has been linked with more aggressive HCCs. The major mechanism for aberrant activation of the signaling in HCC is caused by genetic mutations and/or altered expression of upstream components of the Wnt/β-catenin signaling. This leads to abnormal expression of the β-catenin/TCF-responsive target genes, which regulate cell growth, apoptosis, cell motility, and invasion. Thus, intervention of the Wnt/β-catenin signaling activity can be potential therapeutics for HCC. This review will discuss the identified potential molecular targets related to the Wnt/β-catenin signaling pathway and their potential therapeutic applications.

Keywords: β-catenin, Canonical Wnt, Dishevelled, Frizzled receptor, Glypican-3, Hepatocellular carcinoma, Immunotherapy, Molecular target, Porcupine, Small molecule inhibitor, Tankyrase, Targeted therapy, T-cell factor, Wnt ligand, Wnt signaling.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the third common cause of tumor-related death (~ 1 million deaths per year) worldwide [1 - 3]. The overall prognosis of patients with liver cancer is poor (mortality: incidence index = 0.95). It is often attributable to a late diagnosis with advanced tumor spread at the time of clinical presentation; thus, most patients have inoperable HCC when they seek medical attention. In this setting, existing therapies have only modest benefit. Among systemic therapies that have been tested with advanced disease, sorafenib, an inhibitor for angiogenesis and Ras-Raf-MAPK signaling, has been shown to significantly effect on patients with HCC due to persistent hepatitis B (HBV) or C (HCV) infection [4]. The growing incidence of HCC and the lack of effective systemic chemotherapy have generated an intense search to unravel the cellular and molecular mechanisms responsible for the pathogenesis of this disease in the hope of developing new innovative approaches [5]. The development of HCC takes a long period of time through a complex multistep process. Accumulation of abnormal genetic and epigenetic alterations as well as deregulation of cellular signaling pathways drive normal hepatocytes or progenitors toward malignant phenotype [6 - 15]. Unlike colorectal cancer in which common genetic changes has been found, HCC displays only few general genetic abnormalities among tumors. Considering various risk factors of HCC, the broad genetic heterogeneity is predictable [16]. Thus, gene expression signature and microRNA profiling have been valuable to categorize patients by distinct subgroups and to define possible driver molecules important in hepatocarcinogenesis [12, 17 - 19]. On the other hand, the extensive genetic heterogeneity of HCC led to focus on determining major cellular signaling pathways that may relate to HCCs with different etiologies and molecular mechanisms. Among these pathways, increasing evidence suggests that impaired Wnt/β-catenin signaling pathway could be critical in hepatic transformation [5, 9].

The Wnt signaling pathway is regulated by multiple components and knowledge of how this pathway is controlled will open new approaches for the development of targeted therapies. In addition to HCC, aberrant activation of this pathway has been shown to be important in a large number of tumors to promote cell growth and appears to control characteristics of the malignant phenotype in various

adenocarcinomas of the colorectum [20], breast [21], melanoma [22] and lung [23]. Currently, none of therapeutics targeting the Wnt/β-catenin signaling has been approved for treatment of HCC yet. It is appealing and great potential to target the Wnt/β-catenin signaling to treat HCC. Nevertheless, substantial side effects and risks should be considered since the Wnt signaling pathway play a critical role in normal liver homeostasis and regeneration. In this review, we will summarize recent outcomes to intervene the Wnt/β-catenin signaling pathway and analyze the various therapeutics that target to different stages of the signaling cascade for during HCC development and progression.

THE WNT SIGNALING PATHWAY

Overview of the Wnt Signaling

Wnt signaling regulates diverse developmental and homeostatic functions, including proliferation, differentiation, cell polarity, motility, and migration. The signaling pathways can be classified into two main pathways as canonical (β-catenin) and noncanonical pathway. However, the reality is not straightforward to designate Wnt signaling due to crosstalk between two pathways and other factors involved, rather raises complex nonlinear networks. In human, 19 Wnt ligands and 10 Frizzled (FZD) receptors have been identified [24]. Wnt ligands interact with the extracellular domain of FZD receptors through a conserved cystein-rich domain (CRD). This ligand/receptor complex is associated with several regulators, such as the low-density lipoprotein receptor-related protein (LRP) 5/6 co-receptors required for activation of canonical Wnt signaling [25, 26]. In addition, several alternative/cooperative canonical ligands and receptors have been described. In this respect, norrin protein interacts with FZD4 and LRP5 during vascular development [27], and Rspondin-1 can activate the Wnt/β-catenin pathway *via* Wnt3A in intestinal epithelium [28]. Certain membrane tyrosine kinase receptors can replace FZD receptors; for example, ROR1 and ROR2 interact with the non-canonical Wnt5A and activate the PCP pathway [28, 29]; the Ryk receptor interacts with Wnt1 and Wnt3A of the canonical Wnt [30], and interaction with Wnt5A will activate the non-canonical cascade [31]. Soluble Frizzled-related proteins (SFRPs) can inhibit the Wnt/FZD signal by direct binding with Wnt ligands and by formation of non-functional complexes with

