Biologia Futura

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In-vitro and *in-silico* anticancer potential of taxoids from *Taxus wallichiana* Zucc

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DOI: 10.1556/019.70.2019.33

Introduction: Natural products derived from medicinal plants provide beneficial cancer chemotherapeutic drugs. Bioactive constituents from plants are explored for their anticancer properties. *Methods:* Three known compounds (deacetylbaccatin III, tasumatrol B, and taxawallin J) were isolated from *Taxus wallichiana*. Compounds were screened against four cancer cell lines, such as eA498, HepG2, NCI-H226, and MDR 2780AD. Cytotoxic activity was evaluated using MTT assay against cancer cell lines. *Results:* Tasumatrol B showed good cytotoxic activity conducted for the improvement of inhibiting potential of these compounds against the cancer drug target protein (EGFR tyrosine kinase enzyme). The docking study showed that all compounds have binding affinities and interaction profile with the receptor tyrosine kinase. *Discussion:* The study suggests that these compounds could be used for the discovery of novel inhibitors against the target receptors for the treatment of cancer.

Original Article

Cite this article: Qayum M, Nisar M, Rauf A, Khan I, Kaleem WA, Raza M, Karim N, Saleem MA, Bawazeer S, Uysal S, Zengin G, Jahan S, and Ramadan MF. 2019. *In-vitro* and *in-silico* anticancer potential of taxoids from *Taxus wallichiana* Zucc. *Biol. Fut. 70,* 295–300.

Received: 15 January 2019
Accepted: 26 October 2019

Keywords:
tyrosine kinase enzyme, cytotoxic activity, docking, phytochemicals

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INTRODUCTION

Phytochemicals from medicinal plants have been used to treat various diseases since ancient times (Kinghorn et al., 2003). Plant secondary metabolites exhibited biological activities such as protection against pathogens, growing controlling active compounds including hormone-like constituents that motivate or prevent morphogenesis as well as cell division (Potterat & Hamburger, 2008). An outstanding in the elementary cancer investigation and their clinical oncology clues to the advance of cancer chemotherapy was achieved, but still the treatment of cancer is incomplete. The drug treatment hints to non-satisfactory significances with incurable results for patients due to limitations like an increase of drug resistance (Kuete et al., 2015).

More than 3,000 plants have been reported to show anticancer activities. Plants extracts and isolated bioactive compounds have been tested for their cytotoxic potential using both human cell lines including stomach, liver colon, and prostate and animal cell lines, such as monkey kidney cells (Don et al., 2006). The low molecular weight compounds also inhibited tyrosine kinase phosphorylation block signaling pathway, starting in the extracellular portion of receptors (Manley et al., 2002; Paarakh et al., 2015). The type I tyrosine kinase is a key controller of different cellular pathways.

Taxus wallichiana (Himalayan Yew) is traditionally used for treating pyrexia, acute pains, and epilepsy in northern areas of Pakistan. *T. wallichiana* exhibited pharmacological, anticonvulsant, antipyretic, and anti-nociceptive activities (Nisar et al., 2008). The tree is famous for its taxoids and baccatin-type diterpenoids (Banerjee et al., 1996; Barboni et al., 1993; Miller et al., 1981; Navia-Osorio et al., 2002). Since the discovery of taxol from *Taxus* species (*Taxus brevifolia*), this bioactive natural product is extensively used in the treatment of ovarian, lung, and breast cancers (Phillipson, 2001). Taxoids are used for their inhibitory activity on cancer cell division by arresting cell in mitotic phase. In mitosis, these agents overstabilizes the tubulin dimmers to make such a tubulin fiber that ultimately fails to unwind to release tubulin dimmers (Liu et al., 2004; Sturdikova et al., 1986). The isolated taxane diterpenoids were assessed for their *in vitro* cytotoxic activity *via* arresting the cancer cells in mitosis.

