

# Synergistic Cytotoxic Effect from Combination of Wedelolactone and Cisplatin in HeLa Cell Line: A Novel Finding

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Sadia Sarwar <sup>1,2</sup>

Jun Qing Yu<sup>1</sup>

Humaira Nadeem<sup>3</sup>

Fazlul Huq<sup>1</sup>

<sup>1</sup>Discipline of Biomedical Sciences, Sydney Medical School, The University of Sydney, Cumberland Campus, Sydney, NSW, Australia; <sup>2</sup>Department of Pharmacognosy, Riphah Institute of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan; <sup>3</sup>Department of Pharmaceutical Chemistry, Riphah Institute of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan

**Context and Objective:** Cisplatin is a platinum drug in current clinical use for the treatment of cervical cancer. However, drug toxicity and resistance are its two major limitations. The aim of this investigation was to test the cytotoxic activity of potential phytochemicals alone and in combination with cisplatin in cervical cancer cells.

**Methods:** In this study, cytotoxicity of phytochemicals including wedelolactone (WDL), betulinic acid (BA) and epigallocatechin gallate (EGCG) was investigated in human cervical cancer cell line HeLa through 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) reduction assay. Combined drug action resulting from the combination of cisplatin with WDL and BA was investigated in the same cell line through median effect principle. The combination index (CI) was taken as a measure of combined drug action.

**Results:** BA resulted in synergistic outcome when co-administered with cisplatin at 0/0 time; (bolus administration) while administration of either drug (cisplatin or BA) four hours before the other (0/4 or 4/0) resulted in antagonistic action. WDL, on the other hand, was found out to be synergistic at any of the applied sequence of drug administration (0/0, 0/4 or 4/0).

**Discussion and Conclusion:** This is the first study reporting cytotoxic activity of WDL in HeLa cells either as single agent or in combination with cisplatin. These results support the idea that sequential combination of cisplatin with WDL and BA may work effectively in cervical cancer cells.

**Keywords:** HeLa, wedelolactone; WDL, betulinic acid; BA, MTT reduction assay, combination index; CI, synergism

## Introduction

According to NIH statistics, 13,800 new cases of cervical cancer and 4,290 resulting deaths are expected in 2020. These figures show that cervical cancer is most likely to remain one of the leading causes of death among women. In the US, it makes up to 0.8% of all new cancer cases.<sup>1</sup> Cisplatin (cis-diamminedichloroplatinum II, CDDP), an inorganic platinum compound, is one of the effective chemotherapeutic agents in the treatment of cervical carcinoma. It is in clinical use as a first line monotherapy and also in combination with other chemotherapeutics against local, advanced and recurrent/metastatic cervical cancer.<sup>2-9</sup> The overall response rate is 29.1–67% while median overall survival is almost 12 months in recurrent/advance cervical cancer patients receiving combination chemotherapy. However, development of chemoresistance to cisplatin, intrinsic or acquired, seriously compromises the treatment.

Correspondence: Sadia Sarwar  
Department of Pharmacognosy, Riphah  
Institute of Pharmaceutical Sciences,  
Faculty of Pharmaceutical Sciences,  
Riphah International University,  
Islamabad, Pakistan  
Tel +92-333-5565889  
Email [sadia.sarwar@riphah.edu.pk](mailto:sadia.sarwar@riphah.edu.pk)

Cisplatin is a small molecule originally identified as anti-bacterial agent. Mechanisms underlying the cisplatin mediated anticancer effects are associated with several signaling pathways. The most remarkable effects of cisplatin are changes in DNA conformation and damage induced by DNA-cisplatin adducts. However, DNA damage is non-selective leading to toxicities and adverse side-effects such as nephrotoxicity, gastrointestinal toxicity, neurotoxicity, bone marrow toxicity and ototoxicity.<sup>10</sup> Therefore, it is necessary to continue searching for new anticancer compounds and/or adjuncts to current treatments. Many compounds have been found to inhibit early stages of carcinogenesis in experimental models. Among them are recognized drugs (e.g., nonsteroidal anti-inflammatory drugs abbreviated as NSAIDs) and phytochemicals. When used in combination with cisplatin or other 2nd and 3rd generation platinum drugs, phytochemicals can offer promising benefits in terms of compromising the toxicities or inducing the sensitization towards chemotherapeutics or reducing the drug dose. The number of studies reporting such combinations in basic research is on continuous rise where phytochemicals-induced sensitization of cancer cells to chemotherapeutics has been observed.<sup>11–13</sup>