Collaboration of Epithelial Mesenchymal Transition and Cancer Stem Cells: Sinister Routes for Chemoresistant Recurrent Ovarian Cancer

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Abstract: Overcoming intrinsic and acquired chemoresistance is the major challenge in treating ovarian cancer patients. Initially nearly 75% of ovarian cancer patients respond favorably to chemotherapy, but subsequently the majority gain acquired resistance resulting in recurrence, cancer dissemination and death. This chapter summarizes recent advances in our understanding of the cellular origin and the molecular mechanisms defining the basis of cancer initiation and malignant transformation with respect to epithelial-mesenchymal transition (EMT) of ovarian cancer cells. We discuss the critical role of EMT frequently encountered in different phases of ovarian cancer progression and its involvement in regulating cancer growth, survival, migration, invasion and drug resistance. Using model ovarian cancer cell lines we highlight the relationship between EMT and the 'cancer stem cell (CSC)-like phenotype' in response to drug treatment, and relate how these processes can impact on chemoresistance and ultimately recurrence. We propose the molecular targeting of distinct 'EMT transformed CSC-like cells' and suggest ways that may improve the efficacy of current chemotherapeutic regimens much needed for the management of this disease.

Keywords: Chemoresistance, Differentiation, EMT, Metastasis, Migration,

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Ovarian carcinoma, Recurrence, Stem cell markers.

INTRODUCTION

Epithelial ovarian cancer (EOC) is the fifth major cause of cancer mortality in women and constitutes 90% of all ovarian malignancies. In 2012, worldwide there were an estimated 239,000 new cases and over 140,000 deaths from ovarian cancer [1]. Due to lack of specific signs/symptoms and inadequate awareness about the disease on the part of general practitioners as well as the wider community, the disease is not diagnosed until advanced-stages in most patients contributing to a low overall cure rates. The five year survival for stage 1 patients is 90%, but it reduces to 30% in patients with the advanced disease [2]. While surgery is the primary component of initial therapy in advanced-stage patients, almost all patients are treated with chemotherapy to eradicate the residual microscopic and macroscopic peritoneal metastases. Current treatment with paclitaxel and platinum based combination therapy results in a complete remission in 75% patients. Unfortunately, this remission is short lived lasting only for 6-20 months with subsequent recurrence and death as a consequence of metastatic spread [2]. In this chapter we discuss what is known about the initiation of ovarian cancer and the role of epithelial-mesenchymal transition (EMT) in facilitating the progression of this disease. We also shed light on the role of chemotherapy-induced EMT as one of the potential processes that may contribute to the development of chemoresistance and recurrence. In this context, we discuss the relationship between EMT and the acquisition of 'CSC-like phenotypes' in response to chemotherapy. Emphasis has also been made on the development of new therapeutic approaches and how these would impact on EMT and CSC-like phenotypes with the view for better management of this disease.

PATHOLOGY OF OVARIAN CANCER

The term 'ovarian cancer' refers to a diverse group of malignancies that affect the ovaries [3]. Aberrant differentiation is a unique aspect of ovarian cancer biology as tumors acquire the complex differentiation pattern of fallopian tube (serous carcinoma), endometrium (endometroid carcinoma), endocervix (mucinous carcinoma) and vagina (clear cell carcinoma) [4]. Each subtype has identifiable

precursor lesions and multiple early genetic alterations [5]. Hence, mutations in the early progenitor cells may be the contributing factors in the initiation of ovarian carcinoma.

