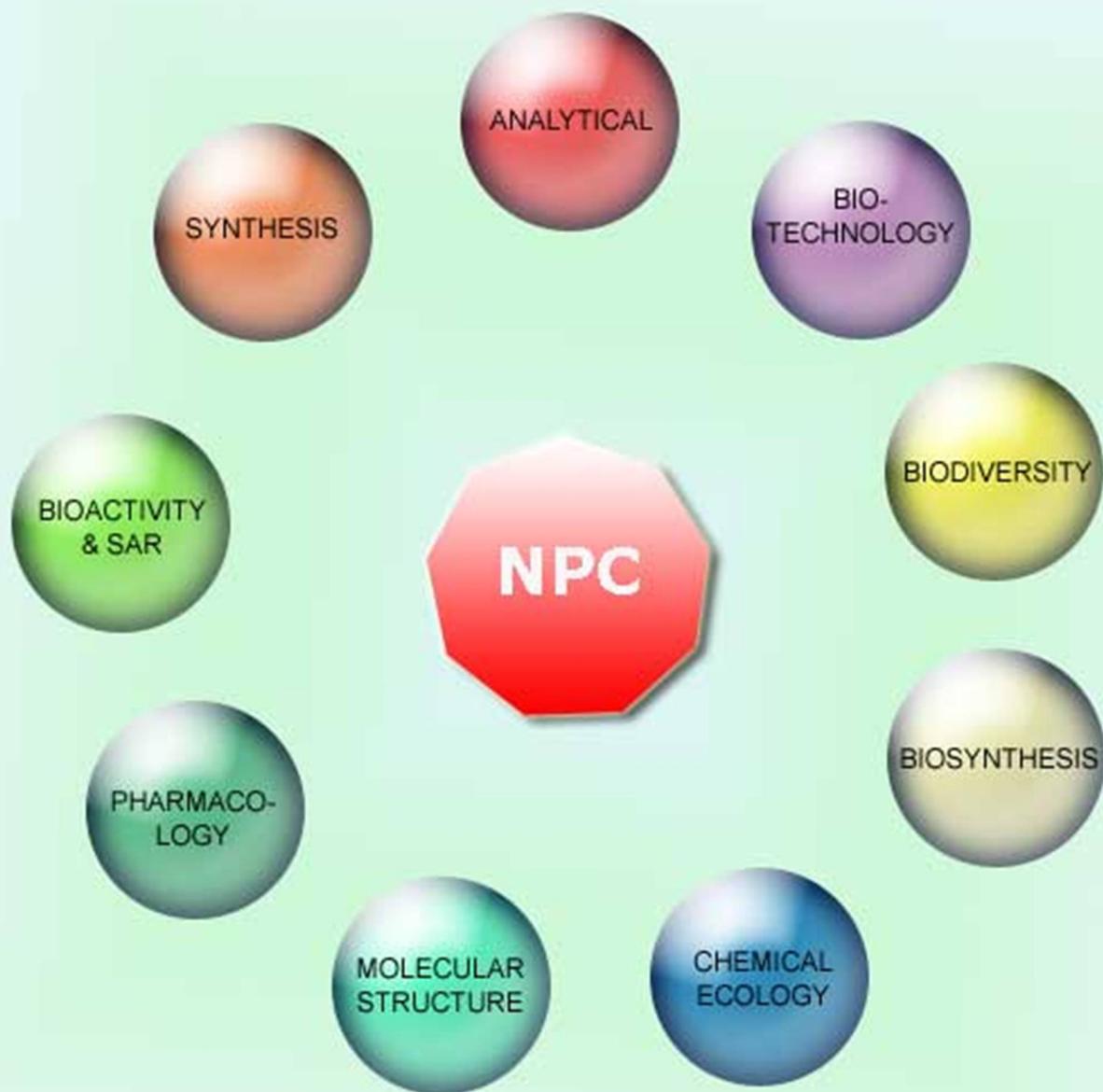


# NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all  
Aspects of Natural Products Research



**This Issue is Dedicated to  
Prof. Dr. Hans-Jörg Schneider  
On the Occasion of his 75th Birthday**

Volume 7. Issue 3. Pages 283-418. 2012  
ISSN 1934-578X (printed); ISSN 1555-9475 (online)  
[www.naturalproduct.us](http://www.naturalproduct.us)

**EDITOR-IN-CHIEF****DR. PAWAN K AGRAWAL**

Natural Product Inc.  
7963, Anderson Park Lane,  
Westerville, Ohio 43081, USA  
agrawal@naturalproduct.us

**EDITORS****PROFESSOR ALEJANDRO F. BARRERO**

Department of Organic Chemistry,  
University of Granada,  
Campus de Fuente Nueva, s/n, 18071, Granada, Spain  
afbarre@ugr.es

**PROFESSOR ALESSANDRA BRACA**

Dipartimento di Chimica Bioorganica e Biofarmacia,  
Universita di Pisa,  
via Bonanno 33, 56126 Pisa, Italy  
braca@farm.unipi.it

**PROFESSOR DEAN GUO**

State Key Laboratory of Natural and Biomimetic Drugs,  
School of Pharmaceutical Sciences,  
Peking University,  
Beijing 100083, China  
gda5958@163.com

**PROFESSOR YOSHIHIRO MIMAKI**

School of Pharmacy,  
Tokyo University of Pharmacy and Life Sciences,  
Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan  
mimaki@ps.toyaku.ac.jp

**PROFESSOR STEPHEN G. PYNE**

Department of Chemistry  
University of Wollongong  
Wollongong, New South Wales, 2522, Australia  
spyne@uow.edu.au

**PROFESSOR MANFRED G. REINECKE**

Department of Chemistry,  
Texas Christian University,  
Forts Worth, TX 76129, USA  
m.reinecke@tcu.edu

**PROFESSOR WILLIAM N. SETZER**

Department of Chemistry  
The University of Alabama in Huntsville  
Huntsville, AL 35809, USA  
wssetzer@chemistry.uah.edu

**PROFESSOR YASUHIRO TEZUKA**

Institute of Natural Medicine  
Institute of Natural Medicine, University of Toyama,  
2630-Sugitani, Toyama 930-0194, Japan  
tezuka@inm.u-toyama.ac.jp

**PROFESSOR DAVID E. THURSTON**

Department of Pharmaceutical and Biological Chemistry,  
The School of Pharmacy,  
University of London, 29-39 Brunswick Square,  
London WC1N 1AX, UK  
david.thurston@pharmacy.ac.uk

**HONORARY EDITOR****PROFESSOR GERALD BLUNDEN**

The School of Pharmacy & Biomedical Sciences,  
University of Portsmouth,  
Portsmouth, PO1 2DT U.K.  
axuf64@dsl.pipex.com

**ADVISORY BOARD**

Prof. Berhanu M. Abegaz  
Gaborone, Botswana

Prof. Viqar Uddin Ahmad  
Karachi, Pakistan

Prof. Øyvind M. Andersen  
Bergen, Norway

Prof. Giovanni Appendino  
Novara, Italy

Prof. Yoshinori Asakawa  
Tokushima, Japan

Prof. Lee Banting  
Portsmouth, U.K.