Tyrosine kinase enzyme possesses multidomain extracellular ligands for particular ligands, a signal-permit transmembrane hydrophobic helix and the tyrosine kinase domain (Bari et al., 2012). Cancer angiogenesis is a key step during which new capillaries grow a vasculature to provide nutrient and eliminate waste material. Consequently, tyrosine kinase inhibitors as antiangiogenic agents may show beneficial effects in cancer treatment. This study aims to evaluate *in vitro* cytotoxic activities of 4-deacetylbaccatin III, tasmatrol B, and taxawallin isolated from *T. wallichiana* as well as investigating their *in silico* activities on EGFR tyrosine kinase enzyme.

MATERIALS AND METHODS

Plant material and extraction

The bark of the plant (*T. wallichiana* Zucc) was collected from the mountain region of Hazara Mansara division of the KPK (Pakistan) during March 2016. It was dried (4 kg) at room temperature, followed by chopping and grinding into a fine powder. The powdered bark was soaked in 15 L of methanol and the extraction was repeated three times (3×48 hr), filtered, and concentrated under vacuum at 40 °C. This process was repeated three times and crude methanol extract (512 g, 12.8% w/w) was obtained. This extract was subjected to normal fractionation protocol resulting in a 211-g chloroform fraction as the major fraction. Dried and powdered bark was macerated with methanol in occasional manual shaking at room temperature. After filtration, the similar procedure was repeated three times using the same volume of methanol every time. The filtrates were evaporated under reduced pressure at low temperature (40 °C). The concentrated methanol extract of bark was obtained. Crude methanol extract of bark was suspended in distilled water and successively extracted with *n*-hexane, chloroform, ethyl acetate, and finally with water. The chloroform soluble fraction was subjected to normal phase column chromatography using silica gel 60 (0.062–0.200 mm; Merch) resulting in the isolation of three taxoids. Tasumatrol B (compound 2, 113 mg) and 18b [4-deacetylbaccatin III (168 mg)] were obtained from subfraction 18a with elution system of 1% acetone:chloroform. Taxawallin J (172 mg) was obtained from subfraction C-19 with elution system of 2% acetone:chloroform (Nisar et al., 2010). The structure of 4-deacetylbaccatin III, taxawallin J, and tasumatrol B were determined and matched with the literature (Arfan et al., 2012; Nisar et al., 2010).

Molecular docking

Docking studies play a significant role in the rational design of drugs. Molecular docking can predict the binding affinity (Li et al., 2011). The three-dimensional (3D) structure of mark protein EGFR tyrosine kinase having PDB four-letter code 2J5F was retrieved from PDB structural database. 3-D crystallographic structure was edited and subjected to energy minimization by Swiss Pdb viewer v4.1.0 software to avoid steric hindrance and all the residues adopt stable conformations. The 2D structures of compounds 1–3 and the standard co-crystallized ligand [N-(4-phenylaminoquinazoline-6-yl)-acrylamide] were drawn in the Chemsketch software (Li et al., 2004). The 2D structures were saved in mol format followed by hydrogen addition and energy refinement was performed through Avogadro's software, then saved in pdb format (Hanwell et al., 2012).

The docking studies were performed through Autodock Vina (Trott & Olson 2010) and i-GEMDOCKv 2.1 software (Institute of Bioinformatics, National Chiao-Tung University, Hsinchu, Taiwan). All the docking software procedures were optimized for reproducibility of docking results. The Autodock vina can be performed using PyRex tools (Yellamma et al., 2013). The solvent molecules were removed from the receptor crystal structure, hydrogens were added, and Gasteiger charges were calculated (Chang et al., 2010). The macromolecule and the compounds 1–3 standard were uploaded in PyRex tool (Jacob et al., 2012). These files were converted into pdbqt format. The grid center was placed on the already co-crystallized ligand of the receptor with a grid box of center $x = 20$, $y = -55$, $z = -20$ and size of $x = 25$ Å, $y = 25$ Å, $z = 25$Å with an exhaustiveness global search algorithm of 8 (Kumar et al., 2013).