The current classification of ovarian cancer divides the malignancy into two distinct tumor types. Of these, type 1 tumors are low-grade, slow growing tumors, generally confined to the ovary at diagnosis. These tumors develop from well-established precursor lesions commonly termed 'borderline tumors' [3 - 7]. These low-grade ovarian neoplasms have been associated with mutations in KRAS, BRAF, PTEN, and CTNNB1/β-catenin and include the major histotype, endometrioid, mucinous and low-grade serous carcinomas [3 - 6]. As these genetic alterations occur early in the transformation process, the contribution of these genes defines the tumorigenic process as well as histological differentiation of type 1 tumors.

In contrast, type 2 tumors are high-grade and rapidly progressing tumors which arise from well-defined precursor lesions [6]. Type 2 tumors rarely contain the mutations in KRAS, BRAF, PTEN, and CTNNB1/β-catenin genes, and vast majority of these neoplasms spread to extra-ovarian sites, specifically, the peritoneum and the fallopian tube early in their development and at a later stage involve the ovary [3 - 6]. Unlike type 1 tumors, type 2 tumors are all high-grade carcinomas, undifferentiated carcinomas or carcinosarcomas [6]. These tumors are composed of large masses of cells with large multinucleated nuclei [6]. They have high mitotic activity and the majority have active DNA damage repair mechanism (DDR) and mutated or ineffective p53 function commonly known as a 'p53 signature' [6, 7]. These tumors may also exhibit gene amplification and over expression of the HER2/neu (10–20%) and AKT2 (10–20%) oncogenes [6]. Gene expression analyses have shown high-grade and low-grade tumors to have a distinct expression pattern which clusters separately, suggesting that the two groups have a different genetic makeup [8].

The initiating events in EOC are poorly understood. Until recently EOC was thought to arise directly from the surface epithelium of the ovary and its associated inclusion cysts or indirectly from benign ovarian lesions derived from the inclusion cysts [9]. The ovarian surface is composed of a thin layer of surface

Oxaliplatin-mediated Inhibition of Survivin Increases Sensitivity of Head and Neck Squamous Cell Carcinoma Cell Lines to Paclitaxel

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Abstract: Oxaliplatin, is a platinum-based antineoplastic agent used in cancer chemotherapy and paclitaxel is one of several cytoskeletal drugs that target tubulin. Paclitaxel-treated cells have defects in mitotic spindle assembly, chromosome segregation, and cell division. Survivin is one of the key anti-apoptotic proteins which is over-expressed in most of the human cancers; and is associated with their biologically aggressive characteristics and drug resistance. Investigations presented deals with the evaluation of the efficacy of oxaliplatin and paclitaxel combination as a potential strategy in controlling HNSCC cell proliferation and the assessment of correlation between occurrence of apoptosis and changes in expression of survivin (IAP) by employing two HNSCC cell lines (Cal27 and NT8e) and one normal cell line (293) panel with differential level of survivin expression in accordance with chemosensitivity. The combined treatment of cells with paclitaxel and oxaliplatin resulted in a significantly higher cytotoxicity compared to individual single drug treatment. Cytotoxicity was prominent in paclitaxel to oxaliplatin (pacl-oxal) sequence treatment with an approximate two-fold increase in apoptosis compared to oxaliplatin to paclitaxel (oxal-pacl) sequence treatment.

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Paclitaxel treatment also significantly increased survivin expression with reduced apoptosis at low concentration. Oxaliplatin, when combined with paclitaxel, decreased the survivin level with increased cell death. Study was further designed to explore the effect(s) of survivin-inhibition by a small interfering RNA (siRNA therapy) method on the apoptosis in HNSCC cells expressing increased sensitivity of the cancer cell lines to paclitaxel whereas over-expression of survivin in the transfected 293-cell line provided resistance. Survivin played a critical role in paclitaxel resistance through the suppression of apoptosis, and a significant induction of apoptosis was observed when oxaliplatin was combined with paclitaxel at least in part by the down-regulation of survivin. In conclusion, the interaction between drugs was synergistic and schedule-dependent.

Keywords: Apoptosis, Chemotherapy, Cytotoxicity, HNSCC, Oxaliplatin, Paclitaxel, Survivin.