*NF- $\kappa$ B* family members regulate hundreds of genes involved in immune-regulation, inflammation, apoptosis and carcinogenesis.<sup>14</sup> It has been established that constitutive activation of *NF- $\kappa$ B* inhibits chemotherapy induced apoptosis in cervical cancer, as well as in other types. In literature, combination of cisplatin with *NF- $\kappa$ B* inhibitors has been suggested to have therapeutic significance.<sup>15</sup> *Bcl-2* family constitutes multiple anti-apoptotic proteins associated to the poor clinical response being over-expressed in cisplatin resistant cervical cancer cells.<sup>16</sup>

Caspases (3, 8, 9) play a pro-apoptotic role and their reduced levels have been found to be associated with cisplatin resistance in cervical cancer cells. Up-regulation of pro-apoptotic effector proteins may contribute to overcome the resistance to cisplatin because in many resistant cases, up-regulation of anti-apoptotic and suppression of pro-apoptotic proteins has been observed.<sup>17</sup> Mitogens activated protein kinases (*MAPKs*) are another class of proteins regulating gene expression in response to extracellular stimuli (stress activated *SAPK/JNK* and extracellular signal regulated kinases (*ERKs*)). Inhibition/down-regulation of *MAPKs* contributes to resistance to cisplatin in cervical cancer while their activation can be a promising strategy to enhance cisplatin-induced apoptosis.<sup>18–20</sup>

Epigallocatechin gallate (EGCG), betulinic acid (BA) and wedelolactone (WDL) are reported inhibitors of *NF- $\kappa$ B*, *BCL-2* and activators of caspases and *MAPKs*.<sup>21–23</sup> In this study, we investigated the effects of these phytochemicals on the survival of resistant cervical cancer cell line HeLa singly as well as in combination with cisplatin in vitro in the hope of finding synergistic combinations to overcome resistance and/or as adjuncts.

## Materials and Methods

### Reagents

Cisplatin was prepared according to the method of Dhara.<sup>24</sup> WDL, EGCG and BA were purchased from Sigma Aldrich, Sydney, Australia. Dimethylformamide (DMF) was purchased from Sigma Chemical Co. (St. Louis, MO USA). All other chemicals and solvents used in the study were of analytical grade. HeLa cell line was a gift from Dr Mu Yao, Department of Endocrinology, University of Sydney, Australia and were purchased from ECACC (93021013).

### Cell Lines and Preparation of Stock Solutions

The experiment was performed at Discipline of Biomedical Science, School of Medical Sciences, University of Sydney, Australia. The cell line was sub-cultured in RPMI 1640 medium that was prepared in 10% fetal calf serum (FCS, 1mM Hepes, 5.6% sodium bicarbonate and 200mM glutamine). 1mM solution of cisplatin was prepared by initially dissolving it in dimethylformamide (DMF) followed by addition of milli Q (mQ) water (ration 1:5). WDL, BA and EGCG were dissolved directly in DMSO to get 1mM, 10mM and 1mM solutions respectively. The solutions were sterilized by filtering using DISMIC-25cs ADVANTEC filter (cellulose acetate, 0.20 $\mu$ m hydrophilic, pressure limitation: 0.51MPa). The stock solutions were serially diluted with freshly prepared RPMI-1640 medium to produce a range of final concentrations used.