The docking studies were also carried out using i-GEMDOCKv2.1 software. The docking study was carried out by setting the software at 70 generations per compound and the population size of 200 random individuals. The best docking conformations were carried out twice implemented by genetic algorithm (Kumar et al., 2013). The receptor urease-binding pocket was recognized with co-crystallized ligand at a distance of 12Å. The scoring function of the i-GEMDOCK is composed of Fitness = vdW + Hbond + Elec. The vdW, H-bond, and Elec terms are van der Waal energy, hydrogen bonding energy, and electrostatic energy, respectively.

Cytotoxicity studies

Cytotoxicity of isolated compounds was tested using MTT procedure. RPMI 1640 medium comprising Gibco BRL was added with 100 µg/ml streptomycin sulfate, 100 µg/ml penicillin sodium salt, 2 mg/ml Na_2CO_3, and 10% fetal bovine serum (FBS; Gibco, Institute of Bioinformatics, National Chiao-Tung University, Hsinchu, Taiwan). The prepared medium was used to keep three human cancer cell lines

including human A498 (renal), human hepatoma (HepG2), NCI-H226 (non-small cell lung), as well as MDR human ovarian cancer 2780AD cell lines. HepG-R and HepG2 cell lines (2×10^4 and 9×10^3) mice hepatocytes stayed planted in 96-well plates. The cells were preserved with the compounds (1.5–100 µM) or vehicle (0.2% DMSO) and hatched for 48 hr. It was tracked by MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) examine (Sigma, St. Louis, MO, USA). Same assay was performed for the remaining cell lines. The IC_{50} of the test compound on various cell lines were obtained from the concentration-effect curves. Paclitaxel (Sigma) was used as positive control (Gomes et al., 2003; Khan et al., 2011).

In vitro cytotoxicity effect was performed using LCMK-2 monkey kidney epithelial cells and mice hepatocytes. The compounds were incubated for 24 hr and the cell practicality was identified using MTT procedures. The cells were preserved in RPMI 1640 medium comprising 10% FBS (Gibco BRL), 110 µg/ml penicillin sodium salt, 2 mg/ml sodium bicarbonate solution, and 100 µg/ml streptomycin sulfate. First seeding of the 7.1×10^3 LCMK-2 cells and 8.6×10^3 mice hepatocytes was showed in 96-well plates. The cells were preserved with test sample at different concentrations as well as with vehicle (0.2% DMSO) and then hatched for 48 hr tailed by execution MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) procedure (Sigma).

RESULTS

Three compounds (4-deacetylbaccatin III, taxawallin J, and tasumatrol B; Fig. 1) were evaluated for cytotoxic activity using different cell lines. Table 1 shows that all compounds had varying degrees of cytotoxic activity. Tasumatrol B had the highest activity compared to the other compounds. Tasumatrol B was active against cell lines A498 ($IC_{50} = 147$ µM), HepG2 ($IC_{50} = 19.4$ µM), NCI-H226 ($IC_{50} = 87$ µM), and MDR 2780AD ($IC_{50} = 0.82$ µM). 4-Deacetylbaccatin III and taxawallin J were inactive against MDR 2780AD. Taxoids have been reported to have good cytotoxic activity (Gordaliza, 2007; Khan et al., 2011).