INTRODUCTION

In modern oncology, in combination chemotherapy, almost all regimens, the majority of drugs used in cancer chemotherapy are cytostatic in use; combine several chemotherapy drugs, their dosage, the frequency and duration of treatments and other considerations. Chemotherapy regimens are often identified by acronyms, identifying the agents used in the drug combination. Different drugs work through different mechanisms in combination chemotherapy, and that the results of using multiple drugs will be synergistic to some extent because they have different dose-limiting adverse effects. They can be given together at full doses in chemotherapy regimens [1 - 8]. A new approach on combitorial therapeutic relevance of lentiviral mediated survivin knockdown combined the both chemotherapy and ionizing radiation having clinical relevance has been reported which may display a requisite for future combined modality approaches in the treatment of cancer particularly HNSCC (Head and neck squamous cell carcinoma) therapy in cancer treatment with modest effects on cell survival and apoptosis [9]. An approach has been used for identifying the molecular targets of the predicted anti-cancer compounds which were mined from reliable sources like experimental bioassays studies associated with the compound, and from protein-compound interaction databases [7]. Therapeutic compounds from DrugBank, and a list of natural anti-cancer compounds derived from literature mining of

published studies, were also used for building partial least squares regression model. The regression model thus built, was used for the estimation of oral cancer specific weights based on the molecular targets [10]. Paclitaxel a microtubule-binding potent cytotoxic agent, treated cells have defects in mitotic spindle assembly, chromosome segregation, and cell division. Paclitaxel has proven clinically efficacious for the treatment of various human cancers including carcinomas of ovarian, breast, head and neck, bladder, lung, and prostate tissues [11 - 15]. There is, however, limited information available on the cellular and molecular mechanisms of the apoptotic effect of paclitaxel on HNSCC cell. Mitosis checkpoint control is important in determining the sensitivity of cancer cell to paclitaxel [16 - 18]. Survivin, a novel anti-apoptotic protein, over-expressed, in most of the human cancers, is reported to be involved in therapy resistance [19 - 24]. We explored the mechanism of HNSCC cell resistance to low concentration of paclitaxel treatment in the present investigations.

Clinically achievable doses of paclitaxel are greatly limited by toxicity to normal tissues, and ranges from sensory neuropathy and gastrointestinal disturbances to severe myelosuppression [25 - 28]. Attempts have been made to overcome this problem by combining paclitaxel with other chemotherapeutic agents [29, 30]. However, paclitaxel-associated neurotoxicity may be exacerbated when used in combination with other neurotoxic agents, such as cisplatin [25, 26]. Therefore, it is important to choose drugs with less toxicity for combination with paclitaxel.

Platinum drugs are one of the important classes of chemotherapy drugs that can induce remissions in various solid tumors [1, 31 - 33]. Oxaliplatin, a third-generation platinum coordination complex, was developed after cisplatin and carboplatin [34, 35]. It lacks such side effects as cisplatin-associated nephrotoxicity and carboplatin induced myelosuppression [34 - 38]. Moreover, clinical studies have demonstrated a chemosensitization effect of oxaliplatin on other drugs, such as 5-FU and folinic acid [39 - 42]. In the present study, the combination of oxaliplatin and paclitaxel was explored as a new strategy for the killing of HNSCC cells and have shown that the addition of oxaliplatin in combination with paclitaxel significantly induced apoptosis, possibly by the down-regulation of survivin.

CHAPTER 6

Melatonin Inhibits the Growth of DMBA-induced Mammary Tumors by Regulating Estrogen Sulfatase Enzyme

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Abstract: Melatonin has oncostatic effects on different neoplasias, particularly on estrogen-dependent breast cancer, by interacting with estrogen-responsive pathways, thus behaving as an antiestrogenic hormone. In MCF-7 (a human breast adenocarcinoma cell line), melatonin reduces both expression and activity of estrogen sulfatase, thus modulating the local estrogen biosynthesis. In order to investigate the *in vivo* sulfatase-inhibitory properties of melatonin, this indoleamine was given to ovariectomized rats bearing DMBA-induced mammary tumors also treated with estrone sulfate. In castrated animals, the growth of estrogen-sensitive mammary tumors depends on the local conversion of biologically inactive estrogens to bioactive unconjugated estrogens. Ovariectomy significantly reduced the size and the number of tumors while the administration of estrone sulfate to ovariectomized animals stimulated tumor growth, an effect otherwise abrogated by melatonin. The uterine weight of ovariectomized rats, which depends on the local synthesis of estrogens, was increased by estrone sulfate, being this effect abolished in those animals also treated with melatonin. The growth-stimulatory effects of estrone sulfate on the uterus and tumors depend exclusively on locally synthesized estrogens, since no changes in serum estradiol were observed in estrone sulfate-treated rats. Melatonin counteracted the stimulatory effects of estrone sulfate on sulfatase activity and expression and incubation with melatonin decreased the sulfatase activity of tumors from control animals.

