# Microtubulin Binding Sites as Target for Developing Anticancer Agents

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**Abstract**: Microtubules (MTs) play important and diverse roles in eukaryotic cells. Their function and biophysical properties have made α–and β–tubulin, the main components of MTs, the subject of intense study. Interfering with normal MT dynamics, for example, by the addition of tubulin ligands, can cause the cell great distress and affect MT stability and functions, including mitosis, cell motion and intracellular organelle transport. It has been shown in the literature that tubulin is an important target molecule for developing anticancer drugs. Tubulin binding molecules have generated considerable interest after the successful introduction of the taxanes into clinical oncology and the widespread use of the vinca alkaloids vincristine and vinblastine. These compounds inhibit cell mitosis by binding to the protein tubulin in the mitotic spindle and preventing polymerization into the MTs. This mode of action is also shared with other natural agents eg colchicine and podophyllotoxin. However various tubulin isotypes have shown resistance to taxanes and other MT agents. Therefore, there is a strong need to design and develop new natural analogs as antimitotic agents to interact with tubulin at sites different from those of vinca alkaloids and taxanes. This minireview provides SAR on several classes of antimitotic agents reported in the literature. The structures and data given are essential to the scientists who are involved in drug design and development in the field of anticancer drugs.

**Keywords:** Microtubules, Tubulin binding molecules, Oncology, Paclitaxel, Chemotherapy.

## Microtubules

Microtubules, the dynamic pipe-like protein composed of α,β-heterodimers, are integral components of the mitotic spindle which is intimately involved in cell division. Microtubules (MTs) are long, hollow structures with 5nm walls surrounding a cavity, usually with internal and external diameters of 15 and 25nm, respectively. They are made up of two globular protein subunits, α- and β-tubulin. The α and β subunits form heterodimers which aggregate in a head to tail arrangement to form long tubes made up of stacked rings with each ring usually containing 13 subunits, which is known as protofilament [1-8]. After an induction period, the protofilaments group together to form a C-shaped protein sheet, which then curl around to give a pipe-like structure known as microtubule.

MTs are the principal components of the cytoskeleton of eukaryotic cells required for cell division, motility, cell form and morphogenesis, secretion, and cell surface specialization.

## Components of MTs [9-38]

α- and β-tubulin, each with a molecular weight of about 50 kDa, are regarded as the main components of MT. The structures of α- and β-tubulin are basically identical: each monomer is formed by a core of two β-sheets surrounded by α-helices. The monomer structure is very compact, but can be divided into three functional domains: the amino terminal domain containing the nucleotide-binding region, an intermediate domain containing the paclitaxel-binding site, and the carboxy-terminal domain, which probably constitutes the binding surface of kinesin Eg5, a motor protein that is essential for production of a bipolar spindle. The N-terminal consists of six parallel β strands alternating with helices. A mixed β-sheet of four strands and three surrounding helices run across the first two domains on the outside surface of the MT.

An aggregation of γ-tubulins, organised in the ring structures within centrosomes, are involved in nucleating MTs. δ-tubulin is implicated in the formation of basal bodies and centrioles. Its loss is associated with a failure to produce the C tubule of the triplet MTs.

MTs grow from discrete assembly sites in cells called microtubule organizing centers (MTOCs). These MTOCs form a focus for MTs growth, and all microtubules initially begin to grow from one of those centers. In most cells there is one major type of MTOC known as the cell center, or centrozome, which contains two microtubular structures known as centrioles. Organisation of microtubule growth at the MOTC involves the presence of γ-tubulin.

Two molecules of energy rich guanosine triphosphate (GTP) are also essential components of MTs. One of these GTP molecules is tightly bound and cannot be removed without denaturing the heterodimer, while the other GTP molecule is freely exchangeable with unbound GTP. GTP is non-exchangeably bound to α-tubulin, whereas GTP and guanosine diphosphate (GDP) can exchange on β. The exchangeable GTP molecule is intimately involved in the regulation of tubulin functions. Binding of this nucleotide to the protein is required for MT polymerization, whereas the hydrolysis of the GTP bound to polymerized tubulin is required for MT depolymerization.

A number of proteins, known as microtubules associated proteins (MAP), each with a mass of 200 kDa, is associated with the formation and stability of MTs. MAPs constitute a

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complex family of proteins, including MAP2, MAP4, Mip-90, tau and STOP, many of which have been shown to regulate tubulin polymerization and function. Kinesins are motor proteins which are essential for production of bipolar spindle rather than tubulin. Kin 1 kinesins are enzymes that are regarded as MT destabilising agents.

**Function of MTs**

1. To form mitotic spindles required for the movement of chromosomes to the poles of the new cell during cell division.
2. To give cells both shape and an organised structure.
3. To provide the tracks for transport of vesicles, organelles such as secretory granules, and mitochondria from one part of the cell to another.

**Role of MTs in Cell Division [39-40]**

Microtubules are mainly responsible for mitosis - a stage in the process of cell division where segregation of chromosomes occurs prior to cell replication. Mitosis is a particularly attractive stage of the cell cycle for interference of normal function.

**MTs Dynamics [39,41-42]**

Microtubules are continuously assembled and disassembled and therefore are dynamic portions of the cell cytoskeleton. The following factors are responsible for the dynamic property of MTs:

1. GTP / GDP: Tubulin is a GTP bound protein required for MT polymerisation, whereas the hydrolysis of the GTP bound to polymerised tubulin is required for MT depolymerisation. The rate of MT nucleation and elongation is thought to be influenced by the free GTP-tubulin concentration.
2. Ion concentration: Polymerisation of tubulin heterodimer is regulated by $Ca^{2+}$ concentration. The low cytosolic $Ca^{2+}$ level characteristic of the resting state of most eukaryotic cells promotes MT assembly, while localised increases in $Ca^{2+}$ cause MT disassembly.
3. Conformational change: Nucleotide dependent conformational change is also responsible for the dynamic assembly/ disassembly property of tubulin.
4. Microtubules are constantly assembled and disassembled by warmth, cold and other factors.

**MTs Isotypes [43-45]**

Tubulin, an α, β-heterodimer, exists as several isotypic forms which differ in their amino acid sequences and their conformations e.g., $\beta_I$, $\beta_{II}$, $\beta_{III}$, $\beta_{IVa}$, $\beta_{IVb}$ and $\beta_V$.

$\beta_I$ and $\beta_{IVb}$ are constitutive; $\beta_{II}$ and $\beta_{IVa}$ are formed in the brain and nervous system; $\beta_{III}$ is restricted to neurons &

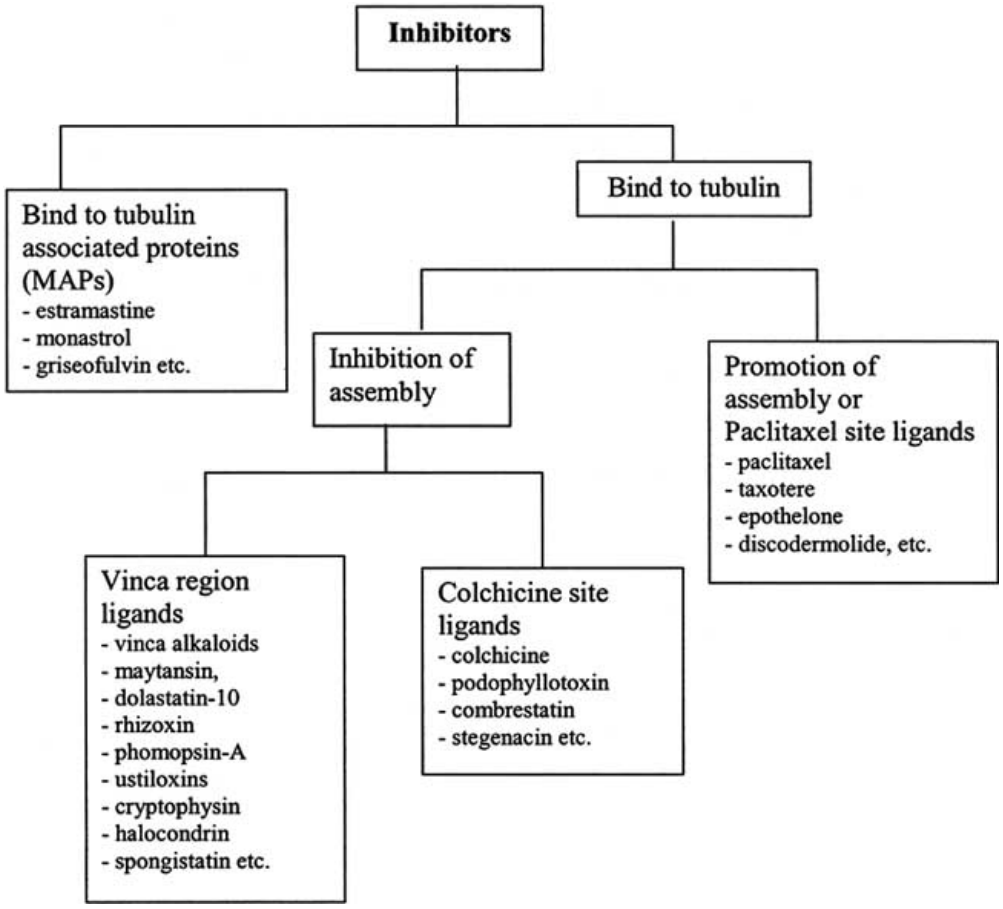



**Fig. (1).** Classification of antimitotic agents (mode of action and binding sites of MT inhibitors).

a small number of other cells; $\beta_V$ is found in hematopoietic tissues and the distribution of $\beta_V$ is unknown. $\alpha$-tubulin mRNAs encode five distinct isotypes.

The different levels of stability for dimers formed with different isotypes may be related to MTs stability eg dimers containing $\beta_{III}$ tubulin are more stable than dimers containing $\beta_{II,}$ but they form more dynamic MTs. Paclitaxel resistant prostate carcinoma cells express higher levels of $\beta_{III}$ and $\beta_{IVa}$ isotypes, with up to nine times the normal levels of $\beta_{III}$ mRNA and MTs formed with $\beta_{III}$ and $\beta_{IV}$ isotypes are over seven times more resistant to paclitaxel. Ovarian tumor cells show an almost fivefold increase in the amount of $\beta_{I,}$ $\beta_{III}$ and $\beta_{IVa}$ isotypes.

## MTs as a Target for Developing Anticancer Drugs

Microtubules are essential for the process of mitosis during cell proliferation. The importance of MTs makes them a sensitive target for developing anticancer drugs.

Mitotic inhibitors act by interfering with the mitosis of cells during the M phase of cell cycle and most drugs that prevent cell division by interfering with mitosis are M phase specific. Cytotoxic tubulin-binding agents are unique among anticancer drugs in that they target the mitotic spindle rather than DNA. The unique dynamic characteristic of MTs provides a basis for the rational design of mitotic inhibitors as antitumor agents and reveals a new way of discovering superior antimitotic drugs, which target rapidly proliferating cancer cells rather than normal cells.

## Classification of MT Inhibitors

Tubulin binding molecules have generated considerable interest among cytotoxic agents due in part to the successful introduction of the taxanes and vinca alkaloids in clinical oncology. Microtubulin inhibitors can be classified according to the mechanism or site of action (Fig. **1**):

1. Drugs binding to tubulin
   a. MT depolymerization inhibitors: taxanes, epothilone and discodermolide.
   b. MT polymerization inhibitors-
      i. Colchicine binding site: Colchicine, podophyllotoxin and combretastatin.
      ii. Vinca alkaloids binding site: Vinca alkaloids, dolastatin and cryptophysin.
2. Drugs binding to other sites
   a. MAPs: Estramustine and monastrol.
   b. Sulfhydril groups: Ethylene bisiodoacetamide and calvatic acids.
3. Drugs binding to unknown site: tryphostin, Moroidin, DZ3358.

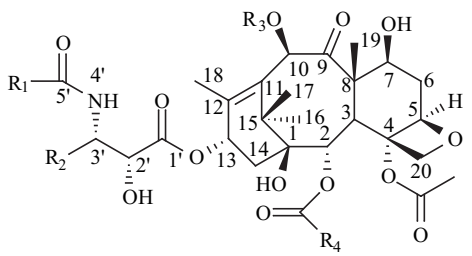
## 1a: Paclitaxel Site Binding Drugs (MT Depolymerization Inhibitors)

Taxoids, a promising group of antimitotic anticancer agents, have a unique mechanism of action, binding mainly to a domain distinct from those of colchicine and vinca alkaloids and inhibiting the depolymerisation of polymerised tubulin [46-48]. The first marketed taxoid drug was paclitaxel (**1**) which was isolated from the bark of the Pacific yew tree *Taxus brevifolia* Nutt. (Taxaceae) [49]. This endangered tree produces low yields of paclitaxel that cannot satisfy the increasing demand. Later paclitaxel was also isolated from hazelnut trees (leaves, twigs, and nuts) and the fungi living on these trees but the concentration is only about 10% of the concentration in yew trees [50]. This drug has now been fully synthesized [51-52] and at present is used extensively in the treatment of breast and ovarian cancers. It is also used for skin, lung, and head and neck carcinomas [53]. Chemistry of this diterpinoid is very complex comprising of an unusual oxetane ring at C4-C5 and an isoserine side chain at C-13 position. Although some therapeutically active paclitaxel analogues modified at C10 and C2 have been developed as advanced second generation taxoids [54], structural variations along the upper part at C6-C12 appear to have less impact on the bioactivity. The lower part, on the other hand, comprising of C14 and C1-C5 appears to be a region which is crucial to the activity. Most changes to the C- and D- rings, including opening of the oxetane ring, lead to loss of activity [55], although some examples of C-ring opened compounds that retain significant activity have been developed [56].

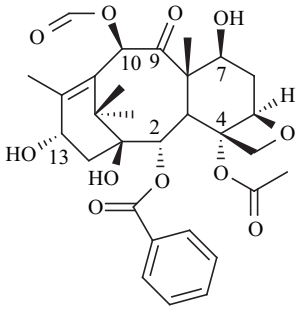
Extensive structure activity relationship (SAR) studies have provided insight into the structural determinants that are important for the activity of paclitaxel. It is now established that the A-ring side chain at C13 with a C2'-OH, the C2 benzoyl group and an intact oxetane ring at C4-C5 are essential for both the cytotoxicity and stabilization of MTs [57-60]. The oxetane ring is a unique feature of paclitaxel and analogues prepared with an opened oxetane ring have a greatly reduced activity [55]. The C4 acetyl group does not appear to play a significant role in the biological activity but may contribute to the defined conformation of the molecule. The C1-OH group makes a significant contribution to the overall bioactivity [55]. The OH group at C-7 is not essential for biological activity [60].

The taxane ring binds near the M-loop; the C2 benzoyl ring in proximity to His227 and Asp224 [62], and the C13 benzamido group within the N-terminal 1-31 amino acids of β-tubulin [63]. The main interaction of the taxane ring with tubulin is at L275 at the beginning of the B8-H9 loop [10].

Paclitaxel is semisynthesized from baccatin III (**4**), an analogue of paclitaxel without the C13 side chain, which is isolated from *Taxus baccata* L [64]. Cytotoxicity of baccatin III is practically insignificant whereas 2-*m*-azidobaccatin III (**5**), a taxol analogue lacking the complete A ring in the C13 side chain but with a *m*-azido benzoyl at the C2 position of the taxane ring, inhibited cell proliferation in human cancer cells at nanomolar concentrations [65]. Recently, it has been demonstrated that baccatin III could cause apoptotic cell death without affecting the cell cycle $G_2$/M phase [66]. Deaminoacetyl taxineA (**6**), isolated from *T. baccata* L, with an unusual tricyclic hexadecane skeleton and an acetate moiety at C13, has no cytotoxic activity [67]. Two other novel open oxetane ring taxoids, taxumairols N (**7**) and O (**8**), have been isolated from the roots of *Taxus mairei* [68]. Two new bicyclic taxanes (**10**,**11**) diterpinoid [69], two *abeo*-taxanes [70], taxumain A (**12**) & B (**13**) from Chinese



**1** : $R_1 = R_2 = R_4 = Ph$, $R_3 = Ac$,
**2** : $R_1 = R_4 = Ph$, $R_2 = t\text{-}BuO$, $R_3 = H$
**3** : R1 = R2 = Ph, $R_3 = Ac$, $R_4$ = m-azidophenyl



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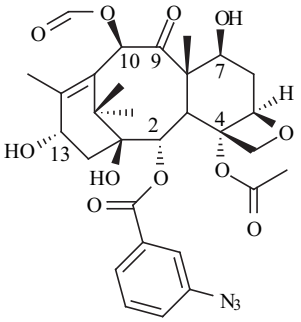

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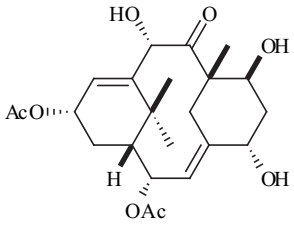

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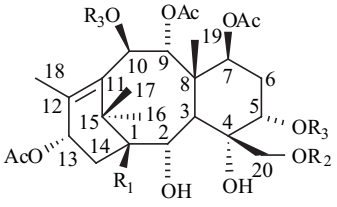
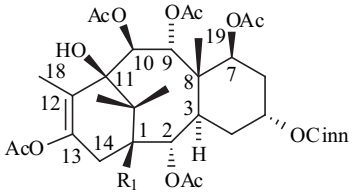


