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Microtubulin Polymerization Inhibitory action of Chalcones, Pyrazoles and Pyrazolines

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ABSTRACT
Microtubulins are formed by the polymerisation of α and β-tubulins, playing majorlyinmaintainance of cytoskeleton, cell signalling, mitosis and gene expression. They mainly function in cell division by forming spindles. These microtubules are potential targets for the development of chemotherapeutic drugs which target rapidly dividing cancer cells.The microtubule targeting drugs inhibits cell proliferation by disrupting the microtubule, causing cell cycle arrest in G_2/M phase and resulting in cell apoptosis. In this review, tubulin polymerization inhibitory activity of chalcones, pyrazoles and pyrazolines were studied to evaluate their anticancer activity.

Keywords: Microtubules, Microtubule Targeting Agents (MTA's), Tubulin Polymerization Inhibition, Chalcones, Pyrazole, Pyrazoline, Multi Drug Resistance (MDR).

I. INTRODUCTION

Microtubules (MT's) are long, hollow cylinders of tubulin dimers that play a major role in the cellular cytoskeleton maintenance and in primary cellular processes such as cell shape organization, cell proliferation, intracellular & vesicular transport, cell signaling, secretion, regulation of motility, mitosis and gene expression[1]. MT's forms mitotic spindles; aggregates of microtubule originating at centrosome (known as the microtubule organizing center) that coordinate the movement of chromosomes throughout mitosis. The main motive of mitotic spindle is to drag the two sister chromatids apart and separate them into two opposite ends of the cell so that cell division can take place[2]. This is why microtubules are potential targets for the development of chemotherapeutic drugs which target rapidly dividing cancer cells.

STRUCTURE OF MICROTUBULINS

Tubulins are cytoskeleton globular protein categorised into five different families, the alpha (α), beta (β), gamma (γ), delta (δ), epsilon (ε) and zeta(z)[2]. Binding of the α and β tubulin protein heterodimers in a head- to- tail fashion results in long polymeric structures called protofilaments and almost 13 these protofilaments forms a cylindrical polymeric tube known as microtubules[3]. In a protofilament, one end has the α subunits exposed whereas the other end has the β subunits exposed, these ends are referred to as (-) and (+) ends, respectively[2].MTs bear irregularstages of growth and shrinkage in a phenomenon known as "dynamic instability"[3]. The binding, hydrolysis, and exchange of a guanine nucleotide on the -tubulin monomer drive the assembly–polymerization and disassembly–depolymerization of microtubules (panel c) (GTP bound to -tubulin is non-exchangeable and never hydrolyzed). GTP hydrolysis is not required for microtubule assembly, but it is required for moving from catastrophe to rescue mode.A pool of GTP-loaded tubulin subunits (part c;(1)) is frequently used to start polymerization.Microtubule

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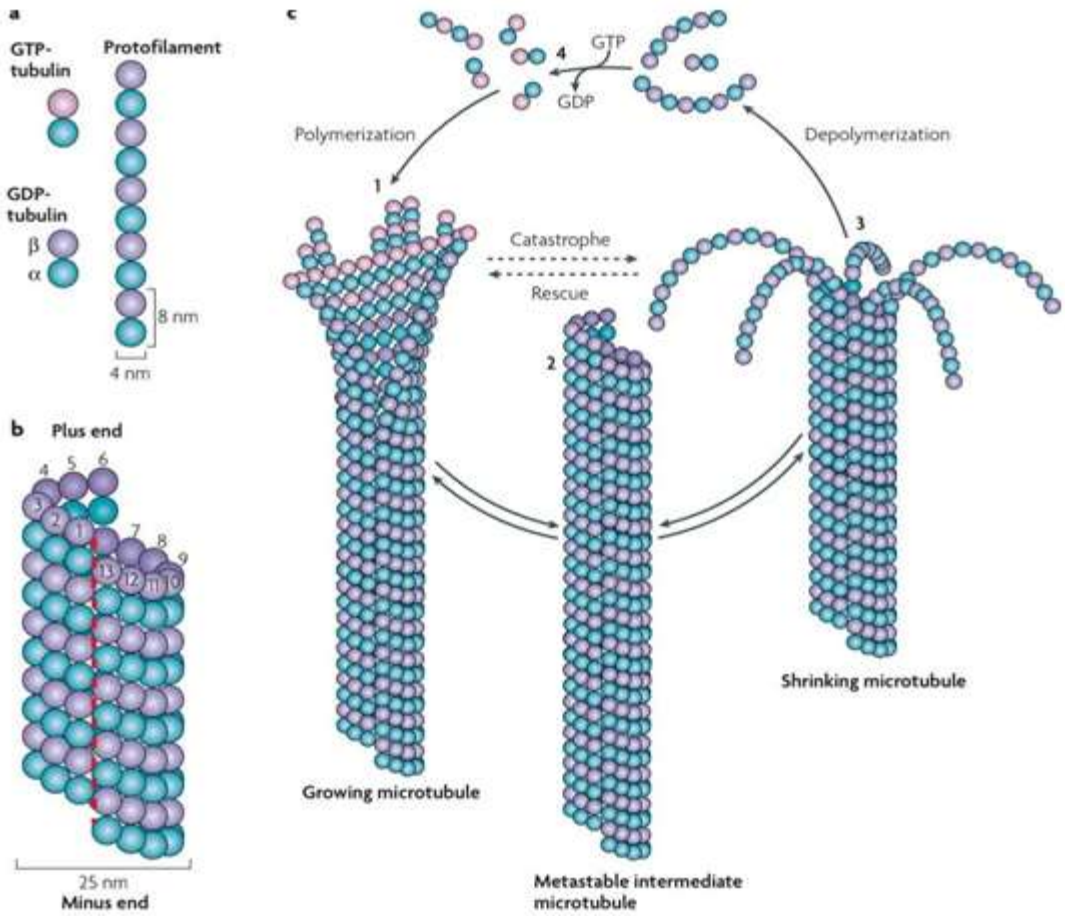