### Cytotoxicity Assay for Single Drugs

The cell survival fraction was determined using the 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) reduction assay. This assay is based on the principle that yellow coloured MTT dye is reduced in living cells to a purple formazan crystals which is measured as absorbance values through spectrophotometer<sup>25</sup> Cells were seeded, at concentration of 4,000 to 5,000 per well in RPMI-1640 medium, into flat-bottomed 96-well

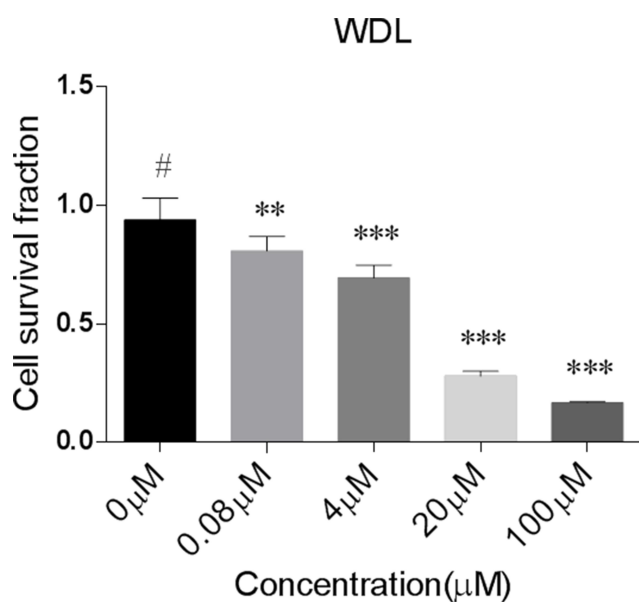
culture plate and allowed to attach overnight. For single treatments, drugs were added at a range of four concentrations (shown in Figures 1–3) to triplicate wells and left in incubator (37°C, 5% CO<sub>2</sub> in air, pH 7.4) for 72 h. After preparation of serial dilutions of the drugs in 10% FCS/RPMI 1640 medium, 100µL of medium was added to cell cultures in triplicate wells. The cells were then left to incubate under normal growth conditions for 72 h at 37°C in humidified atmosphere. After 72 hours, MTT was added followed by DMSO and absorbance was recorded. IC<sub>50</sub> (inhibitory concentration required to bring about death of 50% cells) and cell survival fraction values were calculated as mean ± SD of at least five experiments. Cell survival fraction was calculated as:

Cell survival fraction = absorbance sample/absorbance blank

The one-way analysis of variance (ANOVA) was used to decide whether there were any statistically significant differences between the control and treated cells at \*P < 0.05, \*\*P < 0.005 and \*\*\*P < 0.0005.

## Cytotoxicity Assay for Combination Effect–Median Effect Principle

In this experiment, combined drug action was studied through the median effect principle to determine whether combinations resulted in antagonistic, synergistic or additive effect. The median effect analysis was done to



**Figure 1** Cell survival fraction values as observed in HeLa in response to different doses of WDL. Significantly effective treatments compared to untreated control (shown as #) are indicated as \*\* (P<0.005) and \*\*\* (P<0.0005).

determine combination index (CI) values following the methods of Chou and Talalay. The CI was calculated using the following equation:

$$CI = \frac{D1}{D1x} + D2/D2x$$

Where  $D1$  and  $D2$  represent the concentrations of compound 1 and 2 in combination to achieve  $x\%$  inhibition whereas  $D1x$  and  $D2x$  represent concentrations of compounds 1 and 2 to achieve  $x\%$  inhibition when present alone.  $Dx$  can be readily calculated from the following equation:

$$Dx = Dm[fa/(1 - fu)]^{1/m}$$

Where  $Dm$  represents the median effect dose or IC<sub>50</sub>,  $fa$  is the fraction of killed (affected) cells by the dose,  $fu$  is the fraction of cells remaining unaffected (living cells) so that  $fu = 1 - fa$  and  $m$  is the exponent defining the shape of the dose effect curve. Based on these calculated CI values, dose reduction index (DRI) was calculated. DRI provides an estimation of the extent to which a dose of a single or more drugs or agents in combination can be decreased to get effect levels comparable with single drugs. The CI of < 1, = 1 and > 1 indicates synergism, additiveness and antagonism in combined drug action respectively. Drug combinations that acted synergistically can be identified as those that exhibited significant dose reduction values. The CI,  $D_m$  and linear correlation coefficient  $r$  values were calculated using Calcsyn software (V2) (Biosoft, UK). The  $r$  values indicate goodness of fit for the pooled data (where  $r = 1$  is a perfect fit) and  $r$  of median effect plot for the cell culture system should be greater than 0.95.<sup>26–28</sup>

Investigation of synergism from combination of cisplatin with WDL and BA was the main focus of the study. Drugs were added either at the same time (simultaneous addition, 0/0 h) or one followed by another after 4 h (sequential addition, 0/4 h and 4/0 h). The results of combination studies, analysed using the program CalcuSyn that calculates a quantity called CI as a function of fraction affected  $f_a$  and DRI ([Supplementary Table](#)).

## Molecular Docking Analysis Retrieval of Target Proteins from PDB

The three dimensional (3D) structures of protein targets; protein kinase B (*Akt*), B-cell lymphoma-2 protein (*Bcl-2*), B-cell lymphoma-extra-large Protein (*Bcl-xL*) and nuclear factor kappa-light-chain-enhancer protein of activated B cells (*NF-kB*) having PDB IDs: 1GZK, 4LXD, 4TUH and 2RAM respectively were accessed from Protein Data Bank (PDB).<sup>29</sup> The selected target protein structure were

minimized by using Autodock Tools. Gasteiger charges were added and were saved in pdbqt format.<sup>30</sup>

### Designing of Ligands

The ligands WDL and BA were sketched and energy minimized in Discovery Studio Visualizer and saved in PDB format. Gasteiger charges were added using AutoDock tools in ligand structures to obtain the good structure conformation and were saved in pdbqt format.

### Detection of Binding Site

Binding site for each protein target was detected using DOG Site Scorer. Best pocket depending on the druggability was selected as binding site. The three-dimensional coordinates of the binding site for each protein target were as; protein kinase B (*Akt*) PDB ID: 1GZK; center x = -32.61, center y = 91.69, center z = -64.88 size x = 28, size y = 20, size z = 34. B-cell lymphoma 2 protein (*Bcl-2*) PDB ID: 4LXD; center x = 22.02, center y = 29.36, center z = 6.19 size x = 28, size y = 22, size z = 22. B-cell lymphoma-extra-large Protein (*Bcl-xL*) PDB ID: 4TUH; center x = -21.05, center y = 8.55, center z = 29.72 size x = 22, size y = 16, size z = 28. Nuclear factor kappa-light-chain-enhancer protein of activated B cells (*NF- $\kappa$ B*) PDB ID: 2RAM; center x = 14.72, center y = 35.22, center z = 17.778 size x = 28, size y = 20, size z = 22.

### Docking

Both the ligands were docked within the obtained binding pockets of the target proteins using AutoDock vina via PyRx.<sup>31,32</sup> The exhaustiveness was set 50 and the best pose depending upon the lowest binding energy was obtained.

### Validation

The overall docking protocol was validated by redocking the co-crystallized ligands after sketching them on Discovery Studio Visualizer. The obtained conformations as a result of docking were superimposed on the corresponding co-crystallized ligands extracted from the protein's 3D structure.

## Results

### Cytotoxic Effects of Single Drugs

The IC<sub>50</sub> values of single drugs including WDL, BA and EGCG, as applied to the human cervical cancer cell line HeLa, are given in Table 1. Among tested compounds BA was found to be most active while EGCG was least active. The IC<sub>50</sub> values of WDL, BA and EGCG were 14.85±0.70  $\mu$ M, 12.82±0.37  $\mu$ M and 29.22 ±1.91 $\mu$ M respectively.