**7** : N; 1. $R_1 = H$, $R_2 = H$, $R_3 = H$
**8** : O; 2. $R_1 = OH$, $R_2 = H$, $R_3 = H$



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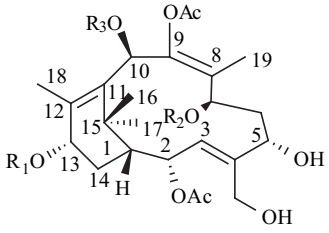
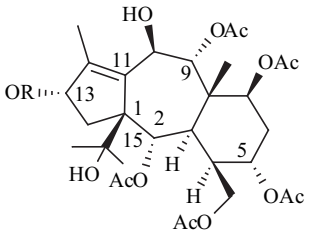
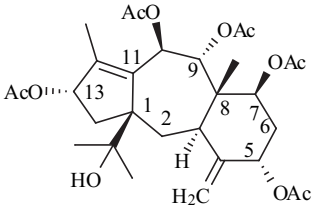


**10** : $R_1 = H$, $R_2 = Ac$
**11** : $R_1 = Ac$, $R_2 = H$



**12** : R = H
**13** : $R = COCH_3$



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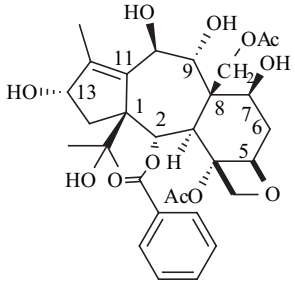

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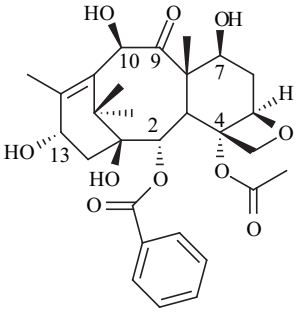

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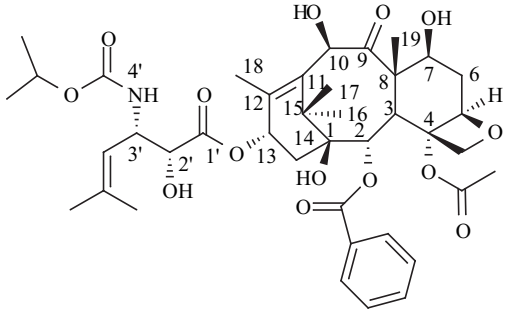


**17** : $R = CH_2CH_2CO$
**18** : R = cyclopropen-CO
**19** : $CH_3CH_2CO$

yew *T. marirei* and a brevefoloiol analogue (**14**,**15**) from the Himalyan yew *T. wallichiana* have been reported [71]. Taxuspine D (**9**), without a C13 side chain or an oxetane ring, isolated from both Japanese yew *T. cuspidate* [72-73] and Canadian yew, *T. Canadensis* [74] was reported to promote the polymerisation of tubulin with a potency corresponding to between half and one-third of the activity of paclitaxel [72]. These results imply that the C13 side chain is not always essential for promoting tubulin polymerisation. When the core structure of taxane is substantially modified, a C-5 side chain may lead to bioactive taxanes [75]. A good number of novel taxanes have also been isolated from Canadian yew [74].

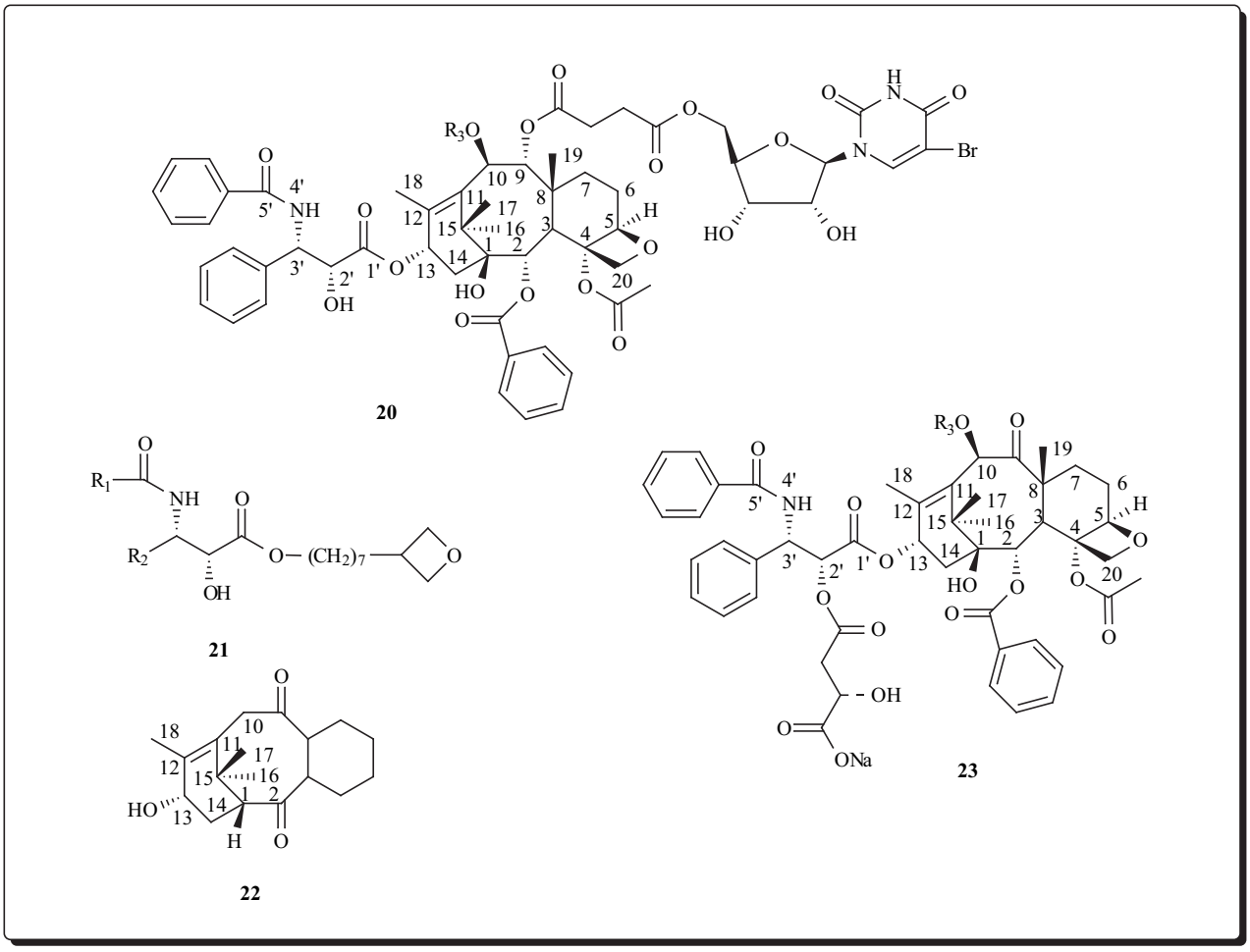
To date, more than 350 taxoids have been isolated from the *Taxus* species, developed and characterized [76-77] but very few are found to be potent cytotoxics. Poor water solubility and a very low yield of paclitaxel motivated researchers to design and develop new analogues of paclitaxel that are more cytotoxic. Docetaxol (**2**), with minimal structural modifications [78] at C13 side chain and C10 substitution showed more water solubility and more potency than paclitaxel, whereas baccatin, without C13 side chain, has no significant cytotoxicity. Clinical trials have shown that patients who develop hypersensitivity to paclitaxel may receive docetaxel without an allergic response [79]. The C13 side chain could be a target for developing new analogues. 2-debenzoyl-2- (m-azidobenzoyl) paclitaxel (**3**), a C2 modified analogue, was found to be more potent ($IC_{50}$ 0.31 ± 0.01 μM) than paclitaxel ($IC_{50}$ 0.42 ± 0.03 μM) [80]. 14β-Hydroxy-10-deacetylbaccatin III (14β-OH-DAB, **16**), first isolated from the needles of Himalayan yew tree, has a higher water solubility [81]. New taxoids derived from it may show improved water solubility, bioavailability and reduced hypersensitivity [54]. Synthesis of second generation taxoids by appropriate modification at C-3', C-2 and acylation at C-10, produces taxoids [82-84] with extremely high potency against drug resistant cancer cells and compounds 17-19 showed two orders of magnitude better activity than those of paclitaxel and docetaxel [54,85]. Our laboratory is involved in developing simple but better paclitaxel like drugs based on receptor topography.

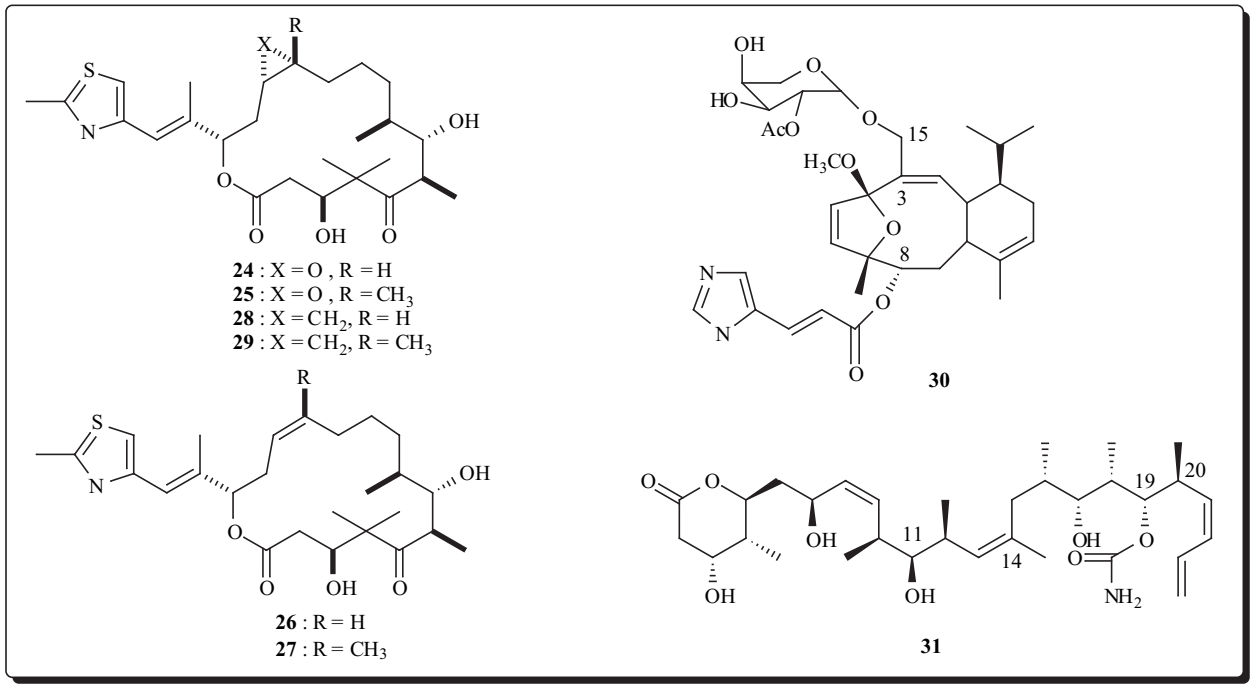
To evaluate the necessity of the taxane core of taxoids, simplified compounds typified by (**21**) with the key pharmacophore properties of paclitaxel, phenylisoserine and an oxetane ring, were synthesised but none showed promising tubulin inhibitory activity. This finding justifies the necessity of the diterpene ring [86]. Novel 7-deoxy-9-dihydropaclitaxel analogues, typified by (**20**), have been synthesised and found to have potent selectivity against human liver cancer cells [87]. Synthesis of a new class of borneol esters (**22**) that might be considered as analogues of paclitaxel has been reported but these compounds showed reduced cytotoxic activity [88].

Design of new taxanes has been directed towards solving the limitations of both solubility and resistance. The paclitaxel ester of malic acid (**23**) has demonstrated improved solubility and enhanced *in vivo* antitumor activity against P388 murine leukemia indicating a higher therapeutic index than paclitaxel [89]. The targeted delivery of paclitaxel has also been reported - octreotide-conjugated paclitaxel demonstrated cell selectivity and exerted the same antitumor effect as free paclitaxel [90].

## Other Paclitaxel Site Binding Drugs

The epothilones A (**24**) and B (**25**), naturally occuring antimitotic agents isolated from the myxobacterium *Sorangium cellulosum* [91-92], exhibit cytotoxicity by the same mechanism as paclitaxel and are active against

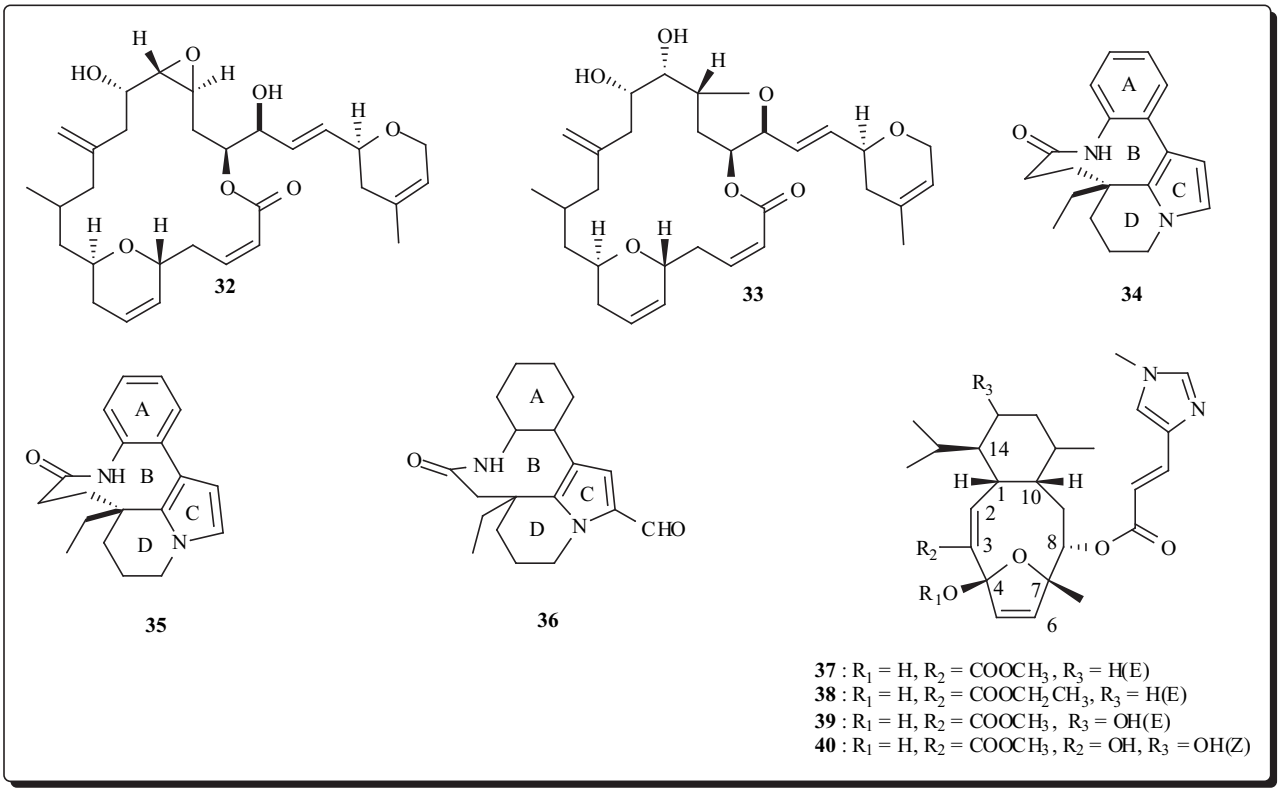





paclitaxel resistant cancer cells [93]. Epothilones have also been successfully prepared by total synthesis [94,95]. Enantioselective total synthesis of these drugs has been achieved [96].

12,13 Desoxy analogues (**26**,**27**) of epothilone B have been semisynthesised and shown a more promising *in vivo* activity profile than either eopthilone B or paclitaxel [97,98]. Cyclopropane analogues (**28**,**29**) of epothilones showed activity in both tubulin polymerisation and cytotoxicity assay [99] but other investigations have indicated that replacement of epoxide moiety of epothilone A with cyclopropyl group leads to total loss of activity [100]. These drugs are not recognised by multi-drug resistant cells and therefore produce much higher potency than paclitaxel in multi-drug resistant cell lines. Epothilones are more water-soluble than paclitaxel thereby offering distinct advantages.

Eleutherobin (**30**), a novel natural glycosylated diterpene isolated from a marine soft coral species *Eleutherobia*, is extremely potent in inducing tubulin polymerization *in vitro*



with $IC_{50}$ similar to that of paclitaxel [101]. This drug is effective against breast, renal, ovarian and lung cancer. SAR profile of eleutherobin analogues revealed that removal or replacement of the C15 sugar moiety alters cytotoxic potency [102].

Discodermolide (**31**), a polyhydroxylated alketetraene lactone marine product, isolated from the Carribean sponge *Discodermia dissolute* [103], inhibited cell mitosis and induced formation of stable tubulin polymer *in vitro* and considered to be more effective than paclitaxel with $EC_{50}$ value of 3.0 μM versus 23 μM [104]. Total synthesis of this drug has also been reported [105,106].

Laulimalide (**32**) and isolaulimalide (**33**), macrocyclic lactoses isolated from the marine sponge *Cacospongia mycofijiensis*, are inhibitors of cell proliferation with $IC_{50}$ values in the low nanomolar range (**32**) and low micromolar range (**33**) respectively [107]. Although their mechanism of action is unknown they are considered to be paclitaxel like microtubule stabilising agent.