Fig. 1: Microtubule structure and dynamic stability [4].

Ends that are still growing alternate between slightly curved and straight protofilament sheets. GTP hydrolysis and inorganic phosphate release occurs quickly after inclusion, and is aided by the head-to-tail assembly of dimers, which promotes burying and locking of the partially exposed nucleotide.GTP hydrolysis is thought to transform a protofilament's conformation from a slightly curved tubulin-GTP structure to a much more deeply curved tubulin-GDP structure.The curved tubulin-GDP is compelled to stay straight once it is a part of the microtubule wall, according to this nucleotide-dependent conformational model.Growing microtubule sheets are thought to maintain a 'cap' of tubulin-GTP subunits within the microtubule lattice, allowing the straight tubulin conformation to remain stable (fig. 1). Closure of the terminal sheet structure results in the formation of a metastable, blunt-ended microtubule intermediate (part c; (2)), which can pause, expand further, or enter the depolymerization phase.Fountain-like arrays of ring and spiral protofilament structures characterise a decreasing microtubule (part c; (3)). This conformational change, which tubulin-GDP is thought to be directing, could weaken lateral connections between adjacent protofilaments. The polymerization–depolymerization cycle is completed when the disassembly products' GDP is exchanged for GTP (part c; (4))[4].

MECHANISM OF ACTION OF MICROTUBULINS

The microtubule targeting drugs or anti-mitotic drugs inhibit cell proliferation by disrupting the microtubule; they cause cell cycle arrest in G2/M phase and make abnormal mitotic spindles resulting in cell apoptosis. Microtubule-targeting agents (MTAs) are divided in 2 categoriesbased on their mechanism of action:

(i) Microtubule stabilizing agents and
(ii) Microtubule destabilizing agents.

Taxanes (paclitaxel and docetaxel) and epothilones are microtubule stabilising medicines

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that enhance polymerization of microtubule polymer, prevent microtubule disintegration, and increase cell masses.On the other hand, Microtubule destabilising drugs such as vincristine and vinblastine, vinorelbine, vindesine, vinflunine, and eribulin, as well as colchicine, maytanine, and pironetin, depolymerize microtubules in the opposite direction. They prevent polymerization and reduce the bulk of microtubule polymer.Both microtubule stabilisers and destabilizers decrease microtubule dynamics at low doses with little effect on the polymer mass. Assuming that these microtubule targeting drugs must be given at very large dosages, they function primarily by reducing spindle-microtubule dynamics, which causes mitosis to be slowed or stopped at the metaphase–anaphase transition resulting apoptotic cell death[1].