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Animals treated with melatonin had the same survival probability as the castrated animals and significantly higher than the uncastrated. We conclude that melatonin could exert its antitumoral effects on hormone-dependent mammary tumors by down-regulating the sulfatase pathway of the tumoral tissue.

Keywords: Breast cancer, DMBA, Melatonin, Pineal, Sulfatase.

INTRODUCTION

Melatonin, the most relevant secretory product of the pineal gland, acts as a regulator of neoplastic cell growth in different kinds of malignant tumors such as prostatic adenocarcinoma, pituitary tumors, leukemia, lung carcinoma, colon carcinoma, neuroblastoma, bladder carcinoma and breast cancer [1 - 3]. The mechanisms that may explain the ability of melatonin to counteract tumor growth were reviewed in Mediavilla *et al.* [4] and include: antioxidant effects [5], regulation of the estrogen receptor expression and transactivation [6], modulation of the enzymes involved in the local synthesis of estrogens [7 - 9], modulation of cell cycle and induction of apoptosis [10], inhibition of telomerase activity [11], inhibition of metastasis [12], prevention of circadian disruption [13], antiangiogenesis [14 - 16], epigenetic effects [17, 18], stimulation of cell diffe-rentiation and activation of the immune system [19]. Perhaps because melatonin was formerly considered as a modulator of the neuroendocrine-reproductive axis, particularly as a controller of seasonal reproduction, the mechanism based on the interplay of melatonin with the estrogen signaling pathway in the context of the hormone-dependent tumors, especially on tumors of the mammary gland, most of which are estrogen-responsive, has been the most thoroughly studied [1 - 3, 20 - 22]. Melatonin reduces the estrogen-mediated growth of breast cancer mainly by two different mechanisms: indirect neuroen-docrine mechanisms, through the down-regulation of the neuroendocrine reproductive axis and the consequent reduction of estrogenic hormones responsible for the normal and pathological growth of the mammary gland, and, direct actions of the pineal hormone over tumor cells. *In vivo* studies on animal models and *in vitro* studies on human breast cancer derived cell lines support the hypothesis that melatonin oncostatic actions on hormone-dependent mammary tumors are mainly due to its antiestrogenic actions and they are consequence of melatonin interactions on the tumor cells'

estrogen-signaling pathway. On tumor cells, melatonin inhibits the estrogen receptor alpha signaling pathway, thus counteracting the effects of estrogens, therefore behaving as a SERM (selective estrogen receptor modulator). However, melatonin has no effect on estrogen-insensitive breast tumor cell lines [22, 23]. In the last years, research has been focused on the ability of melatonin to regulate the activity of some enzymes (aromatase, sulfatase, 17β-hydroxysteroid dehydrogenase, estrogen sulfotransferase) responsible for the local synthesis of estrogens in cultured human breast cancer cells, thus behaving as a SEEM (selective estrogen enzyme modulator) [24 - 26].

The intratumoral metabolism and synthesis of estrogens may play a very important role in the pathogenesis and growth of hormone dependent breast carcinoma. It is surprising that more than two-thirds of breast cancers occur in postmenopausal women when the ovaries cease to produce estrogens. However, despite the 90% reduction in plasma estradiol levels that occur after the menopause, the concentrations of estrogens in normal breast tissue of pre and postmenopausal women are similar. This can be explained by the extragonadal biosynthesis of estrogens, which occurs in several peripheral tissues, including not only breast, but also adipose tissue, muscle, skin and bone. Estrogens are synthesized in the mammary tissue by transformation either from androgen precursors of mainly adrenal origin or from biologically inactive estrogens [27 - 29]. In this situation, plasma levels of estrogens are low, whereas the concentration of estradiol in peripheral tissues such as the mammary gland is high, as a result of the *in situ* biosynthesis [30 - 32]. Yue *et al.* reported that *in situ* synthesis of estrogen is more important than uptake from plasma to maintain adequate estradiol concentrations in breast tissues after menopause [32]. In fact, in postmenopausal women, it has been demonstrated that intratumoral levels of estradiol are 2- to 3-fold higher than those in areas considered as morphologically normal. A major circulating form of plasma estrogen is estrone sulfate, a biologically inactive precursor of estrogens that might represent a form of reservoir, which can be converted to estrone by the sulfatase, present in both normal and tumoral tissues [33]. Plasma concentrations of estrogen sulfates are 5 to 15 times higher than those of unconjugated estrogens such as estrone, estradiol and estriol, and their half-life in plasma (10-12 hours) is considerably longer than