Prof. Julie Banerji  
Kolkata, India

Prof. Anna R. Bilia  
Florence, Italy

Prof. Maurizio Bruno  
Palermo, Italy

Prof. César A. N. Catalán  
Tucumán, Argentina

Prof. Josep Coll  
Barcelona, Spain

Prof. Geoffrey Cordell  
Chicago, IL, USA

Prof. Ana Cristina Figueiredo  
Lisbon, Portugal

Prof. Cristina Gracia-Viguera  
Murcia, Spain

Prof. Duvvuru Gunasekar  
Tirupati, India

Prof. Kurt Hostettmann  
Lausanne, Switzerland

Prof. Martin A. Iglesias Arteaga  
Mexico, D. F., Mexico

Prof. Jerzy Jaroszewski  
Copenhagen, Denmark

Prof. Leopold Jirovetz  
Vienna, Austria

Prof. Karsten Krohn  
Paderborn, Germany

Prof. Hartmut Laatsch  
Gottingen, Germany

Prof. Marie Lacaillle-Dubois  
Dijon, France

Prof. Shoei-Sheng Lee  
Taipei, Taiwan

Prof. Francisco Macias  
Cadiz, Spain

Prof. Imre Mathe  
Szeged, Hungary

Prof. Joseph Michael  
Johannesburg, South Africa

Prof. Ermino Murano  
Trieste, Italy

Prof. M. Soledade C. Pedras  
Saskatoon, Canada

Prof. Luc Pieters  
Antwerp, Belgium

Prof. Peter Proksch  
Düsseldorf, Germany

Prof. Phila Raharivelomanana  
Tahiti, French Polynesia

Prof. Luca Rastrelli  
Fisciano, Italy

Prof. Monique Simmonds  
Richmond, UK

Prof. John L. Sorensen  
Manitoba, Canada

Prof. Valentin Stonik  
Vladivostok, Russia

Prof. Winston F. Tinto  
Barbados, West Indies

Prof. Sylvia Urban  
Melbourne, Australia

Prof. Karen Valant-Vetschera  
Vienna, Austria

**INFORMATION FOR AUTHORS**

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site <http://www.naturalproduct.us>.

Authors may reproduce/republish portions of their published contribution without seeking permission from NPC, provided that any such republication is accompanied by an acknowledgment (original citation)-Reproduced by permission of Natural Product Communications. Any unauthorized reproduction, transmission or storage may result in either civil or criminal liability.

The publication of each of the articles contained herein is protected by copyright. Except as allowed under national "fair use" laws, copying is not permitted by any means or for any purpose, such as for distribution to any third party (whether by sale, loan, gift, or otherwise); as agent (express or implied) of any third party; for purposes of advertising or promotion; or to create collective or derivative works. Such permission requests, or other inquiries, should be addressed to the Natural Product Inc. (NPI). A photocopy license is available from the NPI for institutional subscribers that need to make multiple copies of single articles for internal study or research purposes.

**To Subscribe:** Natural Product Communications is a journal published monthly. 2012 subscription price: US\$1,995 (Print, ISSN# 1934-578X); US\$1,995 (Web edition, ISSN# 1555-9475); US\$2,495 (Print + single site online); US\$595 (Personal online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.

## DNA Binding Studies of Vinca Alkaloids: Experimental and Computational Evidence

Prateek Pandya<sup>a</sup>, Surendra P. Gupta<sup>a</sup>, Kumud Pandav<sup>a</sup>, Ritu Barthwal<sup>b</sup>, B. Jayaram<sup>c</sup> and Surat Kumar<sup>a</sup>

<sup>a</sup>Department of Chemistry, Faculty of Engineering, Dayalbagh Educational Institute, Dayalbagh, Agra-282 110 India

<sup>b</sup>Department of Biotechnology, Indian Institute of Technology, Roorkee-247667 India

<sup>c</sup>Department of Chemistry, Indian Institute of Technology, Hauz Khas, New Delhi - 110016, India

kumar.surat@gmail.com

Received: September 17<sup>th</sup>, 2011; Accepted: January 18<sup>th</sup>, 2012

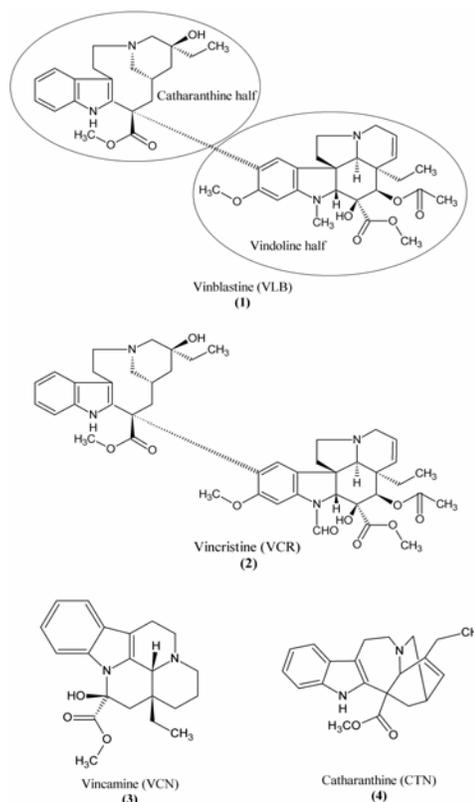
Fluorescence studies on the indole alkaloids vinblastine sulfate, vincristine sulfate, vincamine and catharanthine have demonstrated the DNA binding ability of these molecules. The binding mode of these molecules in the minor groove of DNA is non-specific. A new parameter of the purine-pyrimidine base sequence specificity was observed in order to define the non-specific DNA binding of ligands. Catharanthine had shown 'same' pattern of 'Pu-Py' specificity while evaluating its DNA binding profile. The proton resonances of a DNA decamer duplex were assigned. The models of the drug:DNA complexes were analyzed for DNA binding features. The effect of temperature on the DNA binding was also evaluated.