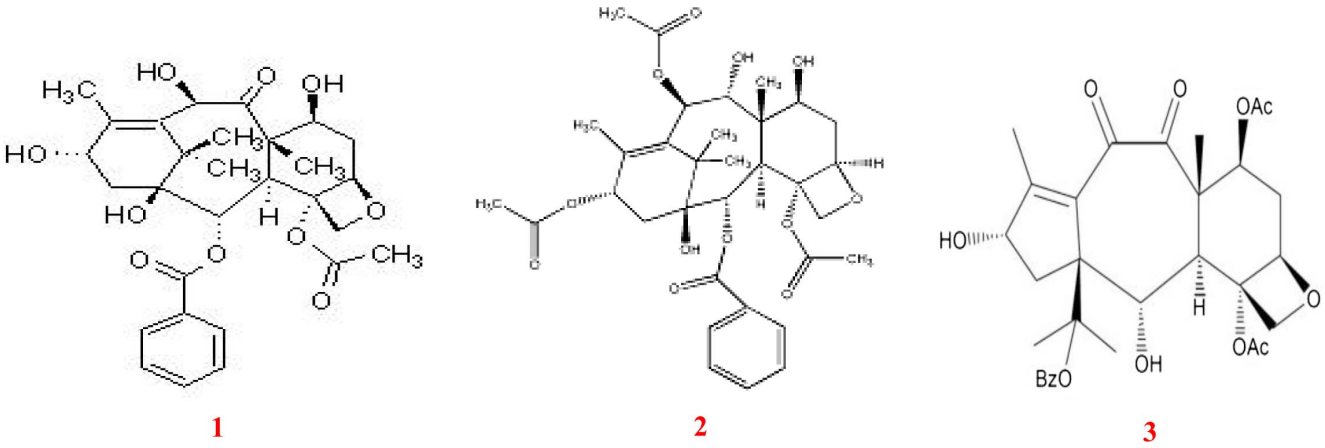

Fig. 1. Chemical structures of deacetylbaccatin III (compound 1), tasumatrol (compound 2), and taxawallin J (compound 3) isolated from *Taxus wallichiana*

Table 1. In vitro anticancer activity of tested compounds

	IC_{50} (µM)			
Compound	HepG2	A498	NCI-H226	MDR 2780AD
4-Deacetylbaccatin III [1]	78.2 ± 0.63	153 ± 0.28	77 ± 0.24	>100
Tasumatrol B [2]	21.3 ± 0.22	182 ± 0.37	106 ± 0.41	>100
Taxawallin J [3]	19.4 ± 0.32	147 ± 0.46	87 ± 0.41	0.82 ± 0.17
Paclitaxel	7.4 ± 0.31	96.3 ± 0.21	61.2 ± 0.3	0.19 ± 0.08

Table 2. Docking energies of tested compounds against tyrosine kinase receptor

	Autodock Vina (kcal/mol)	i-GEM DOCK (kcal/mol)			
Compound	B. Affinity	Total energy	VDW	HBond	Electrostatic energy
4-Deacetylbaccatin III [1]	−6.9	−98	−73	−25	0
Tasumatrol B [2]	−6.1	−83	−69	−14	0
Taxawallin J [3]	−6.4	−90	−83	−7	0
Paclitaxel	−7.1	−102	−89	−13	0

Docking studies play a significant role in the rational design of drugs. This study was conducted to analyze the inhibiting potential of the tested compounds 1–3 against tyrosine kinase enzymes. The docking analysis was based on the hydrogen bond and hydrophobic interactions.

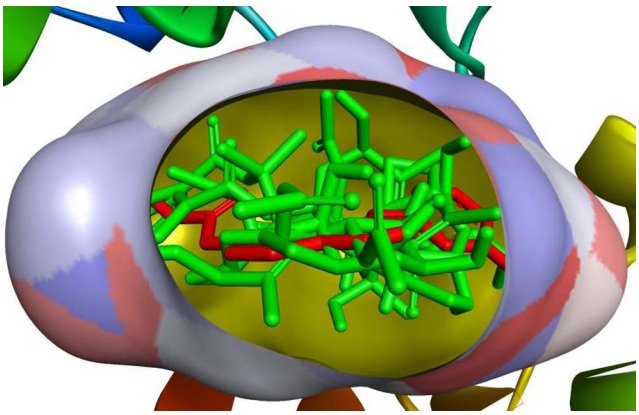

Fig. 2. Binding site predicted for tested compounds in the active site of tyrosine kinase enzyme. Compounds 1–3 represented by green-colored sticks with superimposition on co-crystallized ligand red-colored stick