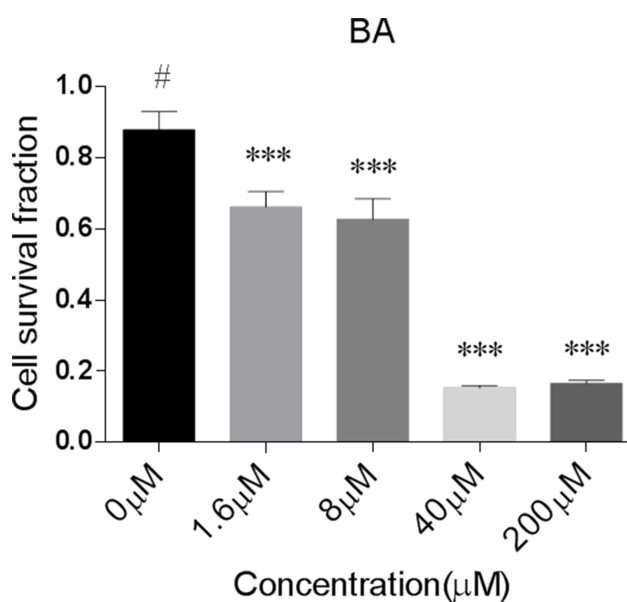
**Table 1** IC<sub>50</sub> Values of WDL, BA and EGCG as Observed in Cervical Cancer Cell Line HeLa

Drugs	IC <sub>50</sub> ( $\mu$ M)
WDL	14.85±0.70
BA	12.82±0.37
EGCG	29.22±1.91

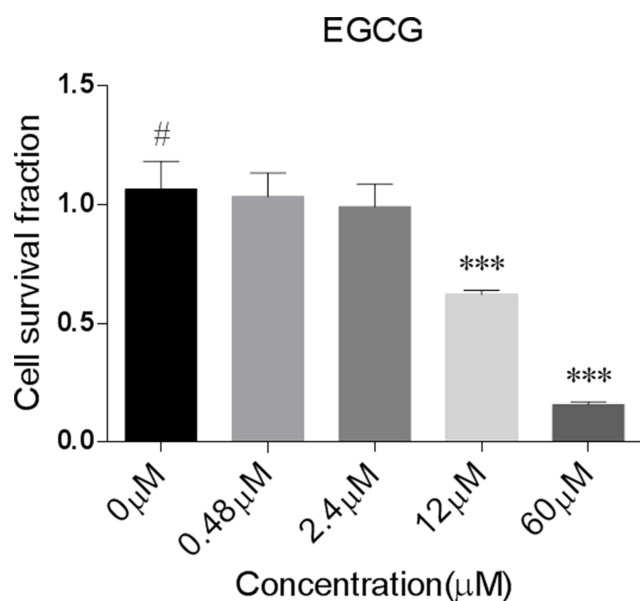
Cell survival fraction values in response to WDL, BA and EGCG are given in the Figures 1–3. The effect was found out to be dose dependent where an inverse proportion (drug dose and surviving cells fraction) is clearly visible in most cases. The reduction in the number of surviving cells at 4 $\mu$ M, 20 $\mu$ M and 100 $\mu$ M of WDL (Figure 1); at 1.60  $\mu$ M, 8 $\mu$ M, 40  $\mu$ M and 200  $\mu$ M of BA (Figure 2) while at 12 $\mu$ M and 60 $\mu$ M of EGCG (Figure 3) was highly significant than respective controls (\*\*P < 0.005).

### Cytotoxic Effects of Drugs in Combination

The study was conducted with the aim to investigate the outcome of the combination of selected phytochemicals with cisplatin in search of possible synergism in cervical cancer cell line HeLa using 0/0, 0/4 and 4/0 (as explained in methodology section) sequence of administration. This binary combination was investigated as function of sequence of drug administration and their concentration.



**Figure 2** Cell survival fraction values as observed in HeLa in response to different doses of BA. Significantly effective treatments compared to untreated control (shown as #) are indicated as \*\*\* (P<0.0005).