**Keywords:** DNA binding constants, DNA interaction, Fluorescence, Drug-DNA binding, Methylene linker, Purine-pyrimidine (Pu-Py) specificity.

Natural alkaloids of different types are known to interact with nucleic acids [1]. Vinca alkaloids, obtained from *Catharanthus roseus*, are an important class of alkaloids that possess extensive therapeutic potential. They are indole based alkaloids having one or two indole rings in their structures. Vinblastine and vincristine (VLB, **1** and VCR, **2**) are used as anticancer agents for the treatment of various types of leukemia. The main activity profile of vinblastine and vincristine alkaloids is due to their reversible binding interactions with tubulin protein. It depolymerises the microtubular assembly, thereby arresting the cell division, resulting in cell death. Vinblastine is mainly useful for the treatment of Hodgkin's disease, lymphocytic lymphoma, histiocytic lymphoma, advanced testicular cancer, advanced breast cancer, Kaposi's sarcoma, and Letterer-Siwe disease [2].

The Vinca alkaloids (**1-4**) (Figure 1) possess features like several H-bond acceptor/donor atoms, planar ring systems, and a large aromatic skeleton that are essential for the DNA binding activity. Vinca alkaloids exhibit fluorescence, which is quenched as a result of drug binding to DNA oligomers. This prompted us to investigate the binding of these alkaloids (**1-3**) with double helical DNA oligomers.

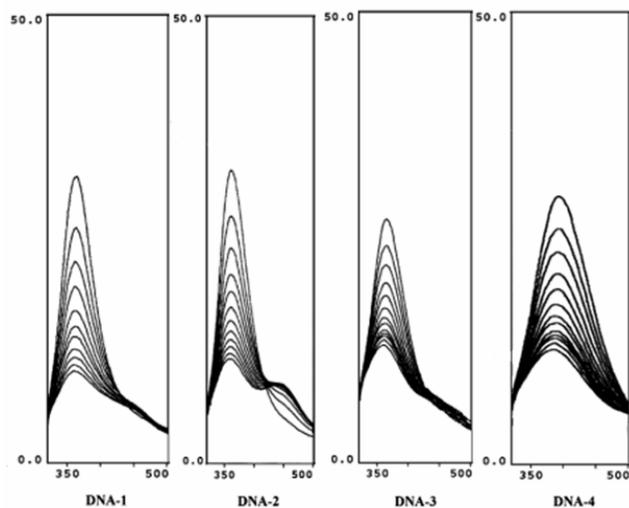
Initially all the compounds (**1-4**) were tested using UV-absorbance spectroscopy and it was found that they gave an absorption maximum in or around 260 nm, a region where DNA oligomers (DNA-1 to DNA-4) also absorb. Due to this overlap of the absorbance maxima of these compounds, it was found that UV absorbance spectroscopy was not very suitable for evaluating drug-DNA binding in the current study. Consequently, UV fluorescence was chosen for evaluating the drug-DNA binding. Fluorescence quenching experiments were employed in order to decipher the DNA binding profile of the Vinca alkaloids. In addition, the structural halves of VLB, catharanthine (CTN) and vindoline (VDL), were also investigated for their individual binding contributions to the overall binding of VLB. Vinblastine (**1**) has 2 or 4 methylene groups attached to an indole nucleus in the nine-



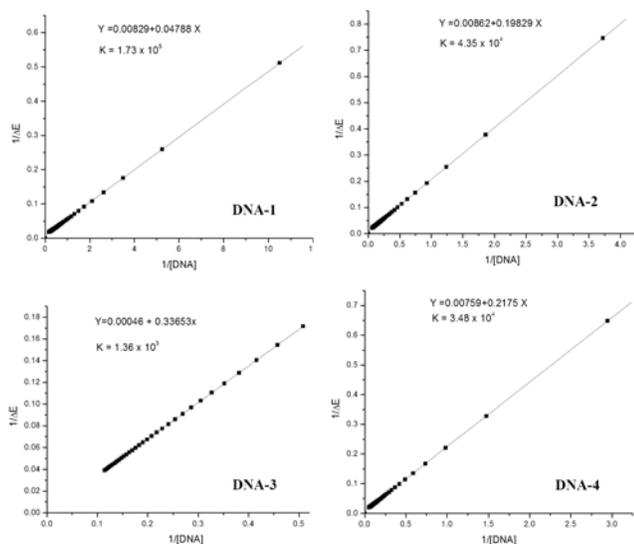
**Figure 1:** Structure of Vinca alkaloids **1-4**.

membered ring of catharanthine. This methylene linker provides extra flexibility to the VLB molecule in the event of substrate binding to furnish a stable drug:DNA complex. The difference between VLB and VCR is the presence of a -CHO group at the indole ring nitrogen in the vindoline half. Vincamine (VCN), on the other hand, possesses only one indole nucleus in its structure.

It was proposed to evaluate the DNA binding nature of VLB, VCR, VCN and the structural halves of VLB, viz. CTN and VDL. It was found that VLB (1), VCR (2), VCN (3) and CTN (4) gave consistent quenching of the fluorescence maxima after each addition of DNA (Figure 2). On the other hand, VDL did not offer any quenching of its fluorescence as a result of DNA binding. Vinblastine sulfate gave a fluorescence maximum at 362 nm when excited at 265 nm. The titration data obtained in fluorescence were fitted by the double reciprocal method (Figure 3) to collect the DNA binding constant  $K$ . As shown in Table 1, the values of the binding constant  $K$  were found to be of the order of  $10^4$  -  $10^5$  for vinblastine sulfate (1), and  $10^3$  -  $10^5$  for the other Vinca alkaloids (2, 3 and 4). This study showed that Vinblastine and other Vinca alkaloids bind with DNA oligomer duplexes suggesting a possible similarity in their DNA binding motifs.



**Figure 2:** Fluorescence spectra of vinblastine sulfate titrations with DNA decamer sequences.



**Figure 3:** Double reciprocal plots of VLB: DNA titrations at 5°C.

The possibility of DNA base sequence specificity in the DNA binding of the vinblastine molecule was further evaluated using DNA decamer sequences designed to have a 4 base pair specific central core. In this study, four DNA decamers were used to assess

the base sequence specificity of DNA binding interaction of vinblastine. To understand the interaction of vinblastine with specific DNA sequences, the drug was titrated with four different DNA decamers viz.