Role of mTOR Signaling in Tumor Cell Motility, Invasion and Metastasis

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Abstract: Tumor cell migration and invasion play fundamental roles in cancer metastasis. The mammalian target of rapamycin (mTOR), a highly conserved and ubiquitously expressed serine/threonine (Ser/Thr) kinase, is a central regulator of cell growth, proliferation, differentiation and survival. Recent studies have demonstrated that mTOR also plays a critical role in the regulation of tumor cell motility, invasion and cancer metastasis. Current knowledge indicates that mTOR functions as two distinct multiprotein complexes, mTORC1 and mTORC2. mTORC1 phosphorylates p70 S6 kinase (S6K1) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1), and regulates cell growth, proliferation, survival and motility. mTORC2 phosphorylates Akt, protein kinase C α (PKCα) and the focal adhesion proteins, and controls the activities of the small GTPases (RhoA, Cdc42 and Rac1), and regulates cell survival and the actin cytoskeleton. Here we briefly review current knowledge of mTOR complexes and the role of mTOR signaling in tumor cell migration and invasion. We also discuss recent findings about the mechanism by which rapamycin inhibits cell migration, invasion and cancer metastasis.

Keywords: Akt, Cell motility, Focal adhesion proteins, GTPases, Invasion, Metastasis, mTOR, mTORC1, mTORC2, Rapamycin, S6K1, 4E-BP1.

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INTRODUCTION

Cancer metastasis, one of the characteristics of malignant tumors, is the primary cause of death in most cancer patients [1]. The metastatic process consists of a series of sequential, interrelated steps including: tumor cells detachment from the primary tumor and invasion of adjacent, healthy tissue, intrusion into the blood and lymphatic vessels, circulation through the bloodstream (circulating tumor cells) to other sites and tissues in the body, extravasation from the vessel of delivery, and metastasis growth in specific distant organs and building a secondary tumor [1 - 3]. Many of these steps are dependent on cell motility and invasion, which allow the cells to change position within the tissues. To spread within the tissues, tumor cells use migration mechanisms similar to that occuring in normal or non-neoplastic cells during physiological processes such as embryonic morphogenesis, inflammatory immune responses, wound healing, and angiogenesis [4]. However, different from the physiological processes of normal cell migration, the tumor cell migration seems to be activated by a variety of promigratory factors without counteracting stop signals, including autocrine motility factors produced by tumor cells, as well as the soluble factors present at the secondary site [5, 6]. Because of this imbalance of signals, cancer cells become continuously migratory and invasive, resulting in tumor expansion across tissue boundaries and the formation of cancer metastasis [6].

Cell migration through tissues results from highly integrated multistep cellular events that are regulated by various signaling molecules, including integrins, Rho family small GTPases, and focal adhesion kinase (FAK) [7 - 9]. In recent years, more proteins and pathways have been identified to be essential for cancer cell motility and invasion, such as phosphatidylinositol 3(kinase (PI3K), protein kinase B/Akt, mammalian target of rapamycin (mTOR), and ribosomal protein S6 kinases (S6K) [10 - 12]. mTOR is known as a key regulator of cell growth, proliferation, differentiation and survival [13, 14]. The mTOR pathway regulates several processes, including protein and lipid synthesis, ribosome biogenesis, autophagy, and metabolism by integrating signals from growth factors, nutrients, oxygen and energy status [14]. Increasing evidence suggests that mTOR pathway also plays an essential role in the regulation of tumor cell motility and invasion, as well as cancer metastasis [11, 15 - 18]. Recent work has identified two

structurally and functionally distinct mTOR-containing multiprotein complexes, mTOR complex 1 (mTORC1) and mTORC2 [14]. The two complexes consist of unique mTOR-interacting proteins which determine their substrate specificity. mTORC1 regulates cell growth, proliferation and survival by promoting anabolic processes, such as protein synthesis, and by limiting catabolic processes [19]. mTORC2 controls the actin cytoskeleton *via* mechanisms by promoting phosphorylation of focal adhesion proteins (focal adhesion kinase, paxillin, and p130^{Cas}) and protein kinase C α (PKCα), and by regulating the activities of small GTPases (RhoA, Rac1 and Cdc42) [20 - 22]. Here, we briefly discuss the insights of mTOR complexes and highlight their roles in the regulation of tumor cell motility and invasion, as well as cancer metastasis. In addition, we summarize recent findings regarding the major mechanisms by which rapamycin inhibit cell migration/invasion and cancer metastasis.