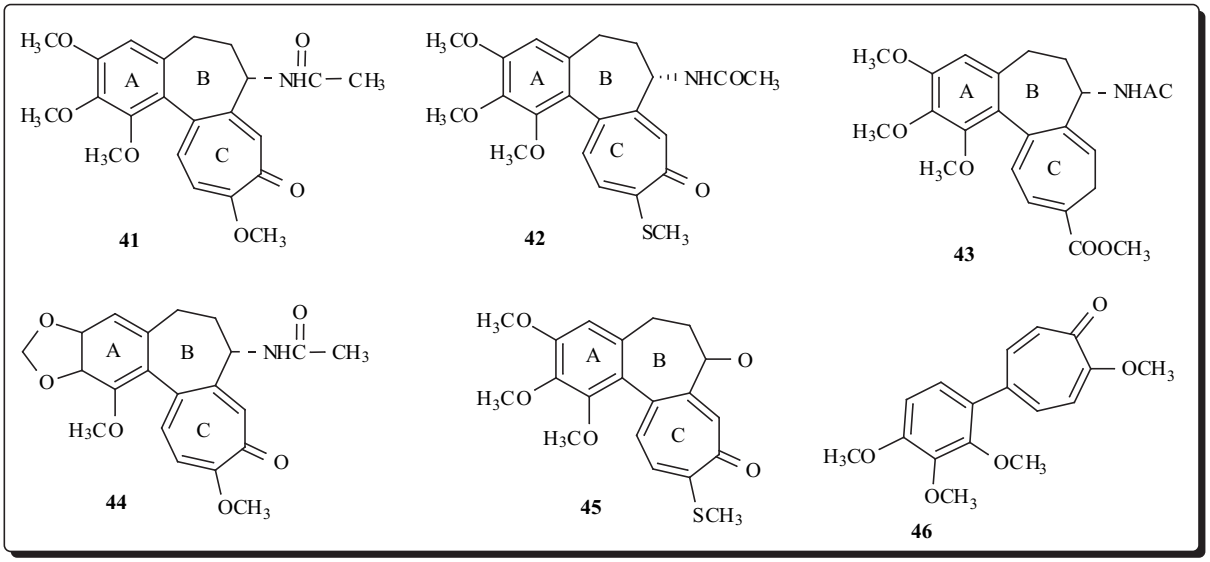
(-)-Rhazinilum (**34**), isolated from *Melodinus Australia* [108], *Rhazya stricta* [109], and *Kopsia singapurensis* [110] has been reported to mimic the effects of both vinblastine and paclitaxel by inducing a non-reversible assembly (spirilization) of tubulin (vinblastine type effect) and by inhibiting the cold-induced disassembly of microtubules (pclitaxel like effect) [111,112]. (-)-Rhazinil (**35**), a formyl derivative of Rhazinilum has also been isolated from a related *Kopsia* species [111]. SAR studies deriving from these analogues suggest that the presence of the phenylated-pyrole unit and the lactam ring as well as restricted rotation about the biaryl axis is required for antimitotic activity [113]. The presence of hydrophobic groups at C14 on D-ring led to a large decrease in the antitubulin activity [114]. (±)-B-Norrhazinal (**36**), a conformationally more constrained analogue of rhazinilum congeners, has been synthesised and found to be cytotoxic against human CA46 Burkitt lymphoma cells with $IC_{50}$ value of 3 μM [115].

Sarcodictyns (**37**-**40**), structurally similar to eleutherobin, have been isolated from marine corals *Sarcodictyon roseum* [116] and *Eleutherobia* species [117] and sarcodictyns A & B showed antiproliferative activity in six human cancer cells with $IC_{50}$ values in the 200-500nM range. Sarcodictyns A &B have also been synthesized [118].

### 1b.i: Colchicine Binding Site

#### *Colchicine and its Analogues*

Colchicine (**45**), a well known alkaloid, obtained from *Colchicum autumnale* and other plants, a classic tubulin binding agent [119], is used in the treatment of gout, familial Mediterranean fever and liver cirrhosis [120-123]. Colchicine binds to soluble tubulin and forms a tubulin-colchicine complex, followed by ligand-induced conformational changes in both tubulin and colchicine itself and thereby causes the MT spindle to disassemble in the metaphase of mitosis. The binding site (s) of colchicine on tubulin have been extensively studied [124,125]. It has been reported that the β-subunit of tubulin is involved in colchicine binding, with the A ring of colchicine lying between Cys-354 and Cys-239 and C ring lying between the peptide region containing Cys-239 and the amino terminal β-tubulin sequence [126] and region β:1-36 [127]. The seven membered B-ring and the C7 side chain are not believed to be crucial for tubulin binding but may affect the conformation of colchinoids and their tubulin binding properties [128]. The trimethoxyphenyl group of the A-ring of colchicine serves as a complex stabilizing anchor on tubulin in the inhibition of MTs assembly and is essential for its activity [129]. An important structural feature of colchicine is the α-methoxytropolone, which constitutes the C-ring portion of the colchicine molecule. The tropolone ring and the C-9 or C-10 substitutes have been implicated in several aspects of the ligand-tubulin binding mechanism [130]. This drug has also been reported to bind to a second, lower affinity site on tubulin in a reversible manner [131]. Although colchicine is one of the oldest antimitotic drugs, its toxicity is similar to its activity. Hundreds of colchicine analogues have been prepared in past decades, including compounds isolated from natural sources, partially synthesized from colchicine, and synthesized *de novo*. Some have been used clinically as antitumor agents, having less toxicity than colchicine. Most modified colchicine analogues share a common binding site on tubuliun, common



mechanisms for tubulin binding and similar pharmacological actions [132].

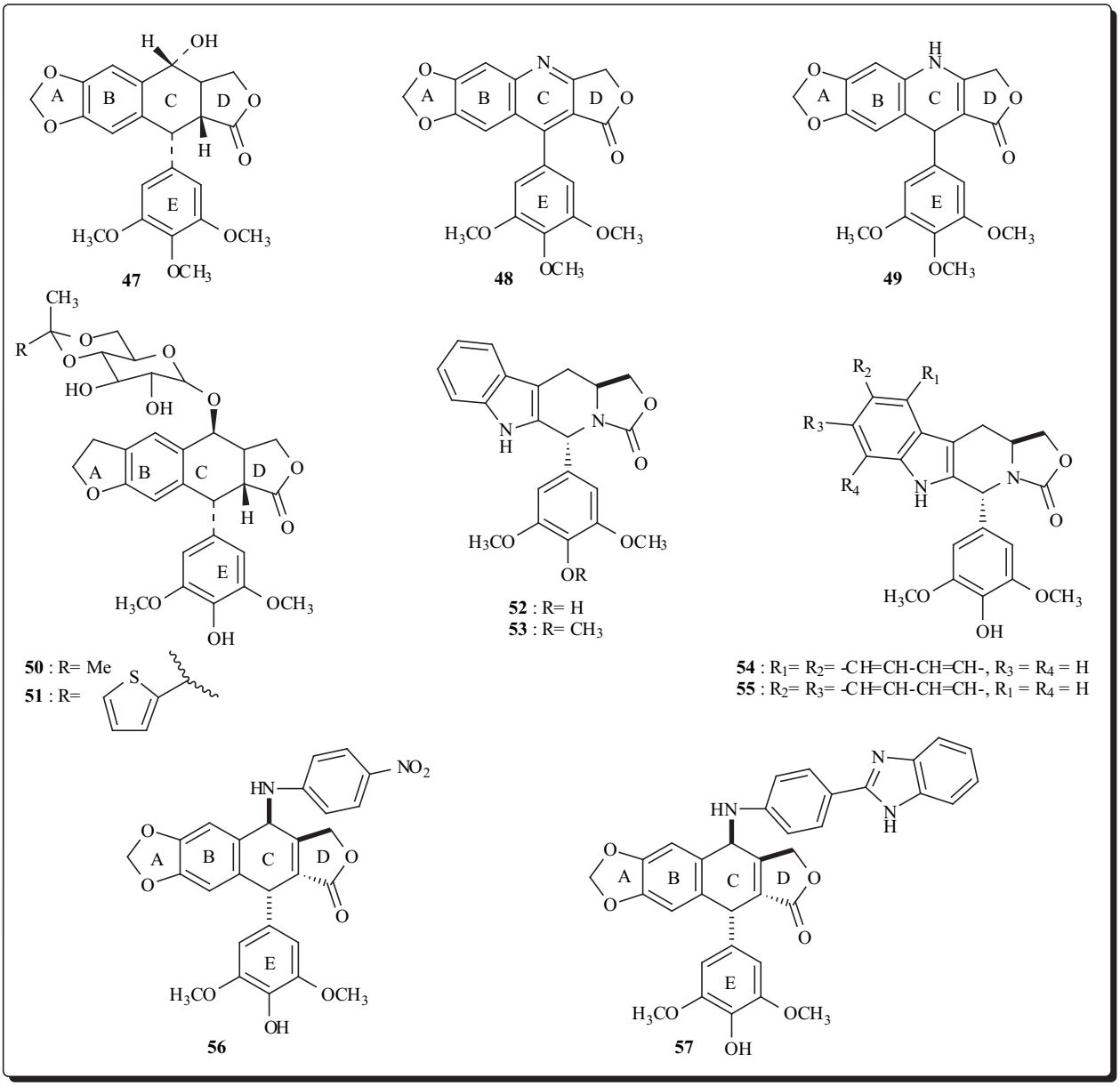
Colchicone (**45**), a non-nitrogen containing natural product isolated from *Colchicum richterii* [133] and synthesised by Banwell *et al*. [134] has been reported to show an inhibitory effect on tubulin polymerisation. Compound **46**, a bicyclic analogue of colchicine binds rapidly and reversibly to colchicine binding site of tubulin, inhibits microtubules assemble and promotes apoptosis in human leukemic cells [135]. This drug produces reversible effects on microtubule disassembly, G2/M phase arrest, more water solubility and has lower toxicity than colchicine [135].

Of the many colchicine analogues, thiocolchicine (**42**) and colchicone (**45**) which are both synthetic, have shown more activity than the parent colchicines [14]. Most natural analogues are modified in the C-7 side chain substituent except allocolchicine (**43**), with an aromatic 6-member ring and a $COOCH_3$ substituent at C-10, and cornigerine (**44**) with an $OCH_3$ substituent at C-10 which have both been obtained from *Colchicum cornigerum* [136]. Allocolcichine (**43**) which has also been isolated from *C. autumnale* [137] and N-acetylcolchinol O-methylether, a semisynthetic analogue obtained by reaction of colchicine with hydrogen peroxide [138], are both more potent inhibitors than colchicine in ITP and ICB assays [139,140].

## Other Colchicine Binding Site Tubulin Inhibitors

### *Podophyllotoxin and its Analogues*

Podophyllotoxin (**47**) and its related analogues were isolated from dried roots of *Podophyllum peltatum* and related species. This compound has been used as a medical treatment of liver sclerosis, constipation, rheumatism and cancer. Its antimitotic activity was first reported at least 50 years ago. Podophyllotoxin has also been reported to use in the treatment of venereal warts but is too toxic to be of clinical value. Podophyllotoxin and its related analogues bind to tubulin protein at the colchicine site and



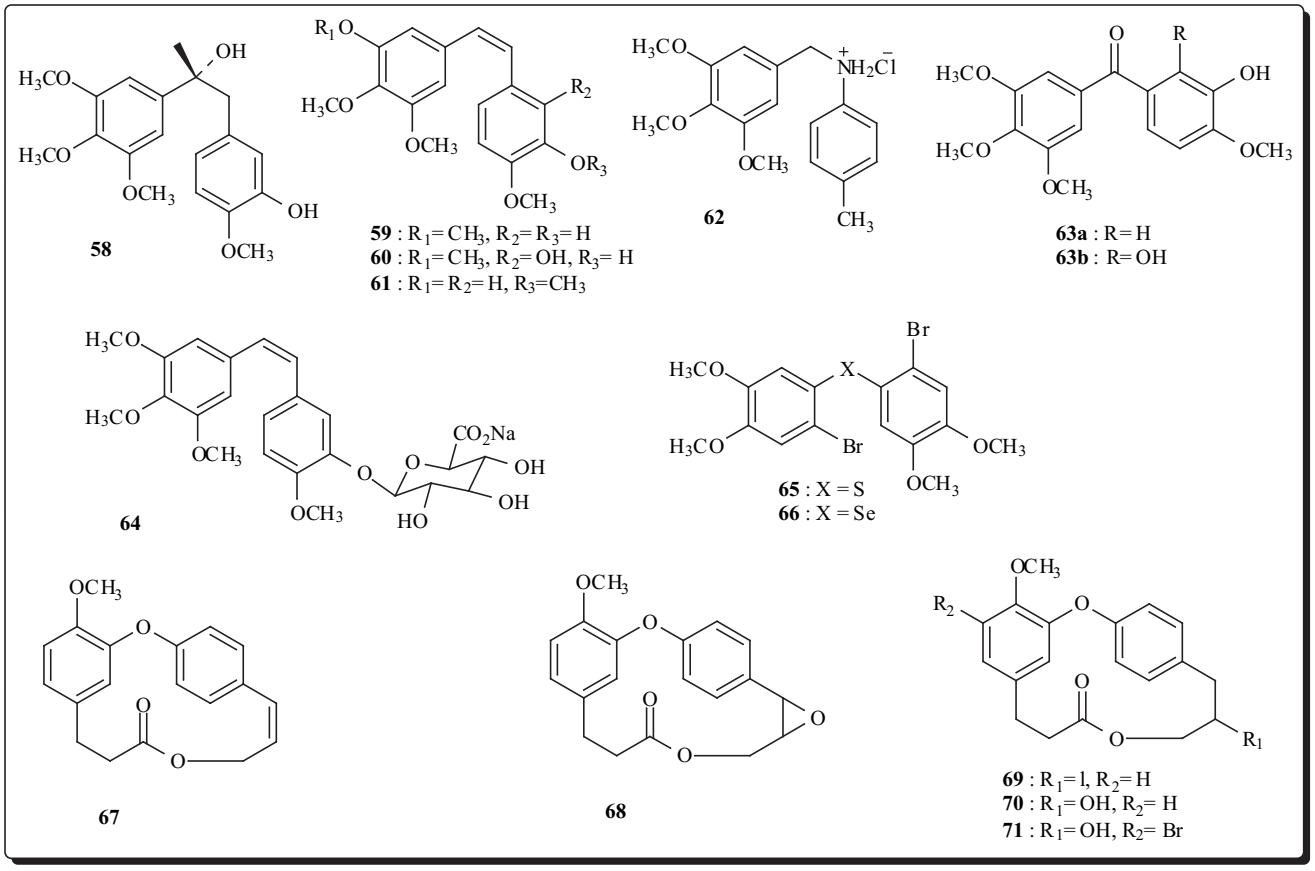
competitively inhibit colchicine binding to tubulin although a recent computer modeling study suggested incomplete overlap of the colchicine and podophyllotoxin binding site (s) [141]. Hitotsuyanagi *et al.* synthesised 4-aza-2,3-dehydro-4-deoxypodophyllotoxins (**48**, **49**) and found that compound 48 is twice as active as natural podophyllotoxin [142]. Etoposide (**50**) and teniposide (**51**), two semisynthetic less toxic analogues of podophyllotoxin have been developed [143]. Etoposide (**50**) is a potent topoisomerase-II (an enzyme involved in the folding and unfolding of DNA during cell replication) inhibitor but a weak tubulin inhibitor [144,145]. It has also been reported that their predominant function is inhibiting Topo II rather than a microtubular interaction [146]. Etoposide is currently used in the treatment of small cell lung, testicular and malignant lymphoid cancers, among others [147]. It is also used in combination chemotherapy. Compound 56 which contains a p-nitroanilino moiety at the 4β position is a topo II inhibitor causing DNA double strand breakage and G2 phase arrest and it is in clinical trial in Taiwan [148]. Compound (**57**), another analogue of etoposide, showed 10-fold more potency than etoposide in both cell killing and topo II inhibition assay [148]. Teniposide (**51**) appears to be a less useful clinical agent though it is undergoing trials in combination therapy for the treatment of metastatic brain tumors [149].

Two interesting synthetic compounds, azotoxin (**52**) and methylazotoxin (**53**) with structural similarity to 4-demethyldeoxypodophyllotoxin were found to be dual inhibitors of Topo-II and tubulin polymerization [150,151].

Azatoxins (**52**,**53**) exhibit dual targeting of both topo II and tubulin [152,153] and 4-methylazatoxin has been found to interact with the colchicine site on tubulin, therefore the effect of structural features on selective inhibition of tubulin and Topo-II provide promise for further azatoxin derivative design [132]. Benzatoxins (**54**,**55**), the congeners of azatoxins, displayed activity greater than that of colchicine for the inhibition of tubulin polymerization [154].

## Combretastatins

Combretastins (**58**), an antineoplastic agents isolated from the South African tree *Combretum caffurum* [155,156], are simple compounds that show antimitotic effects by interacting with the colchicine binding site of tubulin. Combretastin A-4 (**59**) is one of the most potent inhibitors of colchicine binding presently known [157]. Naturally occurring and synthetic combretastatin and its analogues (**59-61**) were identified as antimitotic agents binding to colchicine binding sites [132]. Combretastatin A-4 (**59**), the most potent cancer cell growth inhibitor of the series [125,158,159] is not recognised by the multi-drug resistance (MDR) pump, a cellular pump which rapidly ejects foreign molecules including many anti-cancer drugs [41]. It has also been reported that this compound can inhibit angiogenesis, a process essential for tumor growth [161]. SAR of combretastatin A-4 led to the discovery of the potent cancer cell growth inhibitor phenstatin (**63a**) and hydroxyphenstatin (**63b**), which showed remarkable antineoplastic activity by inhibiting tubulin polymerization with activity comparable to that of combretastatin A1 (**15**) [162]. Synthesized Combretastatin D derivatives (**67-71**) [163] stabilize microtubules to various degrees with the derivatives bearing polar substituents being most active [164]. Various water



soluble benzylaniline hydrochloride salts were synthesised and evaluated for potential therapeutic value in the treatment of cancers [165]. These compounds bind to tubulin in the same manner as combretastatin A-4. The most promising compound in this series was 4-methyl-N- (3,4,5-trimethoxybenzyl)aniline hydrochloride (**62**), with an $IC_{50}$ value of 3.5 μM in the ITP assay and cytotoxicity against a wide variety of cancer cell lines [132]. Combretastin glucuronide (**64**), in which the combretastatin core has been coupled to a small number of sugar moieties, is highly water soluble [166], but about 100-fold less potent than the parent phenol [132].