Temperature dependent DNA binding titrations were carried out in order to investigate if vinblastine sulfate had any DNA base sequence preference and to see the effect of temperature on the DNA binding strength of these drugs. The effects of temperature variation on the VLB: DNA complexes are summarized in Table 2.

**Table 1:** DNA binding patterns of vinblastine sulfate, vincristine sulfate, vincamine, and catharanthine at 25°C.

Alkaloids	Complex	$K_{\text{DN}}$ (mole <sup>-1</sup> )
Vinblastine sulfate (1)	DNA-1	$2.53 \times 10^5$
	DNA-2	$1.29 \times 10^5$
	DNA-3	$1.36 \times 10^5$
	DNA-4	$6.17 \times 10^4$
Vincristine (2)	DNA-1	$2.90 \times 10^4$
	DNA-2	$2.41 \times 10^4$
	DNA-3	$1.22 \times 10^4$
	DNA-4	$4.10 \times 10^4$
Vincamine (3)	DNA-1	$3.37 \times 10^4$
	DNA-2	$2.95 \times 10^4$
	DNA-3	$3.00 \times 10^4$
	DNA-4	$5.27 \times 10^3$
Catharanthine (4)	DNA-1	$1.95 \times 10^4$
	DNA-2	$5.10 \times 10^4$
	DNA-3	$1.35 \times 10^5$
	DNA-4	$3.30 \times 10^4$

**Table 2:** Binding constant values (mole<sup>-1</sup>) of vinblastine with four DNA sequences (DNA-1 to DNA-4) obtained from the double reciprocal method.

Complex	Binding constants at various temperatures		
	5°C	15°C	25°C
DNA-1	$1.70 \times 10^5$	$4.13 \times 10^4$	$2.53 \times 10^5$
DNA-2	$4.35 \times 10^4$	$3.56 \times 10^4$	$1.29 \times 10^5$
DNA-3	$1.36 \times 10^3$	$0.63 \times 10^2$	$1.36 \times 10^5$
DNA-4	$3.48 \times 10^4$	$5.35 \times 10^4$	$6.17 \times 10^4$

**Features of DNA sequences (DNA-1 to DNA-4) in relation to their VLB binding:** The characteristic feature of the selected DNA sequences (DNA-1 to DNA-4) is the presence of six G.C base pairs in each of them. However, DNA-1 possesses two 5'-GpC-3' base steps in each strand. DNA-2 possesses no such base steps from the 5' to 3' ends. On the other hand, DNA-3 and DNA-4 contain four 5'-GpC-3' base steps in their structures.

From the observations of binding constants, it appears that the binding of VLB with DNA sequences does not depend on the 5'-GpC-3' base steps since the binding constants of VLB: DNA-3 and VLB: DNA-4 are smaller as compared with DNA-1 and DNA-2 at lower temperatures, in spite of having a larger number of 5'-GpC-3' base sequences.

Since all four DNA sequences contain equal numbers of G.C base pairs (six) in their structures, it seems likely that their location on the DNA molecule affects the binding of VLB. Moreover, the central core in both DNA-3 and DNA-4 does not consist of G.C base pairs and consequently showed weaker binding with VLB, since the minor groove of the AT sequence is narrower to

accommodate the bulky molecule of VLB. The terminal G.C sequences, unlike the central core, are not contributing significantly to the binding due to their greater flexibility and accessibility to the solution environment. The possibility of a greater flexibility and exposure to water does not allow VLB to stay longer on the terminal G.C sequences leading to weaker drug-DNA interactions.

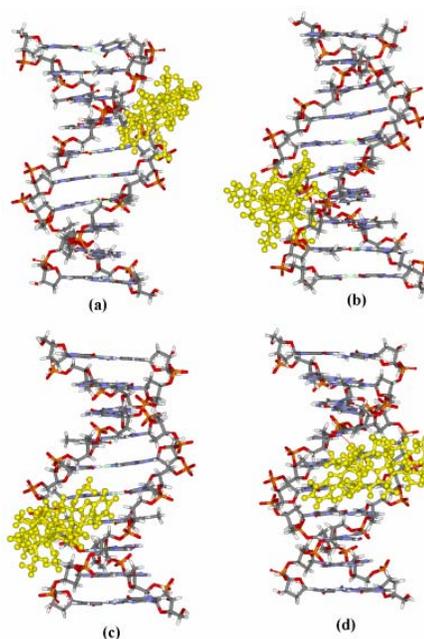
The DNA binding constant for the VLB:DNA-1 complex at 5°C revealed the stronger interaction of VLB with DNA-1 and DNA-2. On the other hand, the DNA binding of VLB with DNA-3 and DNA-4 was found to be weaker, indicating a slight preference for GC-specific sites. Multiple binding modes of VLB with DNA are also possible, as reported in the case of vincristine [3]. However, the exact nature of the multiple binding motifs is still unknown. The overall DNA binding nature of these alkaloids was found to be non-specific in terms of their sequence preference.

The plausible intercalation facilitated by the indole ring moiety in the case of small molecules could occur, as indicated by Schneider and coworkers [4]. Our previous study [5] forwarded the model that the molecule prefers to sit in the minor groove instead of preferentially intercalating between the DNA base pairs. The DNA binding results obtained in the fluorescence titration experiments (Table 1) clearly suggest that vinblastine has some interaction with DNA. However, this does not indicate the mode of interaction of the drug.

The structural features of the alkaloid-DNA complexes were further investigated using a molecular docking method. Minor groove docking was performed due to the fact that VLB and VCR are complex molecules with larger puckered regions, while possessing an indole ring as a planar structure. It is, therefore, suggested that the puckered regions of these molecules will provide hindrance to the entry of the molecules between the base pairs of the DNA double helix and precluding the possibility of complete insertion between the stack of DNA base pairs. In addition, with the absence of any strong positive charge in the ring system, it is quite likely that intercalation is not the favorable mode of binding. However, the possibility of partial intercalation cannot be ruled out, due to the presence of indole rings in the alkaloid structures.

Thermodynamic calculations are essential in order to complement the structural data. Contrary to a large family of DNA binding agents, all the compounds employed in this study were considered as having no formal charge. Consequently, the docking results using the DNADock program showed a large non-electrostatic contribution to the overall binding energy of these molecules.