From the potent compounds, it was observed that they form hydrogen bond and hydrophobic interactions with enzyme active site, which are necessary for enhancing the biological activities. The docking score of the compounds 1–3 is shown in Table 2. The predicted docking poses and superimposition of the compounds 1–3 along with co-crystallized ligand are shown in Fig. 2 to correlate docking results with the *in vivo* study. The interaction analysis of docked compound 1 in the binding pocket of tyrosine kinase (Fig. 3), indicated that compound 1 forms two hydrogen bonding with Gln791 (3.05Å) and Met793 (2.93Å), while nine hydrophobic contacts have been observed from the surrounding residues in the pocket including them Val726, Ala743, Lys745, Met766, Leu788, Leu792, Gly796, Asp800, and Asp855. Similarly docking of compound 2 in the binding pocket of tyrosine kinase (Fig. 4) displayed two hydrogen bonding with the Gln791 (3.05Å) and Thr854 (2.20Å), while eight hydrophobic interactions were observed from the surrounding residues including Leu718, Ala743, Lys745, Leu792, Pro794, Phe795, Gly796, and Leu844. Compound 3 displayed 10 hydrophobic contacts from the binding site of tyrosine kinase (Fig. 5). The residues interacting with compound 3 were Leu718, Val726, Ala743, Thr790, Gln791, Phe795, Asp800, Arg841, Thr854, and Asp855.

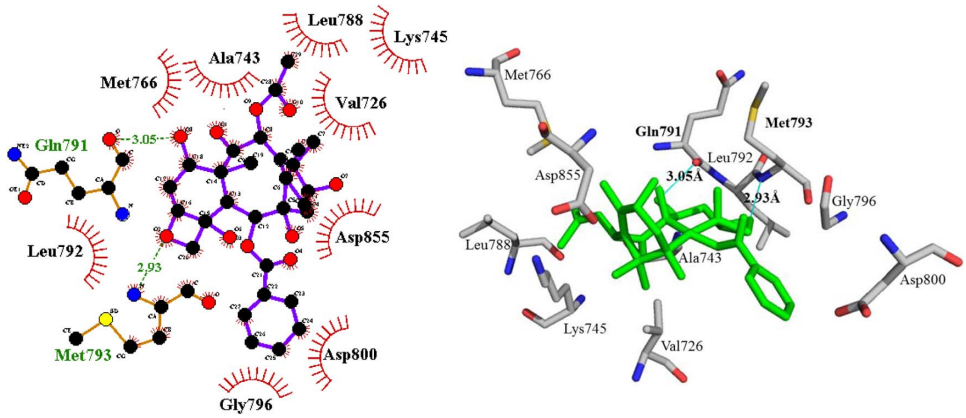

Fig. 3. 2D and 3D representation of interacting amino acid residues of tyrosine kinase against compound 1

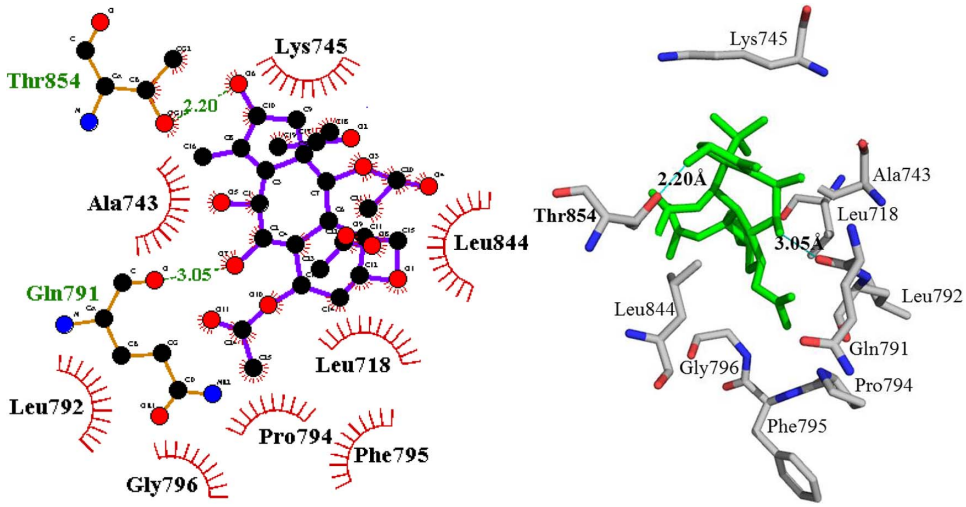

Fig. 4. 2D and 3D representation of interacting amino acid residues of tyrosine kinase against compound 2