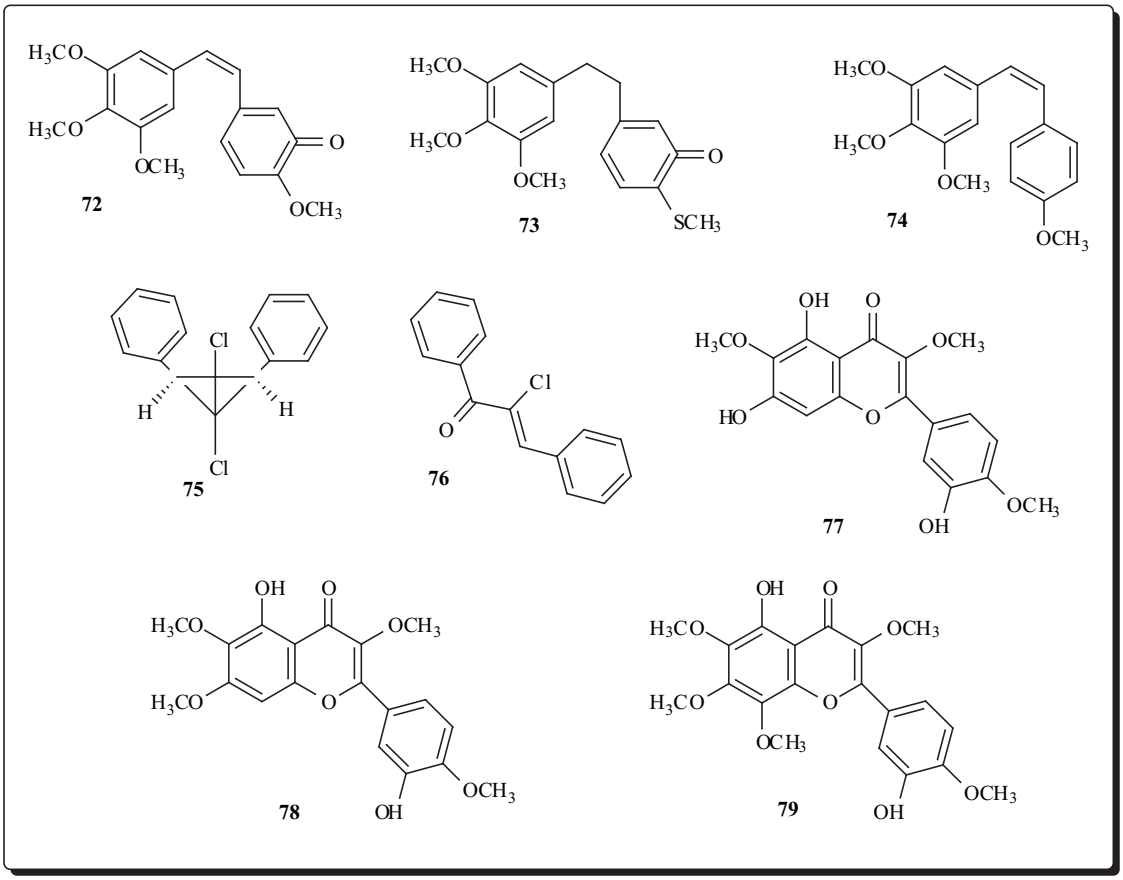
Diphenylselenides (**65**) and diphenylsulfides (**66**), a second class of analogues produced in an attempt to mimic combretastin, do not appear to inhibit MT formation but disrupt the cell cycle and cause mitotic arrest by interaction with microtubules [167]. These agents bind strongly to tubulin dimer and may somehow alter the conformation of the tubulin protein, encouraging polymerization [160]. Combretatropones (**72**,**73**), hybrids of combretastin and colchicine, were synthesized and evaluated by Andres *et al* [168].

In a tubulin related bioassay, **72** inhibited tubulin polymerization with an $IC_{50}$ value of 8.6 μM, and 24 had an $IC_{50}$ value of 12.0 μM. In comparison, colchicine had an $IC_{50}$ value of 3.5 μM [168]. A series of stilbenes related to the combretastin were synthesized and compound 25 exhibited potent tubulin polymerization inhibitory activity with an $IC_{50}$ value of 2.0 μM and was cytotoxic against an array of cancer cell cultures [157,169]. Z-1, 1-dichloro-2,3-diphenylcyclopropane (**75**), a known synthetic agent used for breast cancer has apparent anti-estrogenic activity and remarkably low toxicity in rodents [170,171]. It inhibits tubulin polymerization *in vitro* with an $IC_{50}$ value of 6.7±0.9 μM [172]; however, its metabolite *ZCC* (**76**) was not active *in vitro*.

Surprisingly, both compounds (**75**,**76**) are active against tumor cell growth, arresting cells in the $G_2$/M phase, and **76** is about 10-fold more potent than 26 [173]. The reasons for these differences are presently unknown.

## Flavonols

Flavonols (**77**-**79**) have been reported to have antitumor activity [132] and two flavonols (**77**,**78**) were found to inhibit tubulin polymerization and colchicine binding to tubulin. Centauridin (**77**) was isolated from *Polymnia fruticosa* and identified as a tubulin inhibitor[174]. Flavonol-2 (**78**) was isolated from several plants including *Zieridium pseudobtusifolium, Acronychia porteri, Polanisia dodendra, P. tachysperma, Guttierrezia microcephala and G. sarothra* [132,175,176]. Flavonol-2 and centauridin have been shown to inhibit tubulin polymerization with $IC_{50}$ values of 0.83 and 2.0 μM, and to inhibit colchicine binding to tubulin by 59% and 35% when present at the same concentration as tubulin [176]. Flavonol-2 (**78**) also displayed remarkable cytotoxicity *in vitro* against panels of central nervous system, non-small cell lung, ovarian, colon and renal cancers, melanoma, and leukemia cells with values in low micromolar to nanomolar concentration range [176]. Compound 30 also showed potent cytotoxicity against a panel of human tumor cell lines and was found to be a



strong inhibitor of tubulin polymerization with $IC_{50}$ value 0.83± 0.2 μM and a potent inhibitor of radiolabelled colchicine binding to tubulin [176].

## Aromatic Carbamets

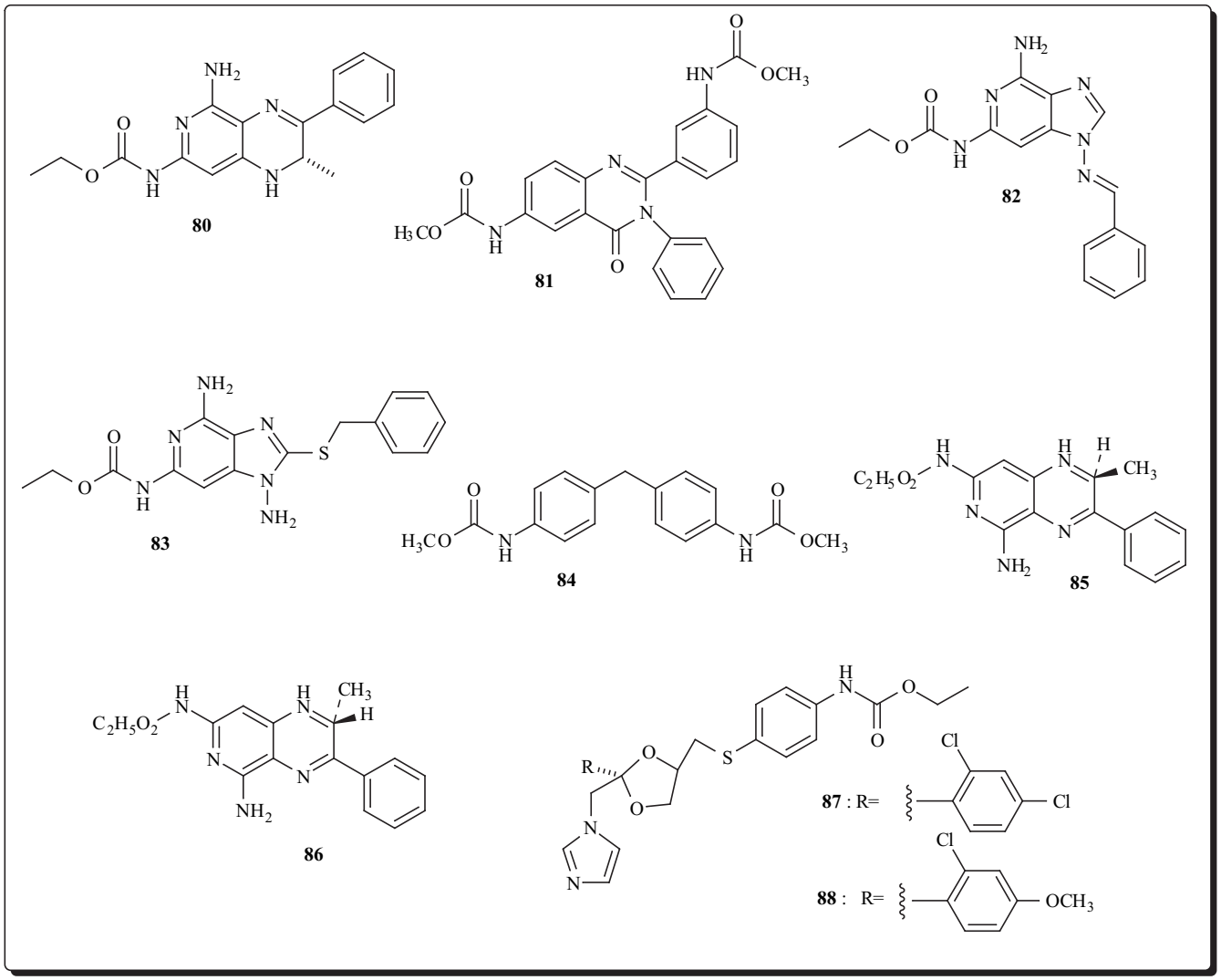
Carbamets are a group of dihydropyridopyrazines (**80**), which have been found to bind to tubulin at nanomolar concentration, prevented the formation of MTs and competitively inhibited the binding of colchicines [177]. Carbamets also showed activity against a number of multidrug-resistant cell lines [178]. Of these compounds, the 2-methyl derivative (**80**), is in clinical trial [179].

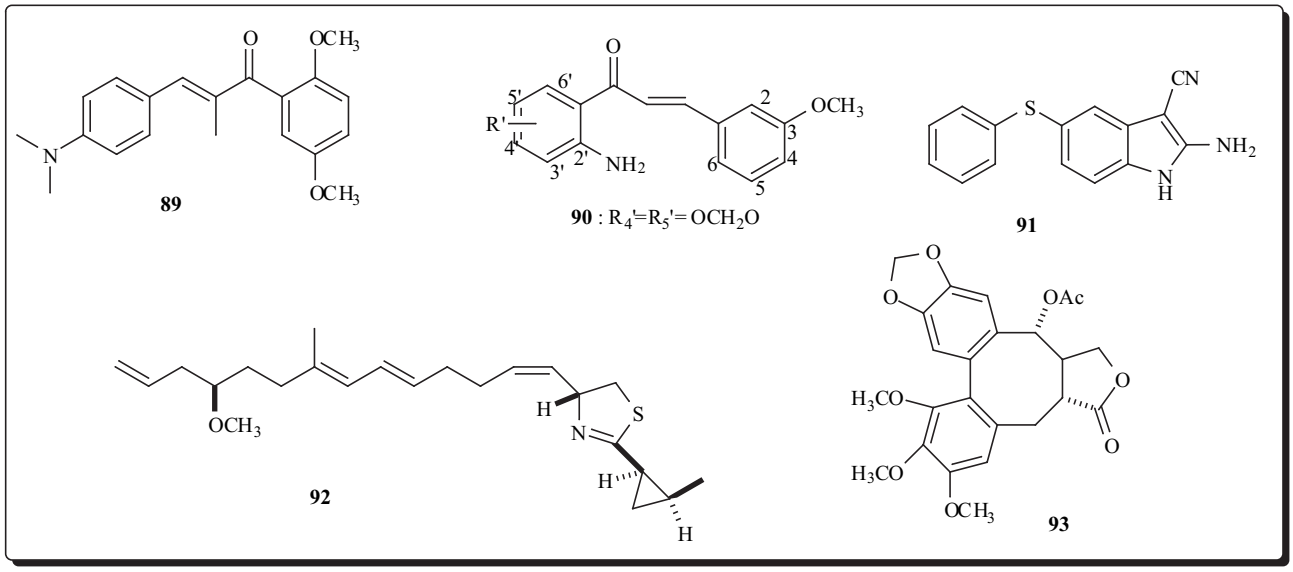
Diphenyl quinazolone (**81**) is also a potent antimitotic agent which has activity *in vivo* [180]. Further investigations on this compound are ongoing [181]. Replacing the pyridine ring with an imidazole ring system resulted in the synthesis of a number of imidazo[4,5-c] pyridines (**82**-**83**) which showed activity against the formation of MTs both *in vitro* and *in vivo*, although these compounds were much less potent than the dihydropyridopyrazines [182]. The synthetic compound *bis*-carbamet (**84**) is also reported to have tubulin inhibitory properties *in vitro* and activity at micromolar concentrations [183]. NSC 613862-S (**86**) and NSC 613863-R (**86**), two isomers of ethyl 5-amino-2-methyl-1, 2-dihydro-3-phenylpyrido[3,4-b]pyrazin-7-yl carbamate, are reported to bind to tubulin in a region that overlaps with the colchicine site and to induce formation of abnormal polymers of GTP-Mg-tubulin [184].

These compounds, along with their related cyclic and acyclic analogues, all contain the aromatic carbamate functionality (Ar-$NHCO_2R$), and are related to nocodazole and tubulazole-C (**87**), which were found to be cytotoxic to mammalian cells and to parasitic organisms [185]. Investigation of this toxicity demonstrated that the drug was an effective MT destabilising agent, being more effective than both colchicine and nocodazole [186]. However, tubulazole-C (**87**), showed poor water solubility which led to the development of the more soluble derivative, erbulazole (**88**) which has a 10-fold increase in activity [187].

## Chalcones

Chalcones (**89**), benzacetophenone molecules containing a trimethoxy phenyl group, have been synthesised and found to be potent cytotoxic agents with $IC_{50}$ values of around 4nM against HeLa cells [188]. Studies in animal tumor models indicated that these drugs showed up to 300 times more potency than that of colchicine in terms of arresting






cell division. Podophyllotoxin was shown to displace the chalcones from their binding site on tubulin, which supported its colchicine binding site [189]. A series of novel 2'-aminochalcones have been synthesised and compound 90 showed potent antitumor activity [190].

### Ampethinile

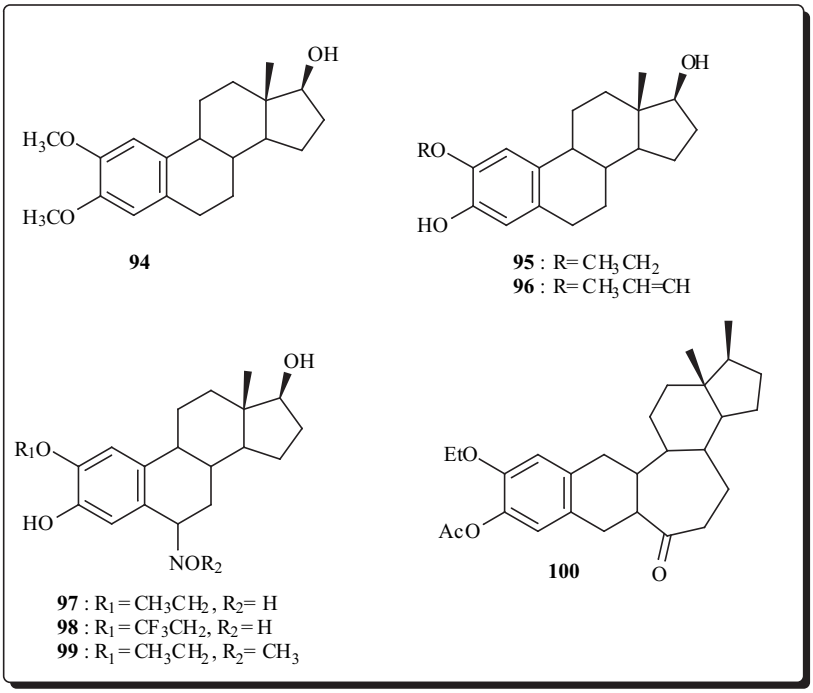
Ampethinile (**91**) has cytotoxic activity and is reported to terminate pregnancy in rats [191]. It competitively binds to colchicine site of tubulin [192] and has undergone clinical trial [160].