Moderate DNA binding constants obtained by both experimental methods and PreDDICTA software indicate that the electrostatic interactions may not have significantly contributed to the DNA binding of these compounds. It was observed in the docking studies that van der Waal's forces and hydrophobic interaction have the most significant contribution in the DNA binding of these molecules. The possibility of the formation of H-bonds also exists due to the presence of H-bond donor (OH) and acceptor (=O) atoms in the indole alkaloids, as well as functional groups present in the minor groove of DNA structures (guanine-NH<sub>2</sub> group at position 2, and cytosine =O atom at position 2). These atoms may provide sufficient anchoring support for drug molecules to remain within the minor groove [7]. Moreover, this flexibility also affords the formation of a few new H-bonds between the drug and DNA atoms providing further stability to the drug-DNA complexes.



**Figure 4:** Docked poses of vinblastine sulfate with DNA decamer sequences. (a) VLB:DNA-1, (b) VLB:DNA-2, (c) VLB:DNA-3, (d) VLB:DNA-4.

#### Purine-Pyrimidine (Pu-Py) Specificity Pattern

This study was designed to find the DNA base sequence specific patterns of small molecules with designed DNA oligomers. However, the docked structures of Vinca alkaloids **1-4** showed purine-pyrimidine specific patterns, which could be important in designing lead compounds for specific sequences (Table 3). Purine-pyrimidine specificity has been observed in the case of the protein-DNA [8] and the drug-DNA complexes [9]. The analysis of docked structures was based on a base proximity model in which the DNA bases in the close immediacy of the bound molecule were taken into consideration. It should be considered in view of the fact that the two groups of nuclear bases, namely, purines (A or G) and pyrimidines (C or T), individually have a similar electron density cloud around them, and, as a result thereof, offering the same electron density and electrostatic potential for the purpose of drug binding.

The binding sites of VLB, VCN and CTN consisted of 3-base pairs while that of VCR consisted of 4-base pairs. Out of three DNA decamer sequences, the binding patterns of any two sequences were grouped based on their similarity. The exact match of purines and pyrimidines of the binding site was defined as 'Same' pattern. '1-Base altered' pattern was assigned to the groups that contain one altered base as purines or pyrimidine in both the docked structures of each group. It was observed that CTN gave binding to all 4 DNA oligomers 'same' pattern of purine-pyrimidine specific binding sites, while other alkaloids gave 'same' and '1-base altered' patterns.

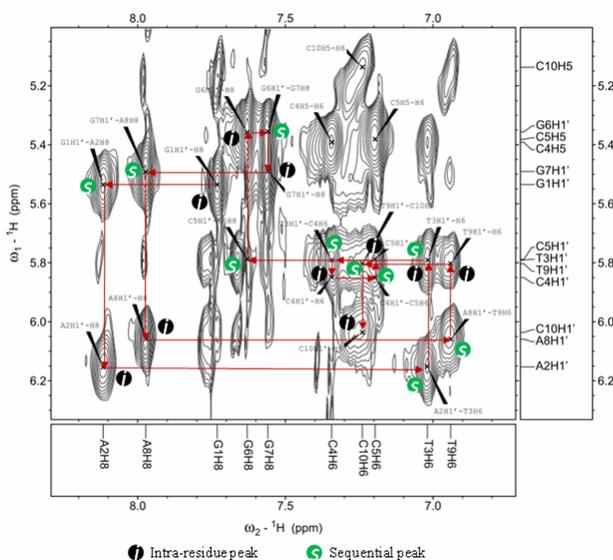
Thus, we can herewith propose that purine-pyrimidine (Pu-Py) specificity patterns can be used as another broader measure of DNA binding specificity exhibited by a drug. In the case of non-specific DNA binding of drugs, the 'purine-pyrimidine' specificity can be employed as a tool to design efficient DNA binding drugs in the management of various disorders.

2D <sup>1</sup>H NOESY NMR data on DNA-2 assisted in the assignment of the individual proton chemical shifts. All the <sup>1</sup>H NMR chemical shift values were secured by deciphering the sequential inter- and

**Table 3:** Purine-pyrimidine specific patterns obtained from drug-DNA docked structures.

Alkaloids	Complex	Binding Site	Pu-Py Specificity	Binding Pattern
VLB (1)	DNA-1	5'-G <sub>4</sub> -G <sub>5</sub> -C <sub>6</sub>	5'-Pu-Pu-Py	Same
	DNA-2	5'-G <sub>7</sub> -A <sub>8</sub> -T <sub>9</sub>	5'-Pu-Pu-Py	
	DNA-3	5'-T <sub>6</sub> -T <sub>7</sub> -G <sub>8</sub>	5'-Py-Py-Pu	1-Base altered
	DNA-4	5'-T <sub>5</sub> -A <sub>6</sub> -A <sub>7</sub>	5'-Py-Pu-Pu	
VCR (2)	DNA-1	5'-G <sub>1</sub> A <sub>2</sub> T <sub>3</sub> G <sub>4</sub>	5'-Pu-Pu-Py-Pu	1-Base altered
	DNA-2	5'-G <sub>1</sub> A <sub>2</sub> T <sub>3</sub> C <sub>4</sub>	5'-Pu-Pu-Py-Py	
	DNA-3	5'-G <sub>1</sub> G <sub>2</sub> C <sub>3</sub> A <sub>4</sub>	5'-Pu-Pu-Py-Pu	1-Base altered
	DNA-4	5'-G <sub>1</sub> G <sub>2</sub> C <sub>3</sub> T <sub>4</sub>	5'-Pu-Pu-Py-Pu	
VCN (3)	DNA-1	5'-A <sub>2</sub> T <sub>3</sub> G <sub>4</sub>	5'-Pu-Py-Pu	1-Base altered
	DNA-2	5'-A <sub>2</sub> T <sub>3</sub> C <sub>4</sub>	5'-Pu-Py-Py	
	DNA-3	5'-A <sub>4</sub> A <sub>5</sub> T <sub>6</sub>	5'-Pu-Pu-Py	Same
	DNA-4	5'-G <sub>1</sub> G <sub>2</sub> C <sub>3</sub>	5'-Pu-Pu-Py	
CTN (4)	DNA-2	5'-A <sub>2</sub> -T <sub>3</sub> -C <sub>4</sub>	5'-Pu-Py-Py	Same
	DNA-3	5'-A <sub>5</sub> -T <sub>6</sub> -T <sub>7</sub>	5'-Pu-Py-Py	
	DNA-1	5'-T <sub>3</sub> -G <sub>4</sub> -G <sub>5</sub>	5'-Py-Pu-Pu	Same
	DNA-4	5'-T <sub>5</sub> -A <sub>6</sub> -A <sub>7</sub>	5'-Py-Pu-Pu	