**Figure 3** Cell survival fraction values as observed in HeLa in response to different doses of EGCG. Significantly effective treatments compared to untreated control (shown as #) are indicated as \*\*\* ( $P < 0.0005$ ).

Combined drug action was measured through dose-response effect (qualitative method; [Supplementary Table](#)) and the combination indices (quantitative; [Table 2](#)) at median effect dose  $ED_{50}$ . In [Table 2](#), dose effect parameters in terms of median dose effect ( $D_m$ ), sigmoidicity or shape ( $m$ ) and conformity or linear correlation coefficient ( $r$ ) are given.

Combination indices show that administration of WDL in combination with the cisplatin resulted in nearly to synergistic effect at all the sequences of combined drug administration; this effect was more remarkable when either of the two drugs is used 4 hours before the other ([Table 2](#) and [Supplementary Table](#)). The combination of BA with cisplatin, however, showed a synergistic trend when administered as bolus (both drugs applied at same time) while moderately antagonistic effect was observed on application of one drug (either) 4 hours before the other ([Supplementary Table](#)).

## Docking Analysis

The docking results of both BA and WDL showed satisfactory binding affinities. WDL appeared to form stronger interactions with *Akt* and *NF-kB* ([Figures 4A–B](#) and [5A–B](#)) as compared to BA ([Figures 6A–B](#) and [7A–B](#)). It showed higher binding affinity with E-value of  $-7.6$  Kcal/Mol and  $-7.7$  Kcal/Mol with *Akt* and *NF-kB* respectively, whereas, BA had the binding energies of  $-5.5$

**Table 2** Combination Indices (CIs) at  $ED_{50}$  in Response to the Binary Combinations of Cisplatin with WDL and BA Using (0/0 h), (0/4 h) and (4/0 h) Sequence of Administration in Cervical (HeLa) Cancer Cell Line

Drugs	Combination Index (CI) Values at $ED_{50}$	$D_m$	$M$	$r$
Cisplatin	N/A	5.21	0.77	0.99
WDL	N/A	58.35	0.59	0.69
0/0	0.80	0.78	1.81	0.98
0/4	0.66	0.64	1.42	0.98
4/0	0.71	0.69	1.33	1
Cisplatin	N/A	1.03	0.77	0.99
BA	N/A	9.3	0.64	0.87
0/0	0.66	0.48	2.63	1
0/4	1.3	0.95	1.81	0.93
4/0	1.24	0.91	1.12	0.94

**Notes:**  $D_m$  is the medium effect dose,  $m$  is the exponent defining shape of the dose effect curve and  $r$  is the reliability coefficient.