### Curacin A

Curacin A (**92**), isolated from the marine blue-green cyanobacterium *Lyngbya majuscula,* inhibits microtubule formation and the binding of colchicine to tubulin dimmers [193-195]. In addition, small amounts of the nearly equipotent curacins B and C (structure are not shown) were obtained from the extracts [196]. Curacin A is the most potent of this family and has an $IC_{50}$ value of 1.8pM in Chinese hamster Aux BI cells [160]. The significant activity of this compound has encouraged great interest in synthesising the compound and total synthesis of this drug has been achieved [197-201].

### Steganacin

Steganacin (**93**) and a number of related compounds were first isolated from the stems and stem bark of the East African tree *Steganotaenia araliacea* [202]. It inhibits mitosis, competitively inhibits the binding of colchicine to tubulin and inhibits tubulin polymerization [203]. It has been reported to bind to tubulin with similar affinity to that of colchicines [204].



### 2-Methoxyestradiol and its Derivatives

2-Methoxyestradiol (2-ME) (**94**), the major endogenous mammalian metabolite of the primary estrogenic hormone β-estradiol, was reported to have cytotoxic effect [205-207]. It has shown cytotoxicity to several tumor cell lines, binding to the colchicine site of tubulin resulting in the formation of abnormal MTs [208]. It also inhibits angiogenesis [209] and it has been postulated that 2-methoxyestradiol perturbs mitotic activity by altering the dynamics of tubulin polymerization [210].

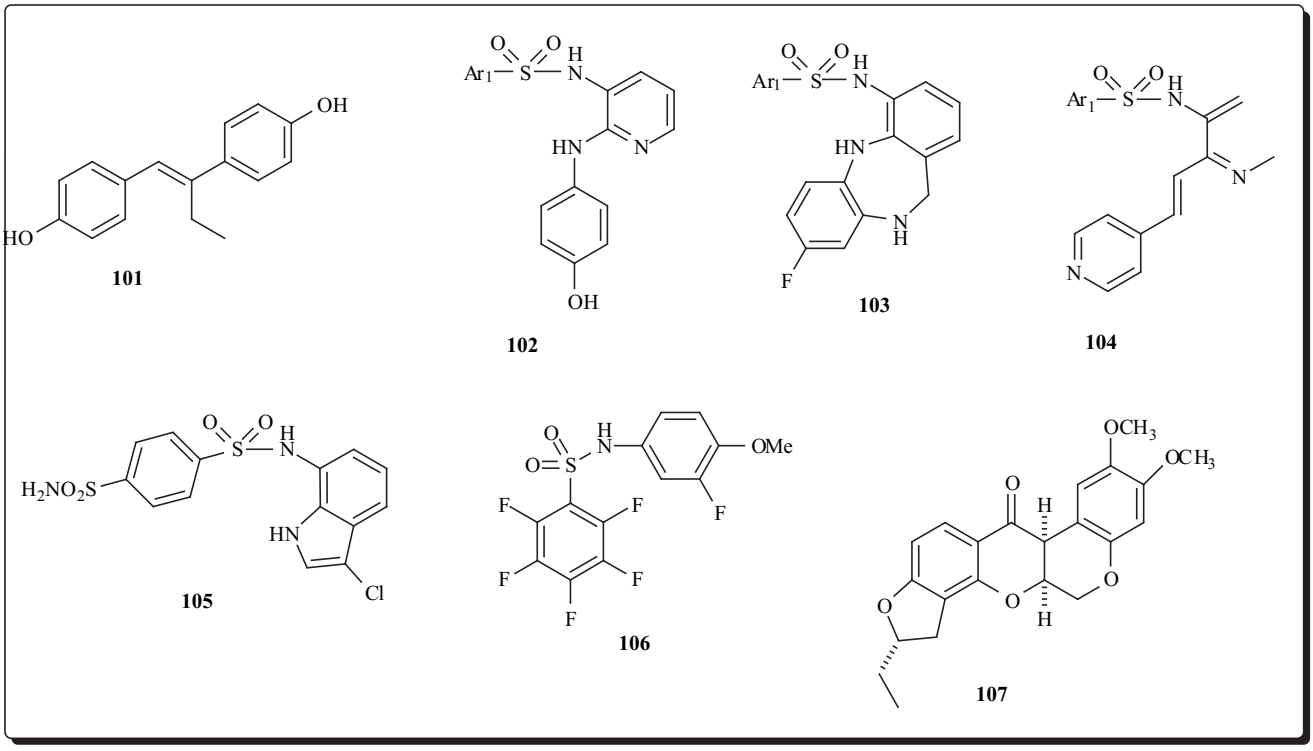
2-ME inhibited β-FGF (Fibroblast Growth Factor) and VEGF (Vascular Endothelial Growth Factor)-induced neovascularization by 39% and 54% respectively, comparable to paclitaxel [211] This compound also showed binding both to unpolymerized and polymerized tubulin [212]. A series of structurally related compounds were synthesized and some compounds (**95-99**) were more potent than 2-ME as cytotoxic agents in cancer cell cultures and as tubulin polymerization inhibitors. The active compounds contained either an ethoxy or a propenyl moiety at the 2-position and /or oxime groups at the 6-position [132]. Based on the hypothetical relationship between the structure of colchicine and 2-methoxyestradiol, a B-ring expanded 2-ethoxyestradiol analogue (**100**) was synthesized in which the B-ring of the steroid was replaced by the B-ring of colchicine and was found to accelerate tubulin polymerization and stabilize the MTs towards cold-induced depolymerization that was produced by paclitaxel [213].

### Diethylstilbsterol

Diethylstilbesterol (**101**), a synthetic estrogen used in the treatment of abortion, was found to induce cervical and vaginal tumors. This compound has been reported to cause depolymerization of MTs and binding to the colchicine site on tubulin [214]. This drug has also been reported to have a reversible disruptive effect on mitotic spindle microtubule organization during maturation [215].

### Sulfonamides

Among sulfonamides, E7010 (**102**) is the most active antimitotic agent, which has been shown to inhibit MT formation by binding at the site of colchicines [216]. It is quite soluble in water as an acid salt. This drug showed good results against a wide range of tumor cells including vinca alkaloid resistant solid tumors [41]. Results from animal studies have indicated activity against colorectal, breast and lung cancer tissues [217]. It is not very potent *in vitro*, with an $IC_{50}$ value of 0.45 μg/ml against colon 38, which is about 100-fold less active than combretastatin A4. However, it showed good efficacy against rodent solid tumors and a wide range of human xenografts [218]. Compound **103**, having a dibenzodiazo group, was the second antimitotic compound in this series. It is two to three-fold more potent than E7010 against various human tumor cell lines *in vitro* [219]. Introducing a pyridivinyl group in place of phenylamino group of E7010 yielded a series of potent antimitotic agents represented by **54**. This compound is 145 times more potent *in vitro* than E7010 against colon 38 cells and is efficacious in the mouse model against colon 26 cells [220]. N- (3-chloro-7-indoyl)-1,4-benzenesulfonamide, E7070), compound 105, blocked the cell cycle progression in G1 phase but not M phase and showed significant antitumor activity against HCT116 human colon carcinoma both *in vitro* ($IC_{50}$ 0.11 μg/mL in cell proliferation assay) and *in vivo* [221]. It has also been reported that tumor cells exposed to E7070 have been found to accumulate at the $G_1$ phase without binding to tubulin [222]. For that reason, E7070 is not categorised as an antimitotic agent, but rather as a cytostatic agent acting on



cell cycle regulation at the $G_1$/S phases [222]. This promising drug was reported to be in phase I clinical trials [223].

T138067, a synthetic compound (2-fluoro-1-methoxy-4-pentafluorophenyl-sulfonamidobenzene) (**106**) has been reported to modify tubulin covalently at the Cys-239 residue of the β-tubulin isotypes, shared by the β1, β2, and β4 tubulin isotypes and thereby disrupt MT polymerization [101]. The investigators found that T138067, in contrast to other known alkylating agents [224-226], significantly prevented the binding of colchicine to tubulin. This compound exhibited cytotoxicity against tumor cell lines that exhibit resistance to vinblastine, paclitaxel, doxorubicin, and actinomycin D. It showed equal effect against multi-drug resistance human tumor xenografts in athynic nude mice. The authors suggested that this compound might be clinically useful for the treatment of human cancers that have developed resistance to standard chemotherapeutic agents [224]

### Rotenone (107)

A natural 6-deoxyclitoriacetal product that has been extracted from *Clitoria macrophyllia*, is used in Thailand as a traditional medicine for controlling pests and skin diseases [227]. It has also been reported as a potent antimitotic agent which interacts with the colchicine-binding site. This drug is reported to have been used in combination with vinblastine [160].

### Sanguinarine, Chelidonine and Chelerythrine

Sanguinarine (**108**), a natural benzophenanthridine, isolated from the seeds of *Papaver somniferum*, has been reported as an inhibitor of the binding of both colchicine and podophyllotoxin to tubulin [228]. This compound inhibits paclitaxel mediated polymerization of MTs at micromolar concentrations [132]. Chelidonine (**109**), isolated from *Chelidonium majus*, is related to Sanguinarine (**108**) and has been noted to have antimitotic activity [229]. Another structurally similar compound Chelerythrine (**110**) has also been reported to inhibit both colchicine and podophyllotoxin from binding to tubulin in higher concentrations [160].

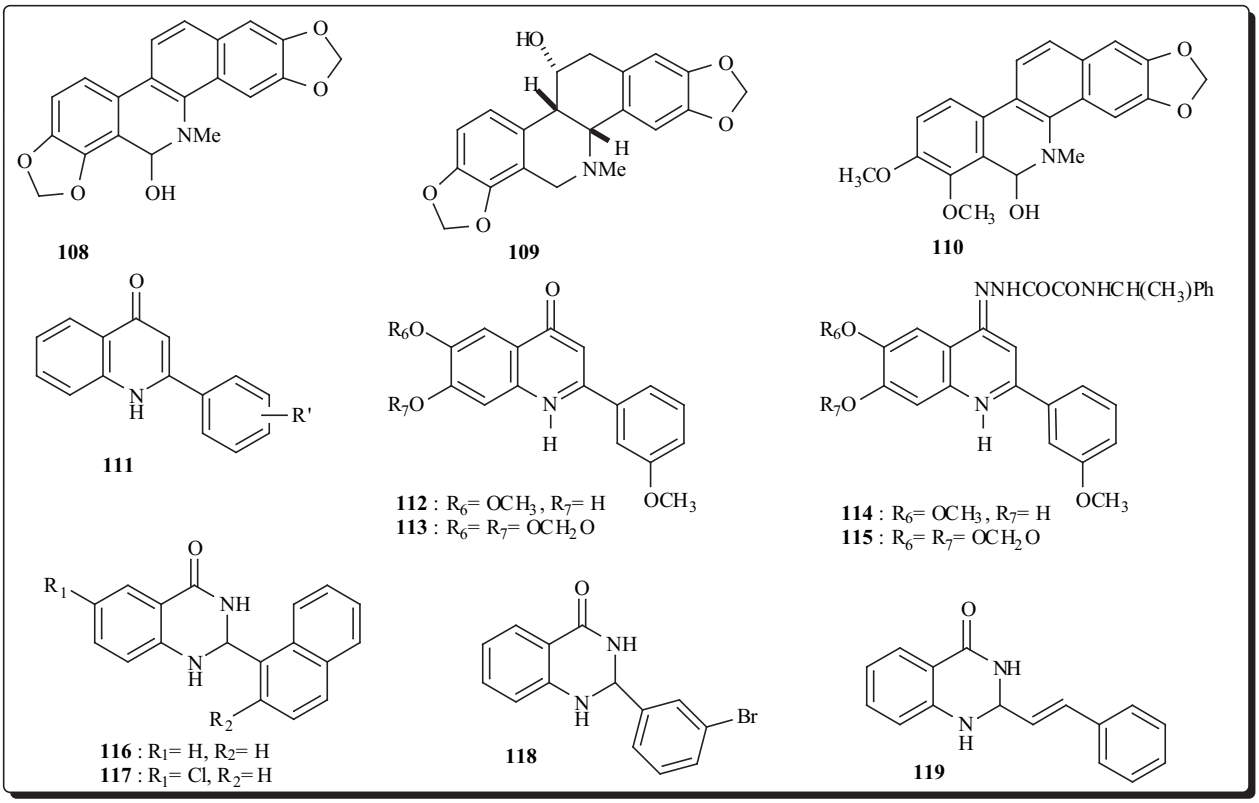
### Quinolones and Derivatives

#### *2-Phenyl-4-Quinolones (PQ)*

Synthesized Quinolones (**111**), the amino analogs of cytotoxic antimitotic flavonoids have been reported to have antimitotic/antitumor activity [230-232]. A series of 2-aryl-4-quinolones were synthesized and evaluated for their interaction with tubulin, including inhibition of tubulin polymerization and colchicine binding to tubulin and cytotoxicity *in vitro* against some tumor cell lines [132].

#### *1,2,3,4-Tetrahydro-2-Phenyl-4-Quinones*

These compounds are another class of quinolone type drugs that have been identified as antimitotic agents. Reduction of the double bond at the 2,3 –position led to a new series of 2,3-dihydro-2-pheny-l-4- (1H)-quinolones (DHPQ, **101**-**104**) which showed potent antimitotic and antitumor activity [132]. 2,3-dihydro-2- (aryl)-4- (1H)-quinazolinones (DHQZ, compounds **116**-**118**) derived by the reduction of the double bond at the 2,3-position together with replacement of C (3) by a nitrogen [11], were shown to have antitumor activity [233,234]. Their tubulin inhibition activity has been identified [235,236]. Compound **69**, a 2-strylquinazolin-4 (3H)-one (SQZ) derivative has been shown



to have antimitotic activity [237,238]. Structure-activity relationship studies of quinolone derivatives led to the discovery of a particularly potent compound, NSC 664171 (**120**), which showed inhibitory activity against tubulin polymerization and radiolabeled colchicine binding to tubulin, suggesting that these compounds are colchicine site binding tubulin inhibitors [132]. This compound also exhibited strong cytotoxic effects against most human tumor cell lines with $GI_{50}$ values in the nanomolar or subnanomolar concentration range [231].

## 2-Aryl-Napthyridinones

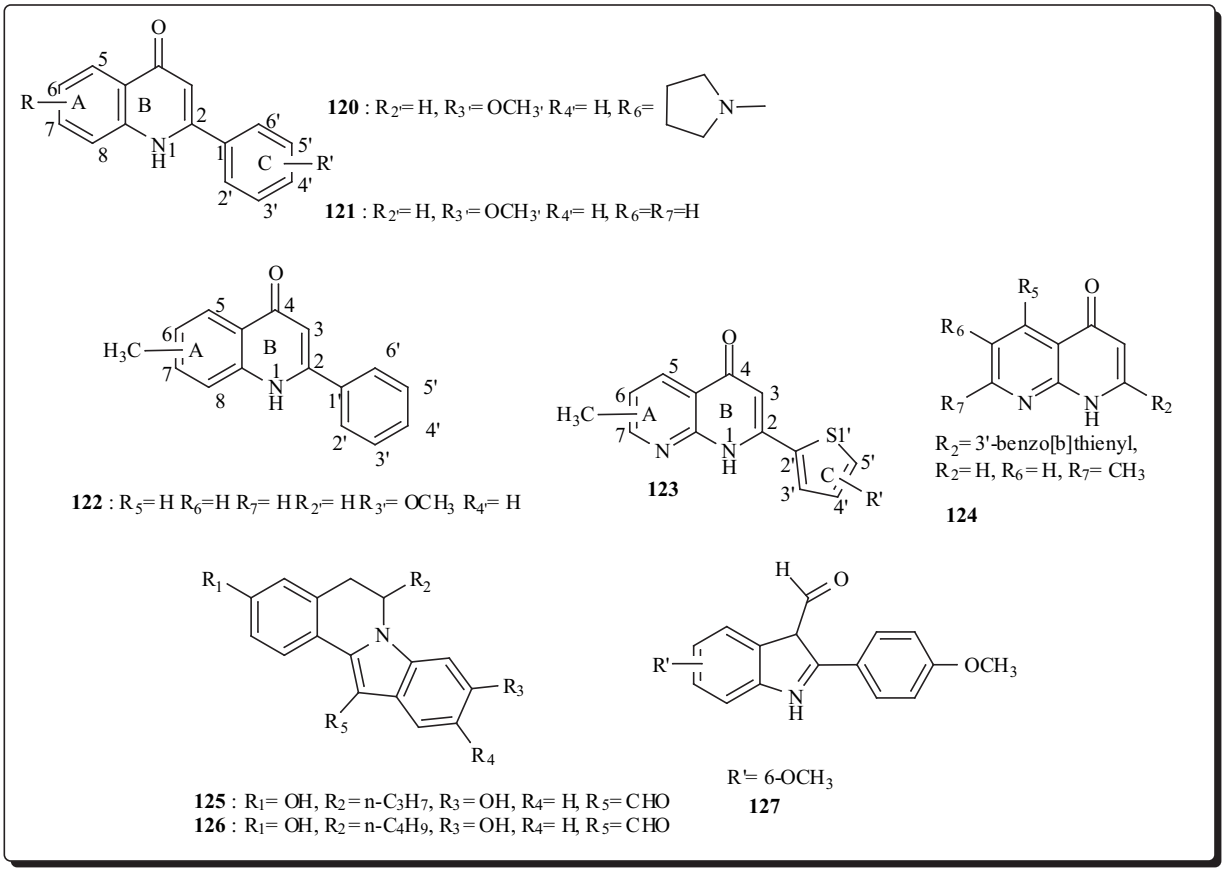
Synthetic derivatives of 2-Aryl-napthyridinones,e.g., 2-(-3-methoxyphenyl)-1,8-napthyridin-4 (1*H*)-one (**122**), showed potent cytotoxic effects sagainst prostate and breast cancer cell lines and inhibition of tubulin assembly and radiolabeled colchicine binding to tubulin [132]. This compound has also been reported to have 100-fold greater activity than the corresponding 2- (-3-methoxyphenyl)-4-quinolone (**121**) [132]. Substituted 2-thienyl-1, 8-napthyridin-4-ones, typified by 123, showed significant cytotoxic effect (log $GI_{50}$<-4.0) against a range of human tumor cell lines. Among these compounds only **124** was a potent inhibitor of the binding of radiolabeled colchicine to tubulin and it was the most cytotoxic and effective inhibitor of tubulin polymerization [239].