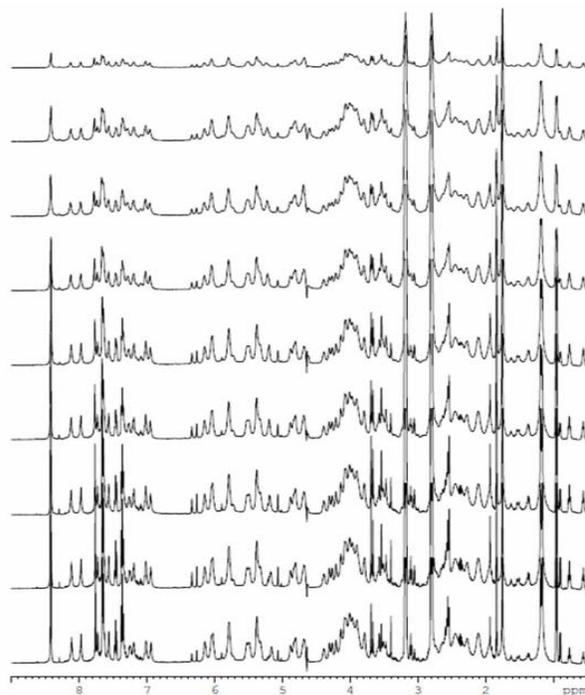
intra-nucleotide cross-peaks between base protons and corresponding H1' protons in the finger print region (Fig. 5). Later, other regions of NOE cross-peaks (base proton-H3'; base proton-H4'; and base proton to H2'/H2'') were also analyzed. An unambiguous assignment of almost all DNA base protons and deoxyribose sugar protons was furnished and listed in Table 4. All the 10 intranucleotide NOE crosspeaks for base-H3' protons were not resolved. In the base proton-H4' region of the 2D-NOESY experiment, however, only 5 intranucleotide NOE cross peaks were assigned for G1, A2, G6, G7, and A8 residues of the DNA-2 decamer duplex.

**Figure 5:** NOESY spectrum of 5'-d(G<sub>1</sub>A<sub>2</sub>T<sub>3</sub>C<sub>4</sub>G<sub>5</sub>G<sub>6</sub>G<sub>7</sub>A<sub>8</sub>T<sub>9</sub>C<sub>10</sub>)<sub>2</sub> (DNA-2) sequence showing finger print region of the spectrum. Symbol *i* indicates the intra-nucleotide cross peaks between base proton and H1' proton, while symbol *s* signifies the inter-nucleotide cross peaks between H1' protons of one nucleotide and base protons (H6 or H8) of the next nucleotide of the DNA backbone.

1D <sup>1</sup>H NMR titration of vinblastine sulfate (1) with DNA decamer sequence DNA-2 was carried out in order to find any chemical shift change in the DNA spectrum after drug binding (Figure 6). The NMR titration was performed to obtain the 1:1 and 2:1 molar

**Table 4:** NOE cross peaks of various protons of DNA2.

Residue	H8/H6	H5	Me	H1'	H2'	H2''	H3'	H4'
G1	7.73	-	-	5.53	2.46	2.63	n.a.	4.35
A2	8.12	-	-	6.15	2.56	2.81	4.89	4.30
T3	7.02	-	1.18	5.79	1.93	2.31	4.89	n.a.
C4	7.35	5.39	-	5.81	1.94	2.29	n.a.	n.a.
C5	7.19	5.33	-	5.38	1.73	2.11	n.a.	n.a.
G6	7.63	-	-	5.36	2.48	2.53	4.83	4.14
G7	7.56	-	-	5.49	2.43	2.57	4.84	4.21
A8	7.97	-	-	6.05	2.41	2.74	4.83	4.26
T9	6.95	-	1.16	5.79	1.78	2.30	4.81	n.a.
C10	7.25	5.15	-	6.04	2.10	2.10	4.36	n.a.

**Figure 6:** 1D proton NMR titration of VLB:DNA-2 complex.

ratio of drug: DNA. However, no changes in the chemical shift values of either drug or DNA proton resonances were observed as a result of the drug-DNA complexation. We may conclude that drug binding to DNA was not observed at millimolar concentration by the NMR method in this case. This needs further experimentation using other NMR techniques.

## Experimental

**General:** Vinblastine sulfate (VLB, 1), and other alkaloids were purchased from Sigma-Aldrich Chemicals Co., USA, and were used after checking for their purity by HPLC. Four DNA decamer sequences were purchased from Sigma-Aldrich as desalted base, viz, DNA-1 to DNA-4.

**Stock solutions:** Stock solutions of Vinca alkaloids (1-4) and DNA were made in 20 mM sodium phosphate buffer (5 mM Na<sub>2</sub>HPO<sub>4</sub>, 5 mM NaH<sub>2</sub>PO<sub>4</sub>, 1 mM (Na)<sub>2</sub>EDTA and 3 mM NaCl) at pH 7.4. Concentrations of alkaloids (1-4) were determined volumetrically and those of DNA decamers (DNA-1 to DNA-4) spectrophotometrically using the molar extinction coefficients for DNA decamer sequences, ε<sub>260</sub> = 95000 for DNA-1, ε<sub>260</sub> = 92600 for DNA-2, ε<sub>260</sub> = 93200 for DNA-3, ε<sub>260</sub> = 96600 for DNA-4. All the compounds obeyed Beer's law in the concentration range employed. The DNA decamers were annealed slowly in the buffer solution. All solutions were freshly prepared and stored below freezing point, duly protected. No change in the optical properties of the compounds (1-4) or DNA was observed.

**Fluorescence measurements:** The fluorescence measurements were recorded on a Hitachi model F4010 spectrofluorimeter (Hitachi Ltd, Tokyo, Japan), where a fixed concentration of vinblastine and other indole derivatives were titrated with increasing concentration of DNA decamers in a fluorescence free quartz cuvette of 1 cm path length. The excitation band pass was fixed at 5 nm while different emission band pass wavelengths were used (5 nm, 10 nm & 20 nm). The scan speed of 240 nm/min was kept fixed during all the experiments. Fluorescence titration experiments of vinblastine and other alkaloids with calf thymus DNA were performed with an excitation band pass of 5 nm, and an emission band pass of 20 nm.