MOLECULES SHOWING TPI ACTIVITY

(i) Chalcones:

Chalcones are a subgroup of flavonoid that are widely distributed in fruits, vegetables, teas, and plants. Chalcones have been found to exhibit various biological properties like anti-inflammatory, antimicrobial, antifungal, antimalarial, antidiabetic, anti-HIV, antiprotozoal, antioxidant, and cytotoxic activities. Amir Faisal et al. showed the identification and characterization of natural chalcones **(1−3)** and the semisynthetic chalcone **4** as a dual FLT3/microtubule polymerization inhibitor. Chalcone **4** has been reported to be preferentially more active in FLT3-ITD-expressing cells and inhibits FLT3 directly in a biochemical assay (fig. 2). It had been additionally determined that chalcone **4** induces mitotic arrest and inhibits microtubule polymerization in vitro.The simultaneous inhibition of FLT3 and microtubules results in apoptotic cell death and overcomes D835Y-mediated FLT3 inhibitor resistance. Two AML cell lines with oncogenic FLT3-ITD mutations (MV-4-11 and MOLM-13) and one AML cell line with wildtype FLT3 were used to assess the GI50 values of chalcones 1-4. Chalcones **1−3** had GI_{50} values ≥ 10 μM in all three cell lines with relative selectivity of chalcones **2** and **3** toward AML cell lines containing FLT3-ITD mutation (GI_{50}> 10 μM) compared to the THP-1 cell line, that contains WT FLT3 (GI_{50}> 100μM). On the contrary, the 2′-allyloxyderivative of natural chalcone **3**, viz., chalcone **4**, was the most selective and potent of the four chalcones toward FLT3- ITD cell lines, with GI_{50} values ranging from 0.20 to 0.24 Mm. Appearantly, the allyloxy group at C-2′ of chalcone **4** completely increased the activity toward MOLM-13 (>130-fold), MV-4-11 (>160- fold), and THP-1 (>45-fold) cells, on comparing with the activity of the parent natural product **3**[5].

Fig. 2: Preparation of Chalcones 1−4 [5].

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(ii) Pyrazole:

Kamal et al. used pyrazole as a core structure coupled to benzimidazole to design general structure **86** as tubulin polymerization inhibitor. Various ketones were used as starting material to synthesize the compound which on treating with hydrazine form phenylpyrazole intermediates which are further coupled with benzimidazole to afford **86** and its analogs (fig. 3). Synthesized compounds have broad spectrum of anticancer activity against numerous antitumour cell lines, GI_{50} values range from 0.34 to 10 mM. Compounds **86d** and **86e** were found most potent.Compound **86d** had a GI50 of 1.65 mM against the leukaemia RPMI-8226 cell line, while compound **86e** had a GI50 of 0.64 mM. Compound **86f** inhibited the proliferation of cell lines OVCAR-4 and OVCAR-5 in the ovarian cancer panel, with GI_{50} values of 1.38 and 1.21 mM, respectively, and compound **86e** had GI_{50} values of 1.24 and 1.15 mM, respectively, against ovarian OVCAR-4 and OVCAR-8[1].

Fig. 3: Pyrazole core coupled to benzimidazole [1].

Hura et al. designed a new series of heterocyclic compounds in an attempt toward overcoming disadvantages including instability and trans-isomerization susceptibility of the Z-double bond of CA-4 and analogues/derivatives. It involved a mixture of the structural features of the medicinal agents and a marketed drug celecoxib, recently approved for treatment of various cancers.The compounds contained 3-(trifluoromethyl)pyrazole as the bridging moiety in which the double bond of CA-4 is replaced,a 3,4,5-trimethoxyphenyl group in ring A, and several relevant (hetero)aryls in ring B. For the synthesis of these compounds, a convenient method with high regioselectivity and yield was made. There were 23 compounds made with relevantly substituted aryls and heteroaryls. All the compounds were tested against MCF-7 cells and Compound **23** was found to be the most effective antiproliferative agent (fig. 4).

Fig. 4: Compound 23 with significant inhibitory activity[6].

Compound **23** also showed significant inhibitory activity against several other cancer cells including the drug-resistant EMT6/AR1cells. Interestingly, Compound **23** showed substantially weaker cytotoxicity toward noncancerous cells than the cancer cells indicating the anticancer potential of the compound. Although many of the tubulin targeting agents have been successfully used in cancer chemotherapy yet toxicity and development of resistance limit their applications. Altered

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isotype compositions, mutations in the drug binding site and drug efflux are thought to be the main causes of the development of drug resistance. In vitro experiments showed that Compound **23** binds to tubulin at the colchicine site and the binding of the compound disrupts the secondary structure of tubulin. It suppressed tubulin polymerization in cells and in vitro, as well as interfering with cell cycle progression by stopping cells at mitosis, resulting in apoptotic cell death[6].