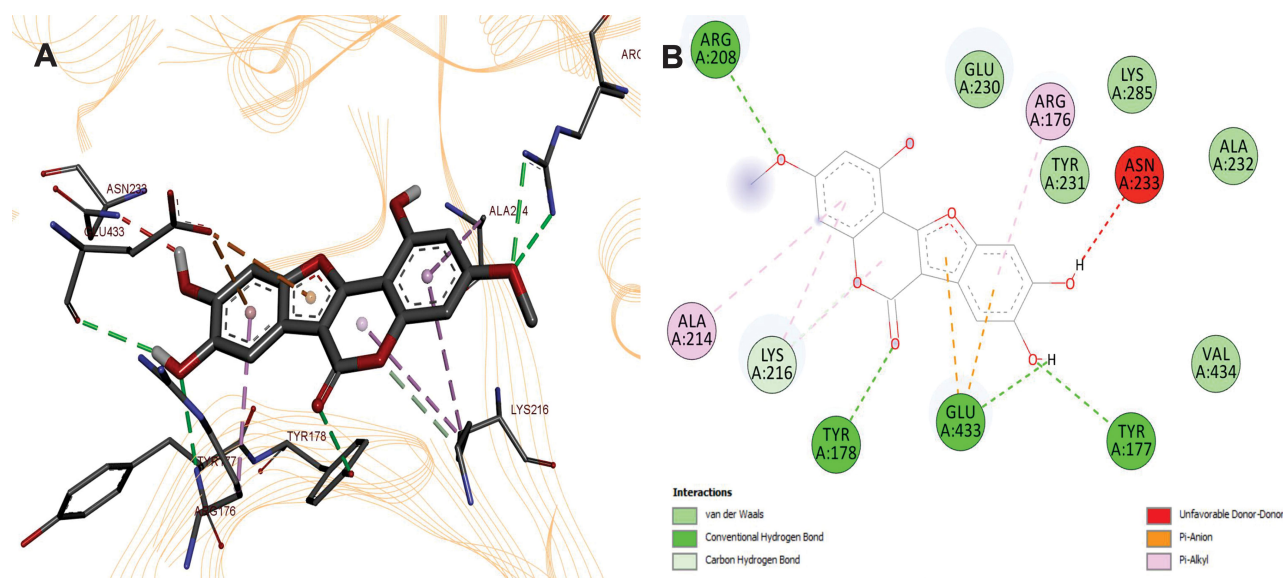
Kcal/Mol and  $-6.4$  Kcal/Mol respectively ([Table 3](#)). The difference in binding energy is clear from the binding interaction. WDL formed 4 hydrogen bonds with *Akt*, whereas, BA 2 hydrogen bonds. Although WDL formed 1 unfavorable donor donor interaction with *Akt*, still the total number and strength of favorable interactions was way more than the single unfavorable interaction. In case of *NF-kB*, both WDL and BA formed 2 hydrogen bonds but the number of other favorable interactions formed by WDL with *NF-kB* was sufficiently high which accounted for the higher binding affinity of WDL with the receptor.

On the other hand, BA appeared to bind more strongly ([Figures 8A–B](#) and [9A–B](#)) as compared to WDL with Bcl-2 and Bcl-xL ([Figures 10A–B](#) and [11A–B](#)). The pattern of binding interactions also confirms the difference in binding energies, as in case of Bcl-2, BA forms 1 hydrogen bond and 6 strong Alkyl-Alkyl and Pi-Alkyl interactions. Whereas, WDL formed no hydrogen bond and 5-Alkyl-Alkyl and Pi-Alkyl interactions.

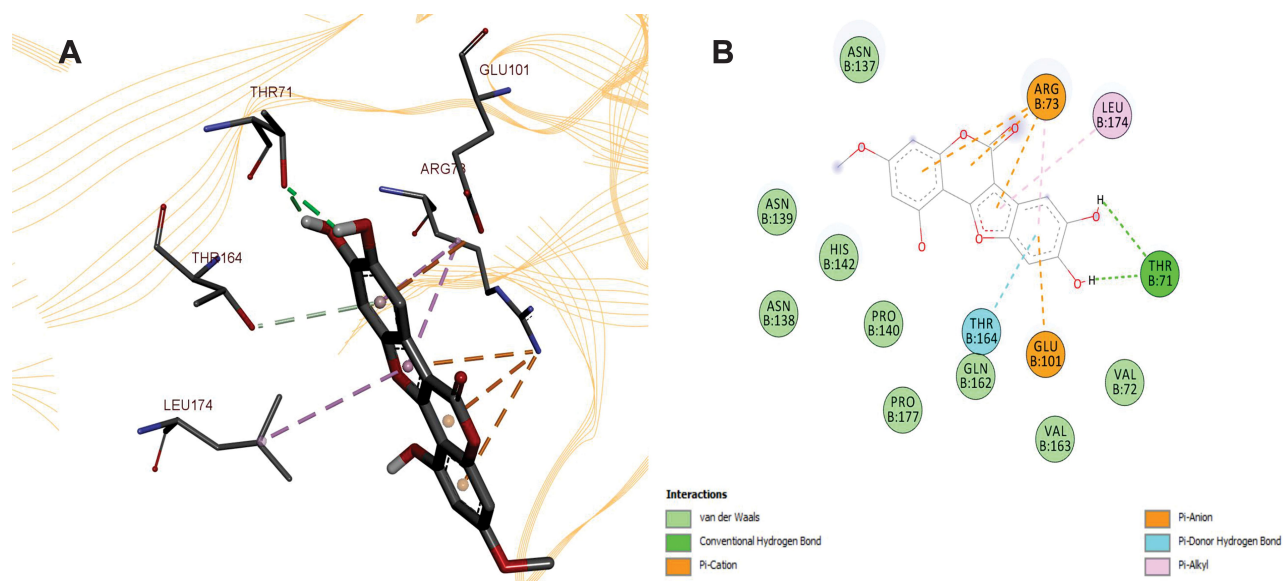
When docked with *Bcl-xL*, both WDL and BA formed 1 hydrogen bond but Alkyl-Alkyl and Pi-Alkyl interactions were higher in number and stronger for BA as compared to WDL. Furthermore, betulinic acid (BA), being a more nonpolar molecule, formed strong hydrophobic interactions with the receptor that resulted in its higher binding affinity as compared to WDL.