**DNA docking:** The DNA binding of Vinca alkaloids was studied by the molecular docking program (DNADock) and the energy calculation program (PreDDICTA) based on a specialized protocol for DNA minor groove binding. In the DNADock program, rigid body docking calculations allowed the drug to remain flexible to obtain a low energy conformation at a specific site in the DNA minor groove (Figure 4), taking into account the thermodynamic aspect of drug-DNA binding [6]. The final drug-DNA complex structures were subjected to binding free energy analysis using PreDDICTA software tool [6].

**NMR methods:** All the NMR experiments were recorded on a Bruker 500-AVANCE Spectrometer. 2D <sup>1</sup>H NOESY NMR data on DNA-2 was obtained with 300 ms mixing time. Chemical shifts are given in ppm. NMR experiments were conducted in a sodium phosphate buffer of pH 7.2 prepared in D<sub>2</sub>O. The water resonance was used as the reference signal. NMR spectra were processed using Topspin software. 2D NOESY assignments were made using SPARKY software from UCSF.

**Acknowledgments** - Authors are thankful to Prof. V. G. Das, Director, Dayalbagh Educational Institute, Dayalbagh Agra, India for providing the laboratory facilities. Authors also acknowledge their gratitude to the Department of Biotechnology (DBT), New Delhi, India for the grant of a Major Research Proposal to SK, under which this work could be accomplished. A Research Associateship to PP by the DBT, New Delhi, India and Project Fellowships to SPG and KP by the UGC, New Delhi are gratefully acknowledged. One of them (SK) acknowledges profound humble gratitude to Revered Prof. P. S. Satsangi Sahab, the Chairman, Advisory Committee on Education, Dayalbagh, Agra, India for the sustained inspiration and constant encouragement.

## References

- [1] (a) Islam MM, Pandya P, Kumar S, Kumar GS. (2009) RNA targeting through small molecule binding: studies on t-RNA binding by cytotoxic protoberberine alkaloid coralayne. *Journal of Molecular BioSystems*, **5**, 244-254; (b) Islam MM, Pandya P, Roy-Chowdhury S, Kumar S, Kumar GS. (2008) Binding of DNA binding alkaloids berberine and palmatine to t-RNA and comparison to ethidium: Spectroscopic and molecular modeling studies. *Journal of Molecular Structure*, **891**, 498-507; (c) Maiti M, Kumar GS. (2007) Molecular aspects on the interaction of protoberberine, benzophenanthridine, and aristolochia group of alkaloids with nucleic acid structures and biological perspectives. *Medicinal Research Review*, **27**, 649-695.
- [2] (a) Svoboda GH, Neuss N, Gorman M. (1959) A note on the alkaloids of *Vinca rosea* Linn. (*Catharanthus roseus* G. Don.). II. Catharanthine, lochnericine, vindolinine, and vindoline. *Journal of the American Pharmaceutical Association*, **48**, 659-666; (b) Paci A, Mercier L, Bourget P. (2003) Identification and quantitation of antineoplastic compounds in chemotherapeutic infusion bags by use of HPTLC: application to the vinca-alkaloids. *Journal of Pharmaceutical and Biomedical Analysis*, **30**, 1603-1610; (c) Sersa G, Krzic M, Sentjurc M, Ivanusa T, Beraus K, Cemazar M, Aursperg M, Swartz HM. (2001) Reduced tumor oxygenation by treatment with vinblastine. *Cancer Research*, **61**, 4266-4271; (d) Struski S, Cornillet-Lefebvre P, Doco-Fenzy M, Dufer J, Ulrich E, Masson L, Michel N, Gruson N, Potron G. (2002) Cytogenetic characterization of chromosomal rearrangement in a human vinblastine-resistant CEM cell line: Use of comparative genomic hybridization and fluorescence *in situ* hybridization. *Cancer Genetics and Cytogenetics*, **132**, 51-54.
- [3] Tyagi G, Jangir DK, Singh P, Mehrotra R. (2010) DNA interaction studies of an anticancer plant alkaloid, vincristine, using Fourier transform infrared spectroscopy. *DNA and Cell Biology*, **29**, 677-683.
- [4] (a) Sartorius J, Schneider HJ. (1995) NMR-titrations with complexes between ds-DNA and indole derivatives including tryptophane containing peptides. *FEBS Letters*, **374**, 387-392; (b) Sartorius J, Schneider HJ. (1997) Intercalation mechanisms with ds-DNA: Binding modes and energy contributions with benzene, naphthalene, quinoline and indole derivatives including some antimalarials. *Journal of Chemical Society Perkin Transactions 2*, 2319-2327.
- [5] Pandya P, Islam, M, Kumar GS, Jayaram B, Kumar S. (2010) DNA minor groove binding of small molecules: experimental and computational evidence. *Journal of Chemical Sciences*, **122**, 247-257.
- [6] (a) Gupta A, Gandhimathi A, Sharma P, Jayaram B. (2007) ParDOCK: An all atom energy based Monte Carlo docking protocol for protein-ligand complexes. *Protein Peptide Letters*, **14**, 632-646; (b) Shaikh SA, Jayaram B. (2007) A swift all-atom energy-based computational protocol to predict DNA-ligand binding affinity and deltaTm. *Journal of Medicinal Chemistry*, **50**, 2240-2244.
- [7] (a) Kumar S, Bathini Y, Zimmermann J, Pon RT, Lown JW. (1990) Sequence specific molecular recognition and binding of a GC recognizing Hoechst 33258 analogue to the decadeoxyribonucleotide d-[CATGGCCATG]<sub>2</sub>: Structural and dynamic aspects deduced from <sup>1</sup>H-NMR studies. *Journal of Biomolecular Structure Dynamics*, **8**, 331-357; (b) Kumar S, Zimmermann J, Joseph T, Pon RT, Lown JW. (1991) Structural and dynamic aspect of binding of a non-intercalating thiazole-lexitropsin to the decadeoxyribonucleotide d-[CGCAATTGCG]<sub>2</sub>: A <sup>1</sup>H-NMR study and molecular modeling study. *Journal of Biomolecular Structure Dynamics*, **9**, 1-21; (c) Singh MP, Kumar S, Joseph T, Bathini Y, Lown JW. (1992) Synthesis and sequence specific DNA binding of topoisomerase inhibitory sequence analogue of Hoechst 33258 designed for altered base and sequence recognition. *Chemical Research in Toxicology*, **5**, 597-607.
- [8] Nikolajewa S, Beyer A, Friedel M, Hollunder J, Wilhelm T. (2005) Common patterns in type II restriction enzyme binding sites. *Nucleic Acids Research*, **33**, 2726-2733.
- [9] Perez JM, Montero EI, Gonzalez AM, Solans X, Font-Bardia M, Fuertes MA, Alonso C, Navarro-Ranninger C. (2000) X-Ray structure of cytotoxic *trans*-[PtCl<sub>2</sub>(dimethyl-amine)]<sub>2</sub> (isopropylamine): Interstrand cross-link efficiency, DNA sequence specificity, and inhibition of the B-Z transition. *Journal of Medicinal Chemistry*, **43**, 2411-2418.