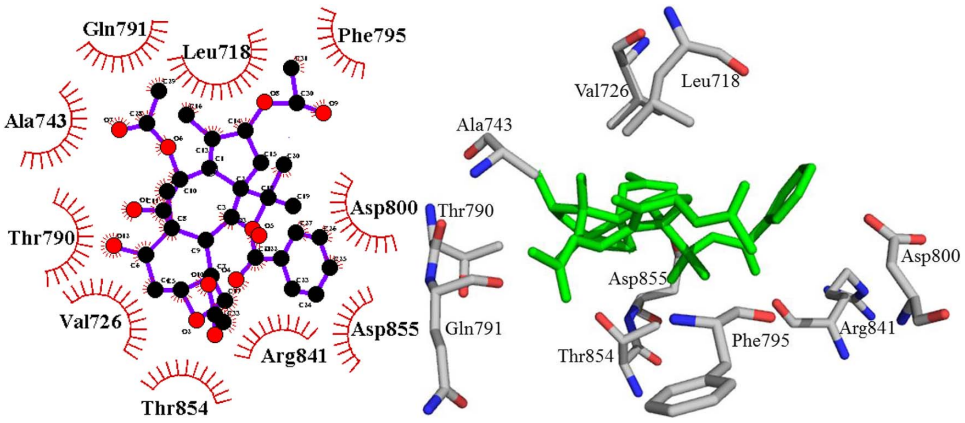

Fig. 5. 2D and 3D representation of interacting amino acid residues of tyrosine kinase against compound 3

DISCUSSION

The Himalayan yew *(T. wallichiana)* is an important medicinal species, which is extensively harvested for Taxol, a potent anticancer drug that has an extraordinary property of inhibiting the development of carcinogenic cells. Taxol is used for the treatment of cancers and other diseases in modern medicine (Mukherjee et al., 2002; Rathore et al., 2019). Six of the top 20 medicines distributed were natural products and clinicians refunded back to drugs like artemisinin, etoposide, and taxol (Phillipson, 2001).

In this study, the inhibiting potential of the tested compounds 1–3 against tyrosine kinase enzymes was studied. Molecular docking is widely employed as a fast technique, both in academic and industrial settings. Molecular docking is a method, which analyzes the conformation and orientation of molecules into the binding site of a macromolecular target. Searching algorithms generate possible poses, which are ranked by scoring functions. Several softwares such as AutoDock, AutoDock Vina, DockThor, GOLD, FlexX, and Molegro Virtual Docker are usually used for molecular docking. Many molecular docking aspects remain challenging, and there is still not an accurate route to readily pinpoint true ligands among a set of molecules, nor to identify with precision the correct ligand conformation within the binding pocket of a given target molecule (Torres et al., 2019). Based on the present results, tasumatrol B could be tested *in vivo* as a natural bioactive compound for the treatment of cancer.

CONCLUSION FOR FUTURE BIOLOGY

In comparison with the reported inhibitors, it could be concluded that compounds 1–3 have similar activities for the tyrosine kinase enzyme. Further studies are needed to analyze the inhibiting potential of herbal origin taxol compounds for the cure of cancer. This study directed the researcher that these compounds could be used for the discovery of novel inhibitors against the target receptors for the treatment of cancer.

Acknowledgments: The authors are thankful to Higher Education Commission of Pakistan for providing financial support.

Ethical Statement: All the study has been approved from ethical committee, University of Peshawar, KPK, Pakistan.

Funding Statement: This study was financially supported by Higher Education Commission of Pakistan.

Data Accessibility: All data generated or analyzed during this study are included in this published article.

Competing Interests: The authors declare no competing interests.

Authors' Contributions: MQ, MN, AR, IK, WAK, MR, NK, MAS, SB, SU, GZ, and SJ conceived and designed the experiment, and contributed regents/materials/analysis tools. MQ performed the experiments. AR, MQ, and MFR analyzed the data. MFR participated in the drafting or revising the article.

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