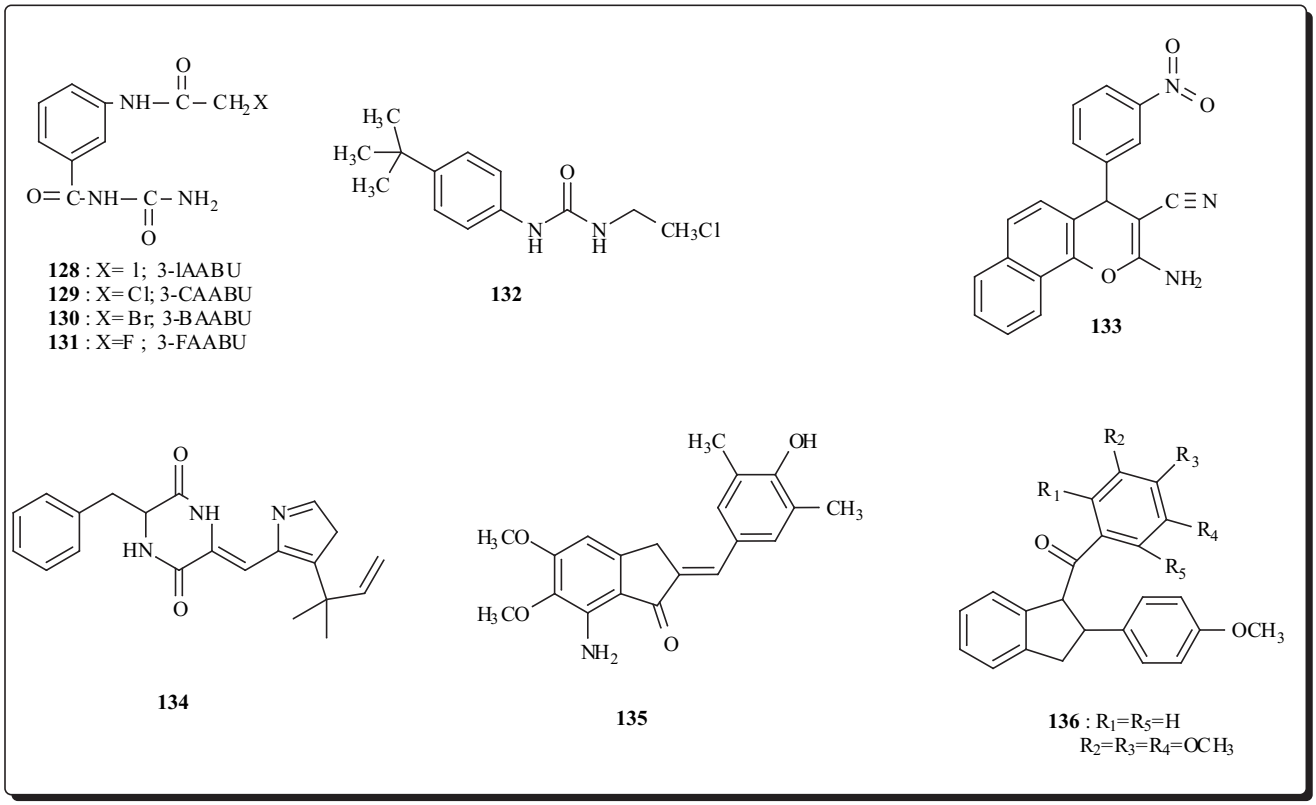
### *5,6-Dihydroindolo[2,1-A]isoquinoline Derivatives*

A number of 5,6-dihydroindolo[2,1-a]isoquinolines with methoxy or hydroxy groups in positions 3,9 and /or 10 and various functional groups such as formyl, acetyl, cyano or alkylamino in position 12 were synthesized and evaluated for both tubulin polymerization and cytostatic activity in MDA-MB 231 and MCF-7 human breast cancer cells. 6-alkyl-12-formyl-5,6-dihydroindolo[2,1-a]isoquinolines, typified by the compounds 125 and 126, have been shown to inhibit the growth of human mammary carcinoma cells by an unknown mode of action [240]. Highest activity was found for the (+)-isomers of 6-propyl- and 6-butyl-12-formyl-5,6-hydro-3,9-dihydroxyindolo[2,1-a]isoquinoline with $IC_{50}$ values of 11 ±0.4 and 3.1 ± 0.4 μM, respectively for the polymerization of tubulin at 37°C [240]. The experiment also showed that hydroxy-substitute indolo[2,1-a]isoquinolines bind to the colchicine binding site and inhibit the polymerization of tubulin [240]. 3-formyl-6-methoxy-2- (4-methoxyphenyl)indoles, typified by 2-phenylindoles derivative **127**, inhibited tubulin polymerization and cell growth inhibition with $IC_{50}$ values of 1.5 μM and 35nM respectively [241].

## Benzoylureas

Among benzoylureas (**128**-**131**), 3- (iodoacetamido)-benzoylurea (3-IAABU) (**128**), a synthetic compound with a much smaller molecular weight than those isolated from natural sources, exhibited anticancer activity in a variety of tumor cell lines with $ID_{90}$ in the range of 0.015-0.29 μM for leukemic cells and 0.06-0.92 μM for solid tumors by inhibiting microtubule assembly [242]. The mechanism of inhibition was similar to that of nocodazole [243]. Of the four (**128**-**131**) compounds, bromine (**130**) and iodine (**128**) derivatives showed strong antimicrotubular activities. The primary action of these compounds is to bind at or near the






colchicine site of tubulin and inhibit microtubule assembly blocking the cell cycle at mitosis [242].

**Chloroethylurea (CEU,132)**

Although the mechanism of CEU cytotoxicity is unknown, evidence was provided that 1-aryl-3- (2-chloroethyl) ureas, a novel class of mild alkylating agents, prevented microtubule depolymerization through alkylation of Cys239 residue of β-tubulin near the colchicine binding site [244]. Among a series of CEU derivatives, compound CEU showed potent cytotoxicity in CHO-TAX 5-6 with $IC_{50}$ value of 4.6± 0.3 μM) [244].

**LY290181 (133)**

2-amino-4- (3-pyridyl)-4H-napthol (1,2-b)pyran-3-carbonitrile, a potent antimitotic agent for a variety of cell types, is mediated by binding to a novel site of tubulin [245] and suppressing the dynamics of mitotic spindle microtubules or tubulin polymerization [246]. Cell cycle analysis showed that LY290181 caused accumulation of smooth muscle cells at the $G_2$/M phase and induced mitotic arrest in Chinese hamster ovary cells and Hela cells [246]. The potency of this compound was comparable to known anti-microtubule compounds such as colchicine or vinblastine [247]. This compound was also found to inhibit vascular smooth muscle cell proliferation [248] and to reduce diabetic-induced endothelial cell dysfunction including blood flow and vascular permeability [249]. (-)-Phenylahistatin (**134**), isolated from *Aspergillus ustus*, showed cyototoxic and cell cycle inhibitory activities during $G_2$/M phase. This fungal diketopiperazine metabolite composed of phenylalanine and dedydrohistidine is reported to bind to tubulin at colchicine site but the precise mechanism is unknown [250].

**Indanosine (135)**

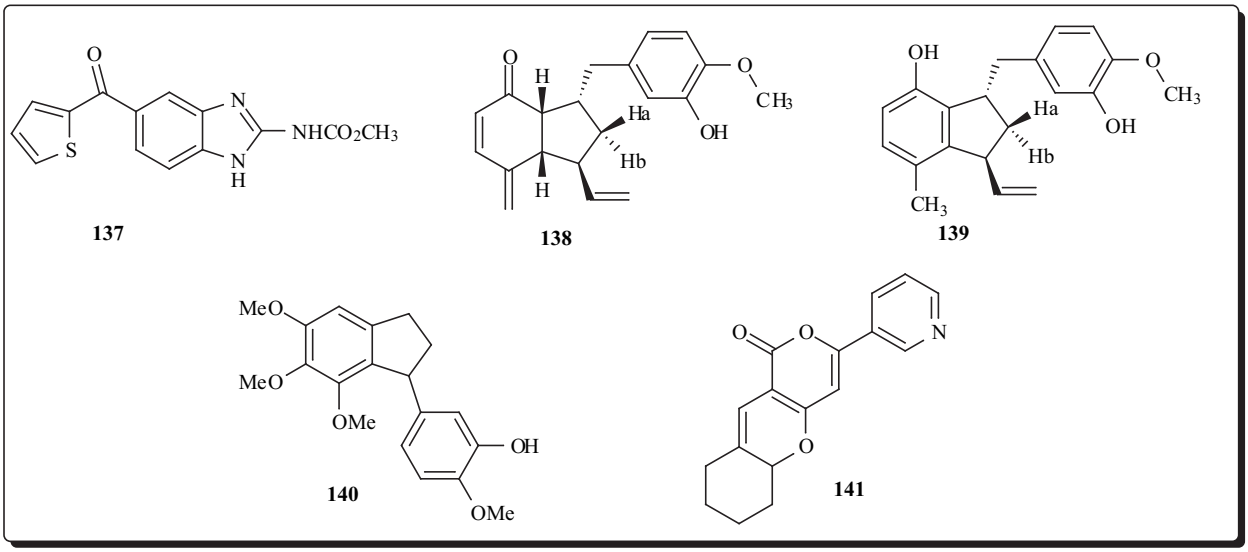
A cytostatic and cytotoxic indanone, potentially inhibited the tubulin polymerization *in-vitro* and disrupted the mitotic apparatus in dividing cells [251]. This drug arrested the growth of multidrug-sensitive cancer cells at the $G_2$/M phase and induced apoptotic cell death in stationary phase cells at concentrations that do not impair the viability of normal nonproliferating cells [251]. Indanosine interacts with tubulin at the colchicine-binding site and inhibits tubulin polymerization with an $IC_{50}$ value equivalent to values obtained with podophyllotoxin and combretastatin A-4 [252]. The investigator suggested that indanosine and related indones may be considered as lead compounds for the development of chemotherapeutic strategies for drug-resistant malignancies.

**Benzothiophene Compounds**

Another type of tubulin polymerization inhibitors were discovered based on the 3-aryol-2-arylbenzo[b]thiophene molecular skeleton. The most promising compound was 2-(4'-methoxyphenyl)-3-(3',4',5'-trimethoxybenzoyl)-6-methoxy-benzo[b]thiophene (**136**), which interacts with tubulin at colchicine binding site and showed significant human cancer cell growth inhibitory activity ($GI_{50}$<10mg/ml) [253].

**Nocodazole (137)**

An effective antihelmintic agent and the most active member of a family of benzimidazole derivatives, competitively inhibits the binding of colchicine to tubulin




[132] and disrupts the dynamic balance between polymerisation and depolymerisation of microtubules.

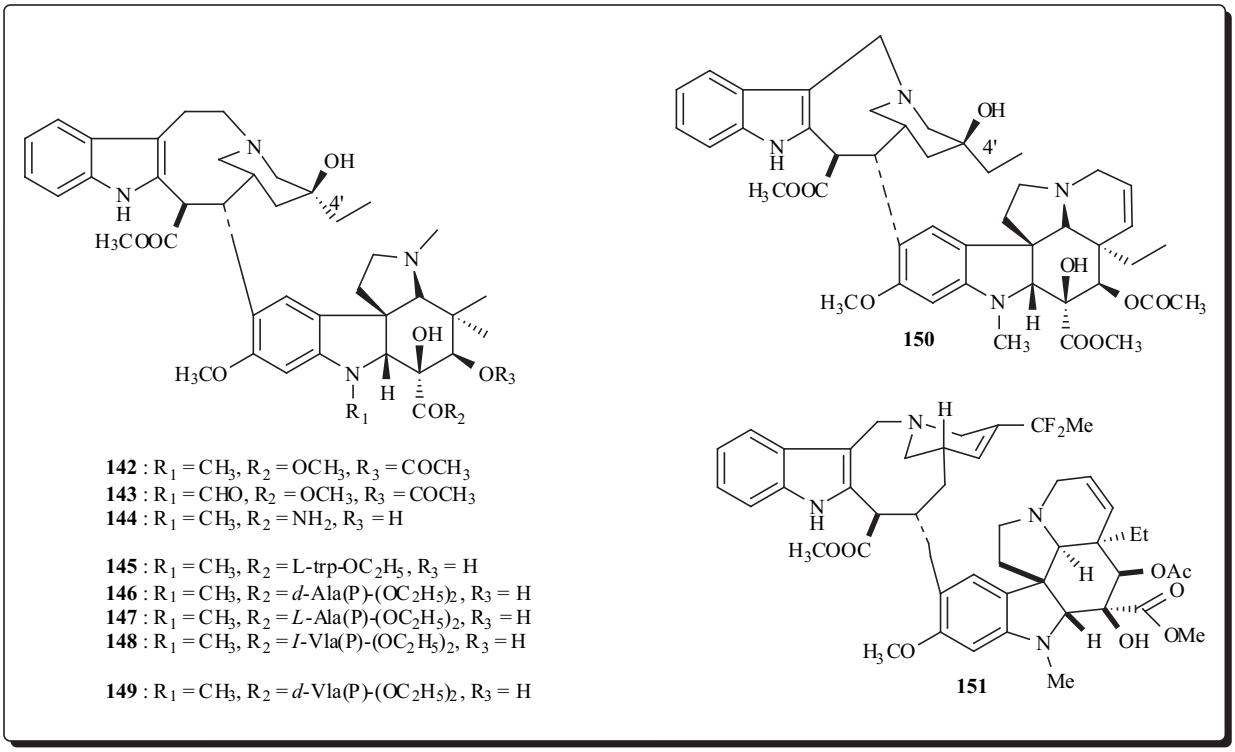
**RPR112378 (138) & RPR115781 (139)**

Two natural classes of antimitotic agent isolated from the Indian plant *Ottelia alimoides*, are effective inhibitors of tubulin polymerization ($IC_{50}$= 1.2 μM) and are able to disassemble preformed microtubules [254]. Compound **138**, 5-fold more active than compound **139**, prevented colchicine binding but not vinblastine binding which indicated that these compounds bind to or near the specific colchicine binding site [254].

Compound (**140**), a temperature dependent potent inhibitor of tubulin polymerization that exerts its cytotoxicity through interaction at the colchicine site, demonstrated highest activity with $IC_{50}$ value of 11.8 μM over the temperature range of 30-37°C [255]. This is a new approach developing anticancer drugs.

**Tricyclic Pyron Analog (TP)**

Typified by (**141**), are a class of bifunctional antimitotic agents that inhibit nucleoside transport ($IC_{50}$ = 6 μM), tubulin polymerisation ($IC_{50}$= 1.5 μM), the viability of leukemic cells *in vitro* ($IC_{50}$ = 0.05 μM) and the growth of solid tumors *in vitro* [256]. Studies suggested that TPs may interact with tubulin at the colchicine binding site to inhibit MT assembly without disrupting the binding sites for GTP or vinca alkaloids [256]. The ability of **141** to inhibit MT assembly and increase the mitotic index of tumor cells




**142** : $R_1 = CH_3$, $R_2 = OCH_3$, $R_3 = COCH_3$
**143** : $R_1 = CHO$, $R_2 = OCH_3$, $R_3 = COCH_3$
**144** : $R_1 = CH_3$, $R_2 = NH_2$, $R_3 = H$

**145** : $R_1 = CH_3$, $R_2$ = L-trp-$OC_2H_5$, $R_3 = H$
**146** : $R_1 = CH_3$, $R_2$ = *d*-Ala(P)-$(OC_2H_5)_2$, $R_3 = H$
**147** : $R_1 = CH_3$, $R_2$ = *L*-Ala(P)-$(OC_2H_5)_2$, $R_3 = H$
**148** : $R_1 = CH_3$, $R_2$ = *l*-Vla(P)-$(OC_2H_5)_2$, $R_3 = H$

**149** : $R_1 = CH_3$, $R_2$ = *d*-Vla(P)-$(OC_2H_5)_2$, $R_3 = H$

indicates that these novel drugs may be considered as cell cycle specific anticancer drugs that are useful for arresting cells in M phase [257,258].