mTOR Strcture and Signaling Complexes

mTOR Structure

mTOR, also known as FRAP (FKBP12-rapamcyin-associated protein), RAFT1 (rapamycin and FKBP12 target), RAPT 1 (rapamycin target 1), or SEP (sirolimus effector protein), is a highly conserved and ubiquitously expressed Ser/Thr kinase [23 - 26]. mTOR and yeast TOR proteins share > 65% identity in carboxy-terminal catalytic domains and > 40% identity in overall sequence [27]. mTOR belongs to the PI3K-kinase-related kinase (PIKK) superfamily since the C-terminus of mTOR shares strong homology to the catalytic domain of PI3K [28, 29]. The different members of this family, such as MEC1, TEL1, RAD3, MEI-41, DNA-PK, ATM, ATR, and TRRAP, are associated with diverse cellular functions, such as control of cell growth, cell cycle and DNA damage checkpoints, recombination and maintenance of telomere length [30 - 32]. Structurally, mTOR contains 2549 amino acids and its domain structure is depicted in (Fig. **1**). The N-terminus of mTOR possesses up to 20 tandemly repeated HEAT motifs including Huntingtin, elongation factor 3 (EF3), A subunit of protein phosphatase 2A (PP2A), and TOR. The C-terminus consists of the FAT (FRAP, ATM, and TRRAP, all PIKK family members) domain, the FRB (FKBP12-rapamycin binding) domain which is a unique feature of mTOR, a

CHAPTER 8

Structure-Activity Studies on Arylamides and Arysulfonamides Ras Inhibitors

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Abstract: This paper reports on the synthesis of a panel of small molecules with arylamides and arylsulfonamides groups and their biological activity in inhibiting nucleotide exchange on human Ras. The design of these molecules was guided by structure-activity data previously collected on similar compounds. Aim of this work is the validation of the hypothesis that a phenyl hydroxylamine group linked to a second aromatic moiety generates a pharmacophore capable to interact with Ras and to inhibit its activation. *In vitro* experiments on purified human Ras clearly show that the presence of an aromatic hydroxylamine and a sulfonamide group in the same molecule is necessary to Ras binding and nucleotide exchange inhibition. The inhibitor potency is lower in molecules in which either the hydroxylamine has been replaced by other functional groups or the sulfonamide has been replaced by an amide. In this case both these moieties, the hydroxylamine and sulfonamide are absent, inactive compounds are obtained.

Keywords: Anticancer agents, Computational chemistry, Ras, Structure-activity relationship.

INTRODUCTION

It is well established that K-, H- and N-Ras proteins play a central role in regulating diverse cellular pathways important for cell growth, differentiation and

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survival [1 - 3]. Deregulation of Ras pathway by activating mutations, over-expression or upstream activation of Ras is common in human tumors [4 - 6]. Of the Ras proteins, K-Ras is the most frequently mutated and is therefore an attractive target for cancer therapy [7, 8]. The complexity of Ras signaling presents many opportunities for therapeutic targeting. A number of different approaches aimed to decrease Ras activity by small molecule inhibitors have been explored in clinical trials [9]. Several of the small molecule therapeutics tested have demonstrated clinical activity, in particular farnesyl transferases inhibitors (FTI), thus supporting the ongoing development of therapies targeting Ras. However, many of the agents currently being evaluated in clinic have multiple targets and their antitumor effects may not be due only to Ras inhibition. To date still no specific and efficient inhibitor of Ras is available for routine clinical use.

In the perspective to develop new molecules directly targeting oncogenic Ras, we have been attracted by small molecules containing the hydroxylamine group and that are able to bind Ras thus interfering with guanine nucleotide exchange, the essential biochemical event leading to Ras activation [10, 11]. We synthesized and tested a first generation of bioactive molecules composed by two aromatic moieties, one of which being a phenylhydroxylamine, connected by a short linker [12]. Some of these compounds showed interesting anticancer properties, unfortunately accompanied by very poor solubility in aqueous buffers and water-solvent mixtures used in biological tests. We therefore decided to project new inhibitors with improved water solubility in which the aromatic moieties able to interact with Ras are linked to polihydroxylated scaffold or polar heterocycles with the aim of improving water solubility [13 - 15]. Some of these compounds were active in inhibiting nucleotide exchange *in vitro* [16]. Docking experiments pointed out that these compounds bind Ras, and experimental NMR data showed that the two aromatic moieties on inhibitors are essential for the interaction with Ras [14, 15].