(iii) Pyrazoline:

Qin et al. found that pyrazole and some other compounds strongly inhibit the tubulin polymerization by binding to the colchicine site of the tubulin. A series of novel pyrazoline-containing derivatives (compounds **5-37**) have been synthesized (fig. 5) and evaluated for their biological activities.In tubulin polymerization and cellular tests, the majority of the drugs demonstrated strong in vitro inhibitory action out of these tiny molecules.Compound **8** was the most effective against tubulin assembling, A549, MCF-7, and HepG-2 cell lines (IC_{50} = 1.88 M, 0.07 M, 0.05 M, and 0.03 M, respectively), and was comparable to the positive control CA-4.

Fig. 5: General synthesis of compounds 5-37, Reagents and Conditions: (i) CH2Cl2, K2CO3, DMF, Reflux; (ii) EtOH, NaOH, rt; (iii)NH2.NH2.H2O, EtOH, Reflux; (iv) EDC.HCl, HOBt, CH2Cl2, Reflux[7].

Moreover, they also showed that compound **8**(fig. 6) was a potent inducer ofapoptosis in HepG-2 cells and it had cellular effects typical for microtubule interacting agents, causing accumulation of cells in the G2/M phase of the cell cycle. Molecular docking observations could provide an important basis for further development of compound **8** as a potent tubulin polymerization inhibitor[7].

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Fig. 6: Compound 8 with most potent activity against tubulin assembling[7].

DRAWBACKS OF MTAs

1. Low water solubility: Most of the MTAs have low water solubility that has become a common drawback. To deal with this problem, Ying-Jie Cui et al. designed, synthesized, and evaluated indenopyrazole derivatives ID01−ID33 for their tubulin polymerization and tumor cell growth inhibitory activities, leading to the discovery of a series of potent MTAs. LL01 was used as a lead and maintained the 1-methyl-1,4-dihydroindeno-[1,2-c] pyrazole core (fig.7) and made systematic changes on the phenolic 6- and 7-positions and the 3- aniline portion.

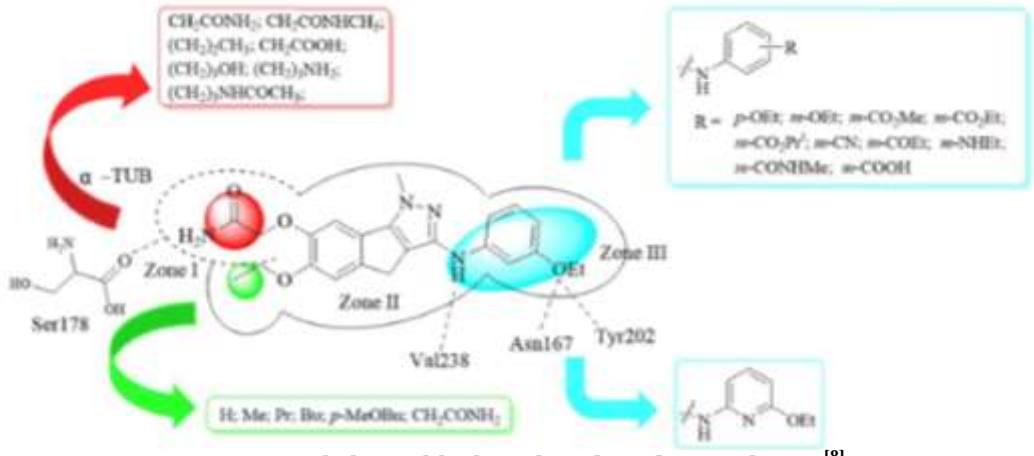

Fig. 7: 1-methyl-1,4- dihydroindeno-[1,2-c] pyrazole core[8].

As the hydrochloride salt(s), ID09 and ID33 (fig. 8)were found to have excellent aqueous solubility and a favourable Log P value, as well as low nanomolar efficacy against a variety of tumour cells, including those resistant to taxol.They caused HepG-2 cell cycle arrest and apoptosis by inhibiting tubulin polymerization and disrupted cellular microtubule networks by targeting the colchicine site.

LL01

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ID09 **ID33**

Fig. 8: Structures of LL01, ID03 and ID33 [8].

At a dose of 25 mg/kg, ID09 and ID33 substantially suppressed tumour growth in the HepG-2 xenograft mouse model. ID09 reduced tumour growth by 68% when given as an intravenous (iv) injection dose of 10 mg/kg every other day[8].