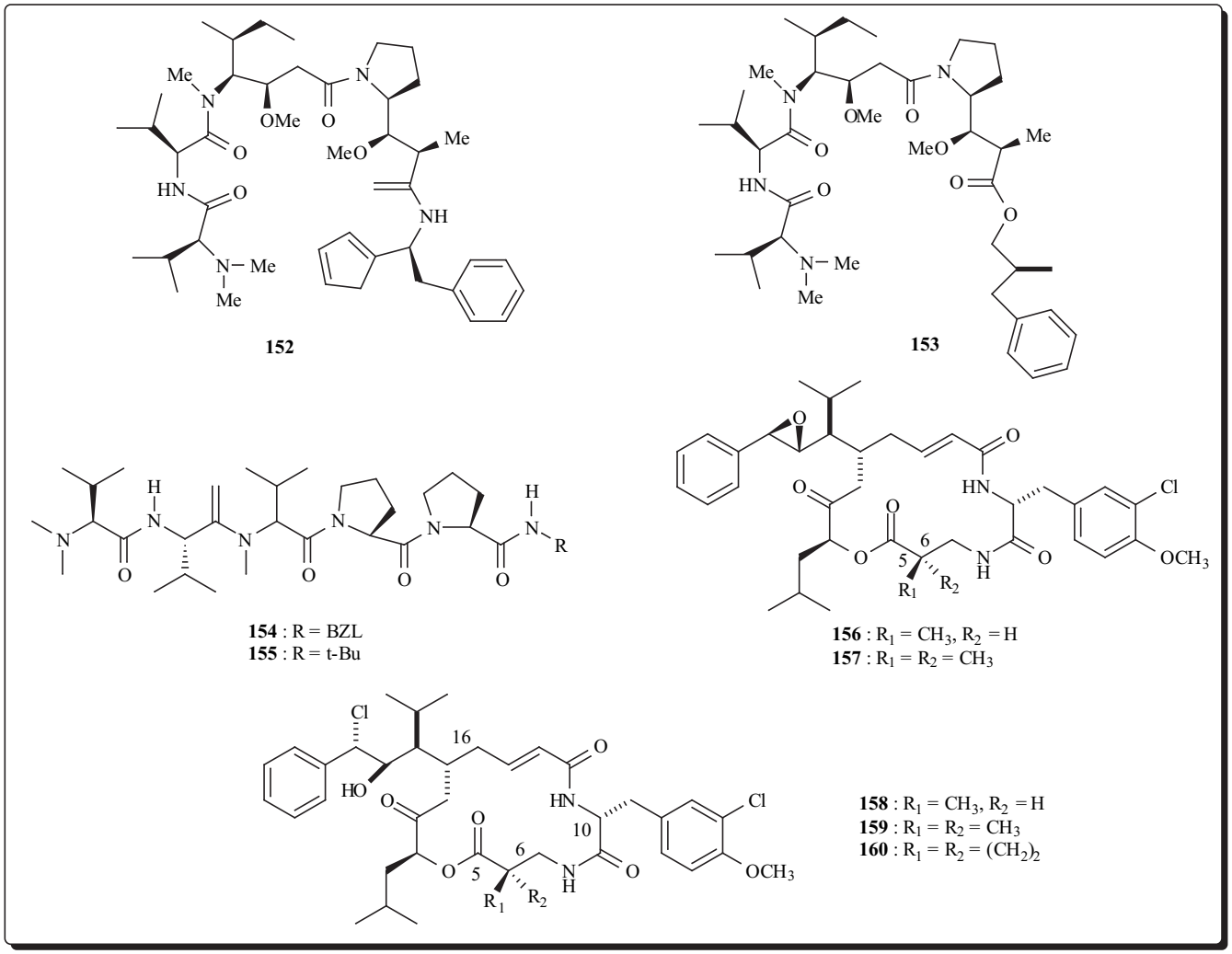
### 1b.ii: Vinca Alkaloid Site Binding Drugs

Vinca alkaloids, vincristine (**142**) and vinblastine (**143**), are the most useful class of antimitotic anticancer agents for the treatment of leukemias, lymphomas and some solid tumors [259]. These agents, isolated from the Madagasker periwinkle *Catharanthus roseus*, are widely used as anticancer drugs. Vinca alkaloids prevent MT assembly by binding to tubulin at a site distinct from colchicine and by blocking the region involved in heterodimer attachment [260]. Vinca alkaloids bind to tubulin at GTP site that is located at both the α and β- subunits with primary sequences around α-339 and β-390 residues [261]. Unlike colchicine, vinca alkaloids bind to tubulin rapidly, reversibly and temperature independently [125,136]. The SAR, pharmacology and clinical uses of vinca alkaloids have been reviewed [132, 160]. After the establishment of vinca alkaloids as promising anticancer agents, efforts have been made to develop new congeners that are more effective, have less side effects and have a broad spectrum of antitumor activity. The basic structure of vinca alkaloids is composed of a catharenthine moiety and vindoline moiety and these alone cannot inhibit MT assembly [262]. Most of the semisynthetic or totally synthetic vinca alkaloids drugs have been developed by the modification of C-4, C-23, C-3' and C-4' position of parent vinca alkaloids [132].

### Vindesine (144)

The first semisynthetic vinca alkaloid, was developed by changing the C-23 acetyl group in vinblastine to an amide [132]. Modifying the vindoline moiety with L-tryptophane at C-23 led to the development of vintripole (**145**) which is in clinical trial [263,264]. Some of its derivatives (**146**-**148**) were found to inhibit tubulin polymerization [265]. The S-isomers were more active [132].

**Vinxaltin** (**149**) is in phase II clinical trial for advanced breast cancer and S-epimer showed more activity than the R-epimer [266]. Another semisynthetic compound vinorelbin (**150**) [267] showed promising activity against breast cancer [268, 269] and is in clinical trial for the treatment of other types of tumors [270]. This drug showed superior antimitotic activity over others with lower reversible neurotoxicity [270-272]. Further modifications in vinorelbin led to the discovery of vinflunin (**151**) which showed significantly superior anticancer activity *in vivo* as compared to vinorelbin [273,274].



## Other Vinca Alkaloid Site Binding Drugs

### *Dolastatin and its Derivatives*

The most promising is dolastatin-10 (**152**), a natural compound isolated from the sea hare *Dolabella auricularia* in 1987. This drug and its related compound dolastatin-15 (**153**) are novel pentapeptides which exhibit powerful antimitotic properties [275]. They are potent inhibitors of tubulin, acting as noncompititive inhibitors of vinblastine binding to tubulin [276]. Dolastatin-10 is 9 times more potent than dolastatin-15 and both are more potent than vinblastine [277]. Dolastatin 10 & 15 (with $IC_{50}$ values of 0.13 to 1.3 pM and 1.3 to 13 pM respectively) are 1000 to 10,000 times more effective than vincristine on various human lymphoma cell lines [278]. Dolastatin10 and 15 showed the most promising antimitotic activity and both have entered clinical trial. Earlier, the discovery of dolastatin 3-15 had been reviewed [279] and SAR, synthesis and pharmacological properties of these agents have been described [280].

Cemadotin (**154**), a water soluble pentapeptide analogue of dolastatin15, is in phase II clinical trials for metastatic melanoma and other solid tumors [281,282]. Cemadotin showed good activity against a broad range of murine and human tumors with $IC_{50}$ values typically in pM range [283]. It also showed excellent efficacy in a number of xenograft and synergeneic tumor models [284]. Compound **155** has oral bioavailability with similar *in vivo* and slightly reduced *in vitro* activity when compared to cemadotin [285].

### *Cryptophysin Analogues (156-160)*

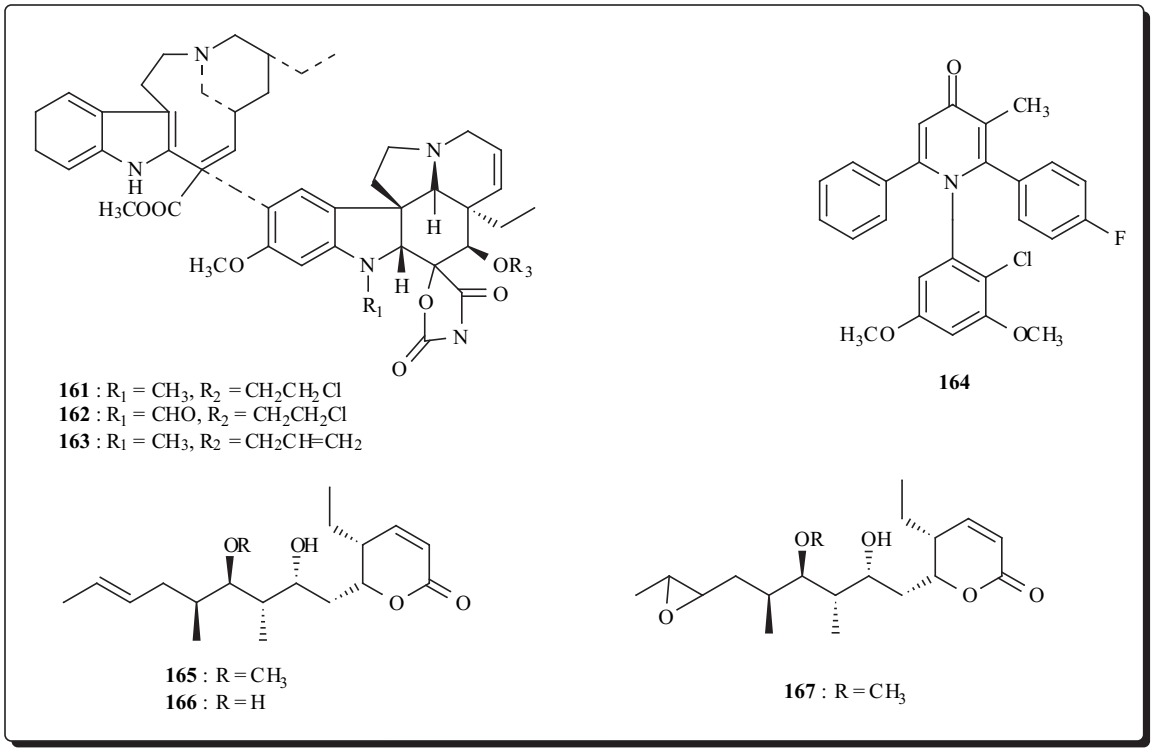
Cryptophysin 1 (**156**), a depsipeptide isolated from the cyanobacterium *Nostoc* sp [286], is a potent antimitotic agent having 100 to 1000-fold greater activity than paclitaxel and vinblastine which induces cell death by apoptosis [287]. This drug does not appear to be a substrate for the p-glycoprotein multidrug transporter [288]. Cryptophysin analogues have been synthesized [289-291] and cryptophysin 52 (**157**) showed the most potent antimitotic activity acting to suppress spindle microtubule dynamics [292]. It blocks cell cycle progression at $G_2$/M phase at lower concentration, causes accumulation of cells at metaphase and kills cells by apoptosis [292]. It demonstrated potent antiproliferative activity in both solid and hematologic tumor cell lines including MDR phenotypes with $IC_{50}$ values in the low picomolar range which is significantly lower than paclitaxel or vinblastine [293]. The synthesised C-6 substituted spirocyclopropyl compound (**160**) has excellent antitumor activity in animal models with $IC_{50}$ value of 0.014nM [294].

### *KAR-2 and Derivatives (161-163)*

KAR-2 (3'-chloroethyl)-2',4'-dioxo-3,5'-spiro-oxazolidino-4-deacetoxy-vinblastine, a bis-indol compound, showed antitimor activity with lower toxicity than vinca alkaloids. Its antimitotic activity was due to inhibition of tubulin assembly [295]. Although it binds to calmodulin, in contrast to vinblastine, it does not exhibit anti-calmodulin activity [296]. It was effective in the mouse leukemia P388 test *in vivo* without significant toxicity [296]. KAR-3 (**162**), and KAR-4 (**163**), two derivatives of KAR, also inhibit tubulin assembly. KAR derivatives displayed their cytotoxic activity at significantly higher concentrations than the mother compounds, although their antimicrotubular activities were similar *in vitro* [296].

## IKP-104 (164)

The 4 (H)-pyrizinone derivative (2- (4-fluorophenyl)-1-(2-chloro-3,5-dimethoxyphenyl)-3-methyl-6-phenyl-4-(pyridinone) is a novel antimitotic drug which inhibits MT



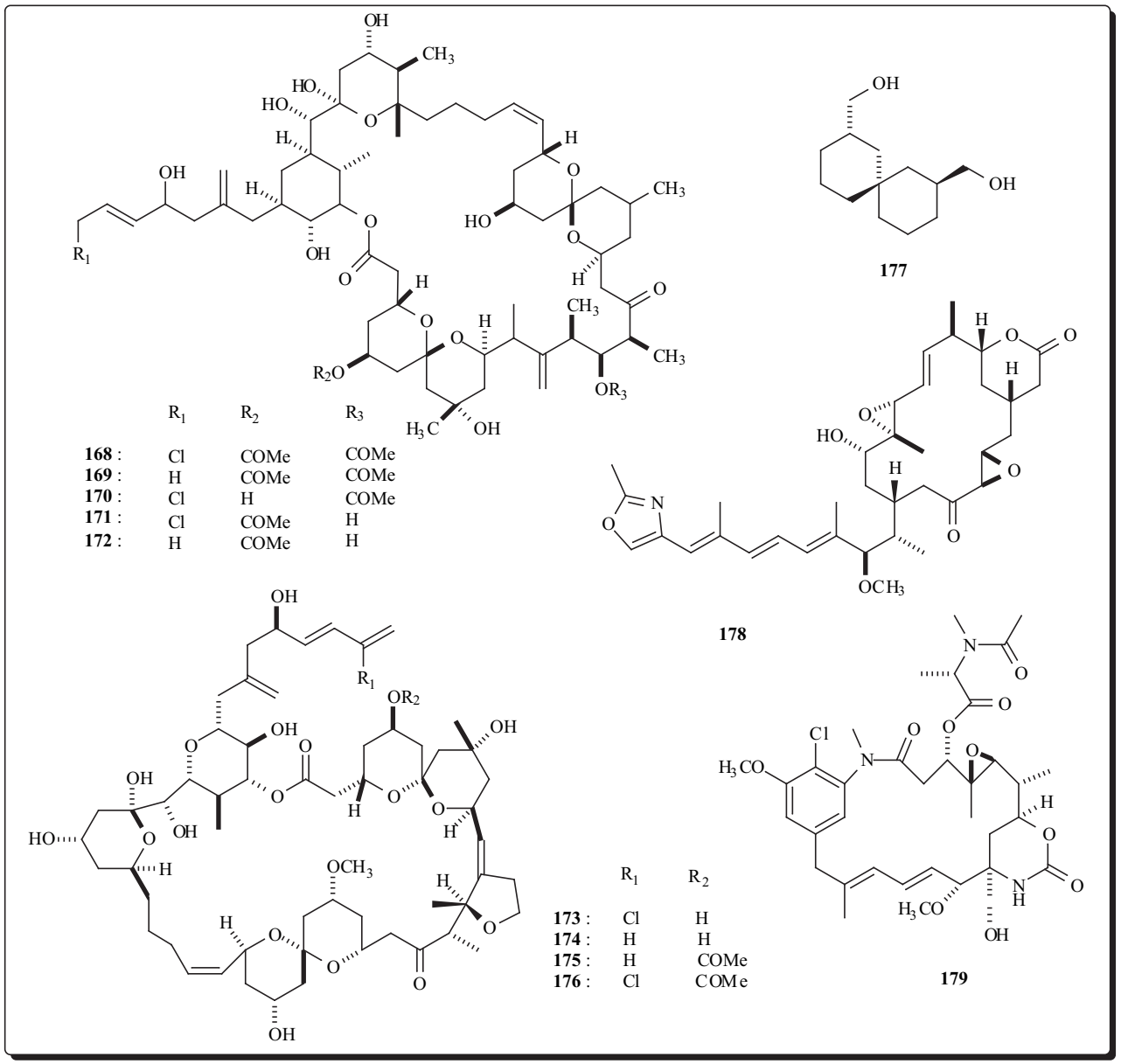
assembly both *in vitro* and *in vivo* and polymerizes tubulin into spiral filaments [297]. This compound also accelerates the decay of tubulin [298].

### *Pironetin and Derivatives (165-167)*

pironetin (**165**) [299,300] and demethylpironetin (**166**) [301] produced potent tubulin assembly inhibition by arresting the cell cycle in M phase ($G_2$/M) and showing antitumor activity against amurine tumor cell line P338 leukemia, transplanted in mice, with $IC_{50}$ value 15 μM. The antitumor activity of these compounds is due to apoptosis caused by the phosphorylation of Bcl-2 and by mitotic arrest [302]. Epoxypironetin (**167**), a derivative of pironatin, showed very weak activity. Pironetin (**165**) was found to inhibit the binding of vinblastine to tubulin. The affinity of pironetin to tubulin is higher than that of vinblastine [302]. Important structural features for MT inhibitory activity of **165** were found to be αβ-unsaturated lactones with chirality at the C-7 position bearing a hydroxyl group and the terminal portion of the alkyl chain [303].

### *Spongistatin*

These complex macrocyclic lactone compounds (**168**-**176**), isolated from the *Spirastrilla spinispirulifera*, have been reported to display potent cytotoxicity with $IC_{50}$ value of 10 μg/ml [304]. Spongistatins showed inhibitory activity against binding of vincristine [305] and dolastatin to tubulin [306]. Spongistatin I (**168**), isolated from an Eastern Indian Ocean sponge *Hyrtios erecta* [307], has been docked in to the 3-D atomic model of the αβ-heterodimer. It was found that the putative spongistatin binding pocket consists of an unusual cluster of 10 aromatic residues situated in close proximity that includes Y 108, W 103, Y 185, W 407, F 399, F 404, F 395, F 418 and H 408 [308]. This compound has also been reported to exert broad spectrum antifungal properties following antimicrotubule activity [309]. A novel synthetic spiroketal pyran, SPIKET-P (**177**) was later rationally designed as a pharmacophore for spongistatin, resulting in tubulin depolymerization in cell free turbidity assays, prevention of mitotic spindle formation, destruction



of the MT organization and induction of apoptosis in human breast cancer cell at subnanomolar concentrations [308]. Docking studies revealed that, when bound to tubulin, the spiroketal ring of SPIKET-P (**177**) would be situated between aromatic residues F 404 and W 407 in the binding pocket and would provide favorable hydrophobic interactions and van der Waals contacts with these residues [308].