# Natural Product Communications

2012

Volume 7, Number 3

## Contents

<u>Original Paper</u>	<u>Page</u>
<b>Efficient Preparation of Incensole and Incensole Acetate, and Quantification of These Bioactive Diterpenes in <i>Boswellia papyrifera</i> by a RP-DAD-HPLC Method</b> Michael Paul and Johann Jauch	283
<b>A Comparison Investigation on the Solubilization of Betulin and Betulinic Acid in Cyclodextrin Derivatives</b> Hai Ming Wang, Codruta M. Șoica and Gerhard Wenz	289
<b>Two New Antimicrobial Metabolites from the Endophytic Fungus, <i>Seimatosporium</i> sp.</b> Hidayat Hussain, Karsten Krohn, Barbara Schulz, Siegfried Draeger, Mamona Nazir and Muhammad Saleem	293
<b>Simulation of Intramolecular Hydrogen Bond Dynamics in Manzamine A as a Sensitive Test for Charge Distribution Quality</b> Dmitry A. Shulga, Dmitry I. Osolodkin, Vladimir A. Palyulin and Nikolay S. Zefirov	295
<b>A Quinoline Based bis-Urea Receptor for Anions: A Selective Receptor for Hydrogen Sulfate</b> Tiffany H. Russ, Avijit Pramanik, Maryam E. Khansari, Bryan M. Wong and Md. Alamgir Hossain	301
<b>DNA Binding Studies of Vinca Alkaloids: Experimental and Computational Evidence</b> Surendra P. Gupta, Prateek Pandya, R Barthwal, B Jayaram and Surat Kumar	305
<b>Natural Flavonoids Interact with Dinitrobenzene System in Aprotic Media: An Electrochemical Probing</b> Nasima Arshad, Naveed K. Janjua, Athar Y. Khan, Azra Yaqub, Torsten Burkholz and Claus Jacob	311
<b>Live Cell Imaging of a Fluorescent Gentamicin Conjugate</b> Jorge O. Escobedo, Yu-Hsuan Chu, Qi Wang, Peter S. Steyger and Robert M. Strongin	317
<b>Molecular Recognition of Carbohydrates: Evaluation of the Binding Properties of Pyrazole-based receptors and their Comparison with Imidazole- and Indole-based Systems</b> Claudia Sonnenberg, André Hartmann and Monika Mazik	321
<b>Transformations of Griseofulvin in Strong Acidic Conditions – Crystal Structures of 2'-Demethylgriseofulvin and Dimerized Griseofulvin</b> Barbara Leśniowska, Said Jebors, Anthony W. Coleman and Kinga Suwińska	327
<b>Rapid Acyl Migration Between Pyrogallyl 1,2- and 1,3-Dipivaloates</b> Yaming Shan, Jimmy (Jingyue) Liu, Nigam P. Rath and George W. Gokel	333
<b>Toward Engineering Intra-Receptor Interactions Into Bis(crown ethers)</b> Martin R. Krause and Stefan Kubik	337
<b>Monitoring Stepwise Proteolytic Degradation of Peptides by Supramolecular Domino Tandem Assays and Mass Spectrometry for Trypsin and Leucine Aminopeptidase</b> Garima Ghale, Nikolai Kuhnert and Werner M. Nau	343
<b>Intracellular Localization of PNA in Human Cells upon its Introduction by Electroporation</b> Eri Noguchi, Narumi Shigi and Makoto Komiyama	349
<b>Fliposomes: pH-controlled Release from Liposomes Containing New <i>trans</i>-2-Morpholinocyclohexanol-based Amphiphiles that Perform a Conformational Flip and Trigger an Instant Cargo Release upon Acidification</b> Yu Zheng, Xin Liu, Nataliya M. Samoshina, Vyacheslav A. Chertkov, Andreas H. Franz, Xin Guo and Vyacheslav V. Samoshin	353
<b>Competition of Ester, Amide, Ether, Carbonate, Alcohol and Epoxide Ligands in the Dirhodium Experiment (Chiral Discrimination by NMR Spectroscopy)</b> Jens T. Mattiza, Vera Meyer, Gülsüm Özüdüru, Tanja Heine, Joerg Fohrer and Helmut Duddeck	359
<b>Synthesis of Acetylene Bridged Germanium Phthalocyanines</b> Felicitas Plenzig, Alexey Lyubimtsev and Michael Hanack	363
<b>Host-Guest Chemistry of Alkaloids</b> Anatoly K. Yatsimirsky	369
<b>Proanthocyanidins: Oligomeric Structures with Unique Bio-chemical Properties and Great Therapeutic Promise</b> Zhanjie Xu, Peng Du, Peter Meiser and Claus Jacob	381
<b>Aurones: Interesting Natural and Synthetic Compounds with Emerging Biological Potential</b> Clemens Zwergel, François Gaascht, Sergio Valente, Marc Diederich, Denyse Bagrel and Gilbert Kirsch	389
<b>Sulfur and Sulfur Compounds in Plant Defence</b> Ifeanyi D. Nwachukwu, Alan J. Slusarenko and Martin C. H. Gruhlke	395
<b>The Effect of Diallyl Polysulfanes on Cellular Signaling Cascades</b> Mathias Montenarh and Nathaniel. E. B. Saidu	401
<b>Interactions of Calix[<i>n</i>]arenes with Nucleic Acids</b> Miao Li, Max Sena Peters and Thomas Schrader	409