2. Multi-drug Resistance: One of the important hurdles that still limits current anticancer agents is the development of drug resistance. Multidrug resistance (MDR) is the simultaneous resistance to a variety of structurally and functionally unrelated chemotherapeutic drugs and is a substantial obstacle preventing the success of anticancer agents. Several cancers initially respond well to chemotherapy early on during treatment but afterwards develop acquired resistance, and more than 90% of patients with metastatic cancer fail to reply to or relapse from chemotherapeutics. For these reasons, rigorous efforts have been devoted to determine both inherent and acquired MDR mechanisms over the past few decades.

Two significant mechanisms have been proposed: Noncellular and Cellular mechanisms.The ability of tumour cells to withstand chemotherapy is known as Noncellular drug resistance. It is always mediated by tumor microenvironment and is often associated with solid tumors having distinctive properties, such as heterogeneous tumor vasculature, high interstitial fluid pressure, increased existence of noncycling tumors caused by lack of nutrients and oxygen and acidic environment. Cellular MDR mechanisms arise within solid tumors. Compared with noncellular MDR mechanisms, cellular MDR mechanisms are necessary for novel drug development.

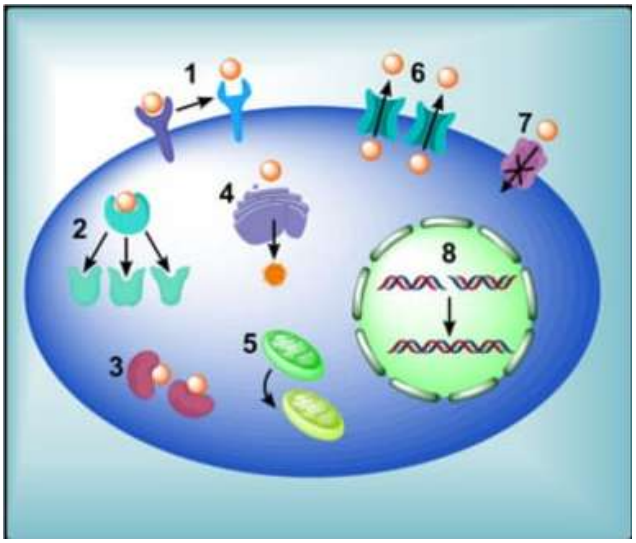

Fig. 9: Drug- resistance mechanisms in tumor cells. 1. Loss or change of surface receptor. 2, Mutations in drug targets, 3. Enzymatic deactivation, 4. Altered drug metabolism, 5. Change in apoptotic pathways, 6. Increased drug efflux, 7. Decreased drug influx (solute carriers), 8. Increased DNA repair [3].

Some examples in which cellular mechanisms are changed to escape drug effect include: elevated DNA repair, increased drug metabolism, altered apoptotic pathways to bypass drug targets, loss or change of drug target proteins, and increased efflux of anticancer drugs (eg, altered activities of membrane transporters such as ATP- binding cassette [ABC] transporters) (Fig.9).

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STRATEGIES TO OVERCOMEMDR

One of the most common techniques to overcome Pgp-mediated MDR is to use ABC transporter inhibitors, which make tumour cells more sensitive to chemotherapeutics.

Some methods to overcome MDR include:

- developing novel anticancer drugs that don't seem to be the substrates of P- gp,
- suppressing the MDR- related genes through destruction of messenger RNAs (mRNAs) by utilising microRNA and RNA interference,
- To restrict glutathione synthesis, hammerhead ribozymes against glutamate cysteine ligase or L-buthionine-(S,R)-sulfoxime are used to reduce intracellular GSH levels.
- targeting P- gp to reverse MDR with anti- P- gp monoclonal antibodies (eg, MRK16 and MRK17), and using nanotechnology to send anticancer drugs to specific targets efficiently[3].

II. CONCLUSIONS

Microtubules are a "Privileged" target for the development of potent antitumour/anticancer agents. Numerous analogues of important classes like combretastatin derivatives, chalcone derivatives, pyrazole derivatives and pyrazoline derivatives may serve as a possible lead for the synthesis of clinically neccessary candidates in coming future. A variety of compounds have been designed and synthesized bearing numerous heterocyclic scaffolds proving to modulate its activity and have emerged as potent tubulin inhibitors.

ABBREVIATIONS

AML; Acute myeloid leukemia, FLT3-ITD; FLT3-internal tandem duplication, THP-1 ;human acute monocytic leukemia cell line, MV-4-11; human AML cell line, MOLM-13; human leukemia cell lines,MCF-7; human breast cancer cell line, HepG-2; human liver cancer cell line, RPMI-8226; Cellosaurus cell line, OVCAR-4 & 5; Human Ovarian Cancer Cell Line.

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