We then developed water-soluble Ras inhibitors by linking natural mono- and disaccharides to phenylhydroxylamine [17]. The interaction between these compounds and Ras was characterized by means of NMR techniques.

We present here a structure-activity characterization of compounds **1-17** as shown

in Fig. (**1**). This small library of compounds has been generated by progressive structural variations starting from other Ras inhibitors previously developed by us [11] and other groups [9, 10].
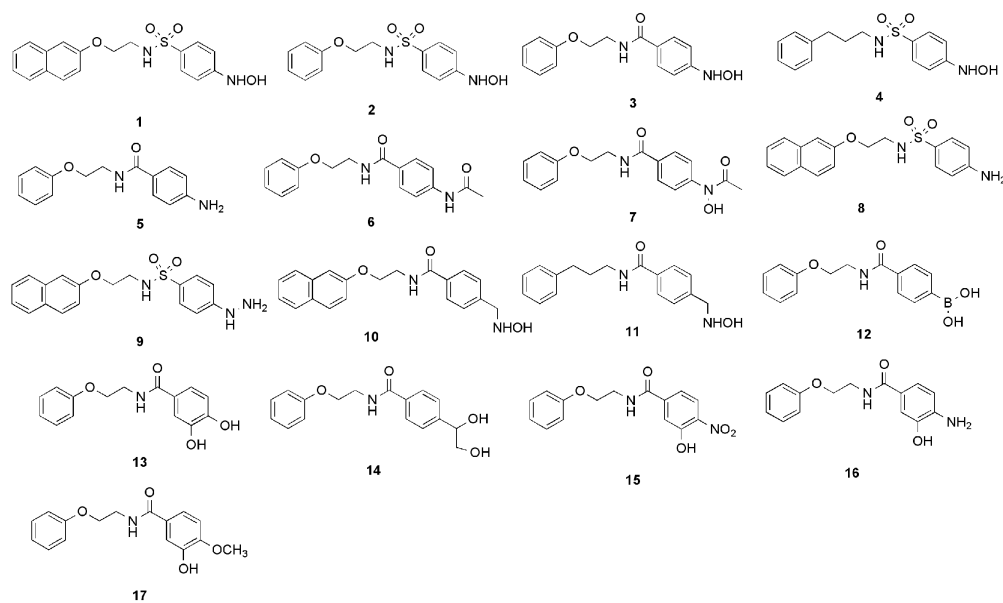

Fig. (1). Structural variations on an original common motif: chemical structures of rationally designed Ras ligands **1-17**.

The activity in inhibiting *in vitro* the guanine nucleotide exchange and dissociation on purified human Ras and the ability to interfere with the growth of normal mammalian cells and of k-Ras transformed has been evaluated for all compounds. The biological data were complemented with an *in silico* analysis to clarify molecular details of the interaction of these molecules with Ras. In compounds **1-4** a phenylhydroxylamine moiety is linked to either a phenyloxy or a naphtyloxy group through amide or a sulfonamide bonds. In compounds **5-17** the hydroxylamine group has been replaced by other bioisoster groups that mimic some interaction properties of hydroxylamine in terms of hydrogen bond formation (the nitrogen and oxygen atoms of -NHOH group are hydrogen bond donors, while the two hydrogens are acceptors). We therefore replaced hydroxylamine with an amine group in compounds **5** and **8** with an acetamide or

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ATTA-UR-RAHMAN, *FRS*

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Prof. Rahman won the UNESCO Science Prize (1999) and was elected as Fellow of the prestigious Royal Society (London) in July 2006. He has been conferred honorary doctorate degrees by many universities including (Sc.D.) by the Cambridge University (UK) (1987), He was elected Honorary Life Fellow of Kings College, Cambridge University, UK, conferred the TWAS (Italy) Prize and the Austrian government has honoured him with its high civil award ("Grosse Goldene Ehrenzeischen am Bande") (2007). He is Foreign Fellow of Chinese and Korean Academy of Sciences, Foreign Fellow of the Chinese Chemical Society and former President of Pakistan Academy of Sciences.