Rhizoxin (**178**), isolated from the fungus *Rhizopus chinensis*, an antifungal agent, has antimitotic activity. Although there is some controversy over the exact binding site of this compound it may be regarded as a vinca alkaloid site binding drug because it can affect the binding of vinca alkaloids to tubulin [310]. This compound has been reported to be effective against many human cancer cell lines and some vinca alkaloids resistant tumors [311].

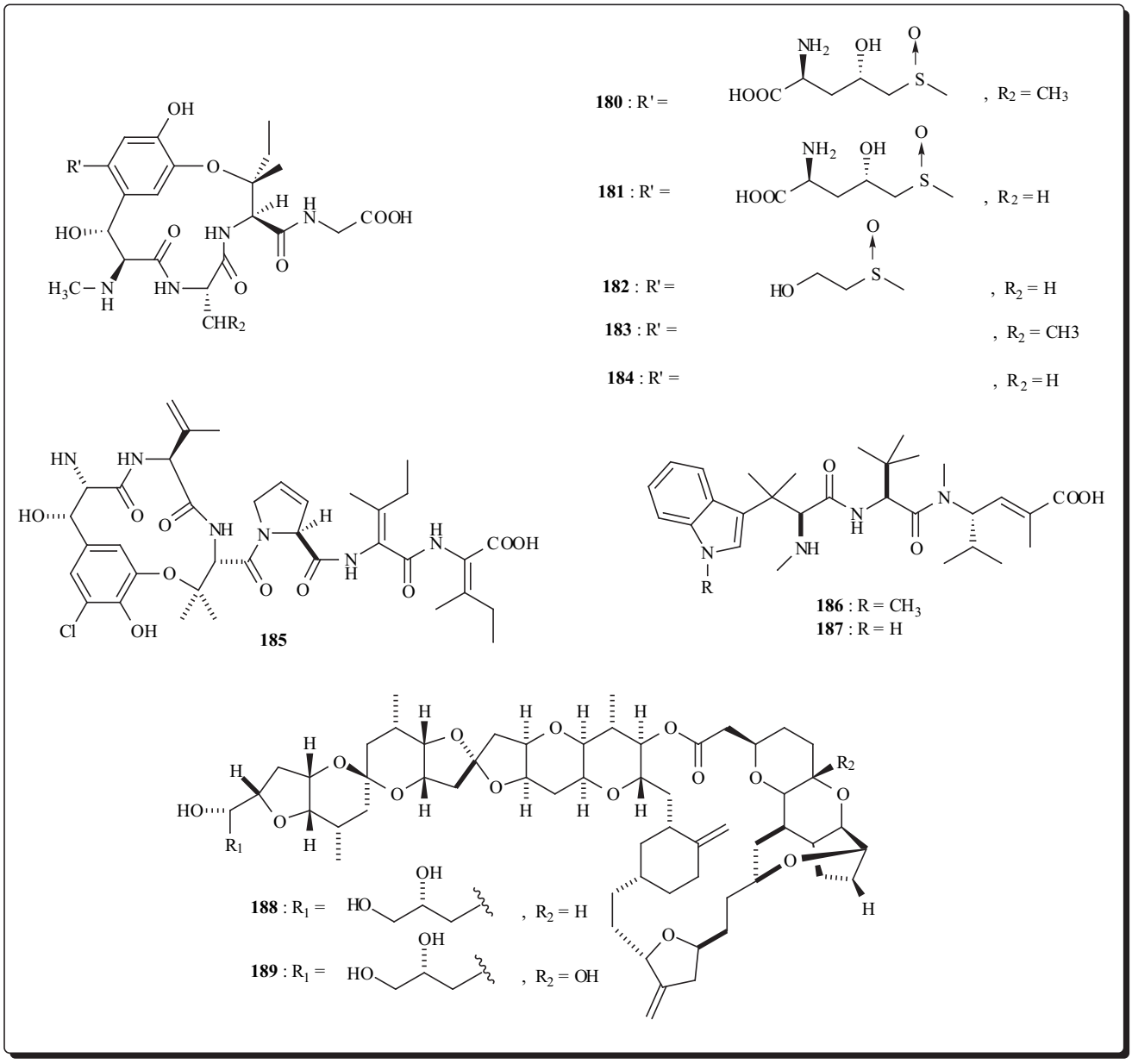
Maytansin (**179**), a family of highly cytotoxic macrolides isolated from *Maytenus* species, binds to tubulin in a reversible manner and can competitively inhibit the binding of vinblastine and vincristine [312,313]. Maytansin binds to assembled tubulin and causes disassembly of microtubules and prevents tubulin spirilization [314].

Ustiloxins (**180**-**184**), a family of four cyclic peptides, isolated from the water extract of false smut balls on rice panicles caused by the fungus *Ustilaginoidea virens*, have been found to strongly inhibit the formation of MTs [315,316]. Ustiloxin A (**180**), the most potent member of the family shows similar biological activity to rhizoxin and prevents mitosis at concentrations below 1 μg/ml [317]. Ustiloxin A (**180**), B (**181**), C (**182**), D (**183**) and F (**185**) have shown $IC_{50}$ values of 1.0, 1.8, 2.5, & 10.3 μM respectively [318].

### *Phomopsin A*

Phomopsin A (**185**) produced by the fungus *Phomopsin leptostromiformis*, causes disassembly of microtubules and prevents further polymerisation of tubulin [319].

Halicondrin B (**188**), a complex polyether macrolide isolated from *Halikondria okadai*, *Axinella carteri*, and



*Phankella carteri*, has been reported to arrest cell growth at subnanomolar concentrations. This compound, which has been synthesized, and its related compound halistatin (**189**) are noncompetitive inhibitors of the binding of both vincristine and vinblastine to tubulin [160].

### *Hemistarlins (186-187)*

These compounds, isolated from the marine sponge *Cymbastela* sp., show potent inhibitory activity against the P338 cell line with $IC_{50}$ value 0.4ng/ml [320].

## Drugs binding With Other Sites:

### *MAPs*

Estramustine Phosphate (EMP, **190**) [321], a synthetic antimitotic agent, has been found to alter dynamic stability of MTs by binding to the MAPs, specifically MAP-2. This compound also caused drug induced apoptosis in the human malignant glioma cell line [322]. Oral EMP in combination with etoposide is a treatment regimen for patients with hormone-refractory prostate cancer and thereby deserves further investigation [323]. Clinical evaluation of this drug and vinblastine in patients with hormone-refractory prostate cancer has also been reported [324].

### *Motor Proteins*

Monastrol (**191**) arrested cells in mitosis by binding to kinesin Eg5, a motor protein that is essential for production of a bipolar spindle. Monastrol inhibits the Eg5 driven microtubule motility with an $IC_{50}$ value of 14 μM [325]. It was suggested that this drug, with limited clinical benefits, would be useful as a tool for studying mitotic mechanism.

## Drugs Binding to Sulfhydryl Groups

The sulfhydryl groups of tubulin are highly reactive entities, especially a thiol group on cysteine residues. Tubulin has 20 cysteine residues, of which 12 are in the α-tubulin and 8 in the β-tubulin subunit [326,327]. These cysteines can be exploited to study the conformational changes of tubulin by binding with ligands. Some compounds inhibit MT assembly by interacting with certain sulfhydryl groups which can be considered as the target for these compounds. The reactivity of these groups with tubulin ligands makes them excellent probes for the interaction of tubulin with ligands. Sulfhydryl directed reagents that prevent MT formation may also be classified as antimitotic agents. Some ligands having antimitotic activity are illustrated below.

N,N'-Ethylenebis (iodoacetamide) (**192**) is a bifunctional agent that interacts with tubulin by forming cross-links between sulfhydryl groups of tubulin and thereby prevents assembly of tubulin into microtubules [328]. This drug is a specific sulfhydryl directed reagent that is being used extensively in various experiments to ascertain the nature and function of sulfhydryl groups of tubulin.

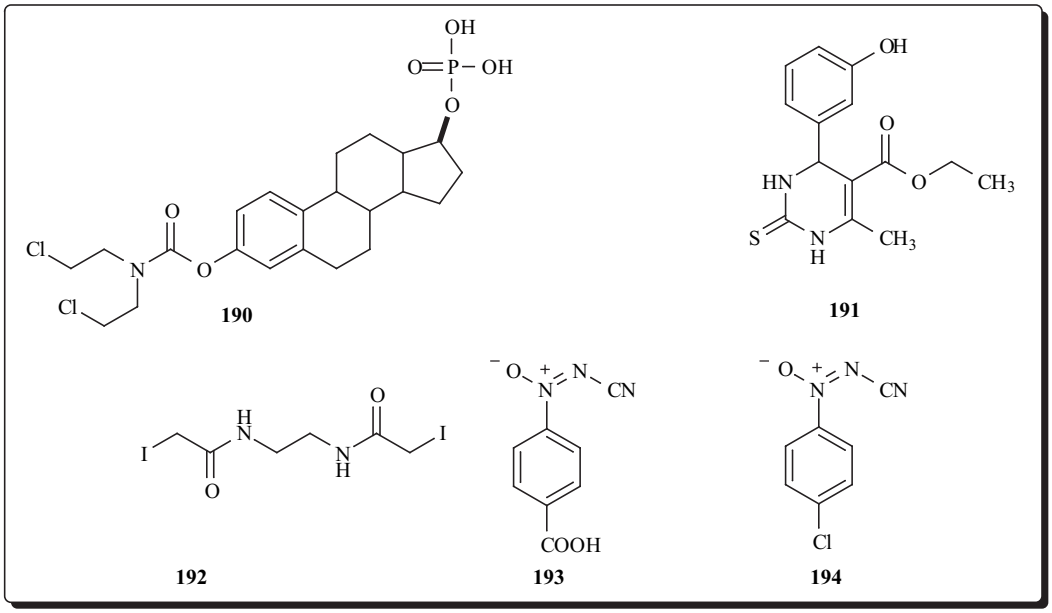
Calvatic Acid (**193**), an antibiotic and cytostatic compound isolated from *Calvatica liacina* [329,330], prevents microtubules assembly by binding with SH groups of tubulin [160]. It has been reported that chloro derivative (**194**) of this compound is more active and prevented the binding of colchicine to tubulin [331].

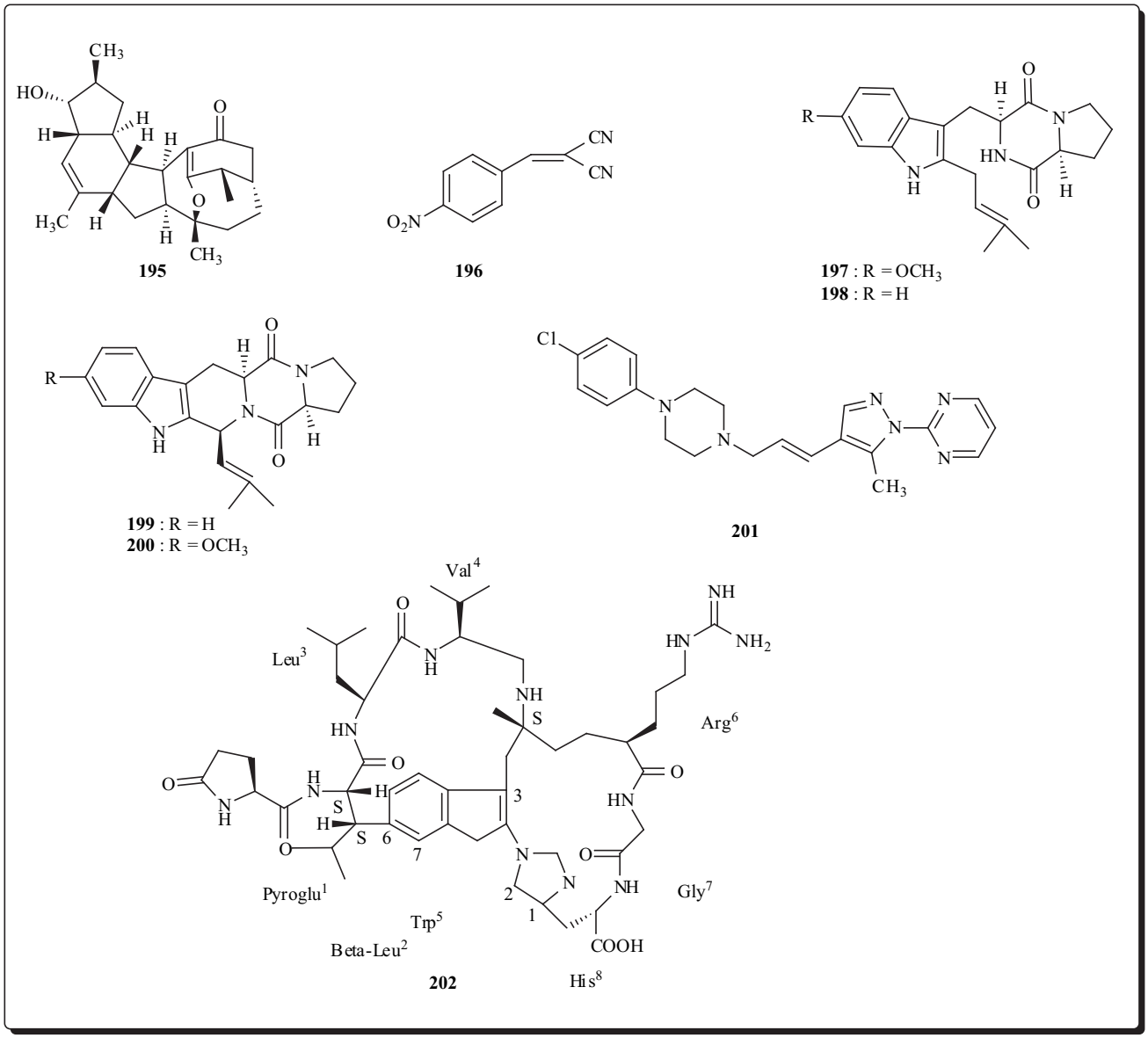
**Methylmercury** (MeHg) specifically promotes the depolymerisation of MT and inhibits cell proliferation in mouse glioma cells [332]. The disruption of MT by MeHg results in the inhibition of the synthesis of tubulin through autoregulatory repression in posttranscriptional process as in the case of colchicine. It has been reported that MeHg depolymerises MT by binding to sulfhydryl groups in the tubulin subunit and increases the pool size of unpolymerised tubulin subunit [333]. It is suggested that MeHg is capable of depressing tubulin synthesis through the increase of unpolymerised tubulin subunits by MT disruption [332].

## Drugs that Bind to an Unknown Site of Tubulin

### *FR 182877 (195)*

A new hexacyclic antimitotic compound isolated from the culture broth of *Streptomycis* sp. No. 9885 [334], exhibited potent antimitotic activity with $IC_{50}$ value of 600






ng/ ml against murine ascitic tumor and human solid tumor *in vivo* [335].

Tryphostin AG-1714 (**196**) and several other related compounds have been reported to disrupt microtubules ($IC_{50}$ = 1-10 μM) in a variety of cancer cell lines. These agents do not affect the polymerization or stability of microtubules *in vitro*, which implies that the effect on microtubules is indirect. However, microtubules disassembly induced by AG1714, was blocked by paclitaxel. The authors also revealed that tyrosine phosphorylation is involved in the regulation of microtubules dynamics [336].

***Tryprostatins (197-200)***

Tryprostatin A (**197**), the first natural diketopiperazine alkaloid isolated from the *Aspergillus fumigates*, inhibits microtubule assembly by interfering with the interaction between MAPs and the C-terminal domain of tubulin and inhibits cell cycle progression at the M phase by disrupting microtubule spindle [337]. Tryprostatin B (**198**) showed cell cycle nonspecific inhibition on cell growth. 198 and demethoxyfumitremorgin C (**199**) have been synthesised and the synthetic compounds showed more potency than that of natural products [338].

**DZ3358** (**201**), a pyrimidinyl pyrazole derivative, exhibited potent antiproliferative activity against several cancer cell lines *in vitro* and antitumor activity *in vivo* [339]. Although the mechanism of action is unclear it has been found to cause cell cycle arrest in G2/M phase and to block tubulin polymerization [340]. The inhibitory effect of this drug on tubulin polymerisation was as potent as that of colchine whereas the antiproliferative activities were weak [340].

Moroidin (**202**), a bicyclic ring system isolated from the seeds of *Celosia argentea* (Amaranthaceae), has been reported to strongly inhibit the polymerisation of tubulin [341]. The seeds of this Chinese herbal medicine are used in the treatment of eye and hepatic diseases in China and Japan [342,343]. The inhibitory activity ($IC_{50}$ value of 3.0 μM) of the tubulin polymerisation by moroidin was more potent that of colchicine ($IC_{50}$ value of 10 μM) whereas the hydrolysate compound of moroidin showed less activity

than the parent compound but was comparable to colchicines [344].

Deuterium Oxide ($D_2O$), a modulator of MT dynamics, is known to promote the assembly of tubulin into MTs *in vitro* to increase the volume of mitotic spindle and the number and length of MTs spindles [345]. The mechanism responsible for the ability of $D_2O$ to stabilise MT dynamic may involve the enhancement of hydrophobic interaction in the MT lattice and/or the substitution of deuterium bonds for hydrogen bonds [345]. $D_2O$ may inhibit cell proliferation during interphase by stabilising the interphase MT cytoskeleton [345] or prevent disassembly that is a prerequisite for the formation of mitotic spindles.

## ACKNOWLEDGEMENTS

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