Overall, both molecules showed satisfactory binding energies and can be considered as good candidates for the selected targets.





**Figure 4** (A) 3D and (B) 2D poses of WDL with Protein kinase B (Akt) PDB ID: 1GZK.

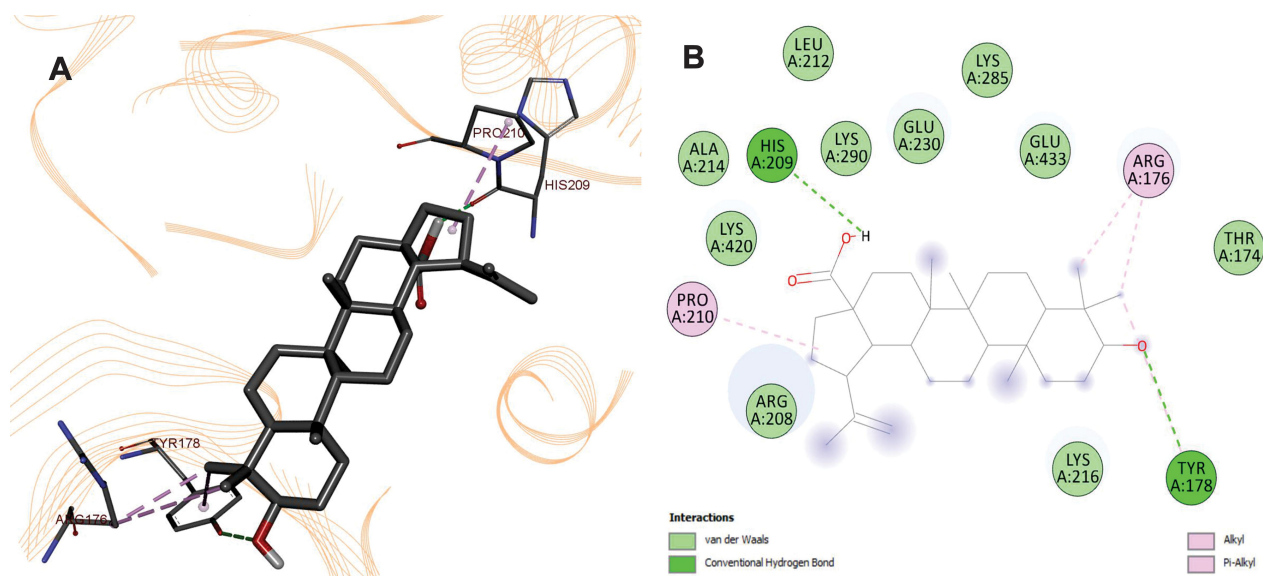


**Figure 5** (A) 3D and (B) 2D poses of WDL with Nuclear factor kappa-light-chain-enhancer Protein of activated B cells (NF-kB) PDB ID: 2RAM.

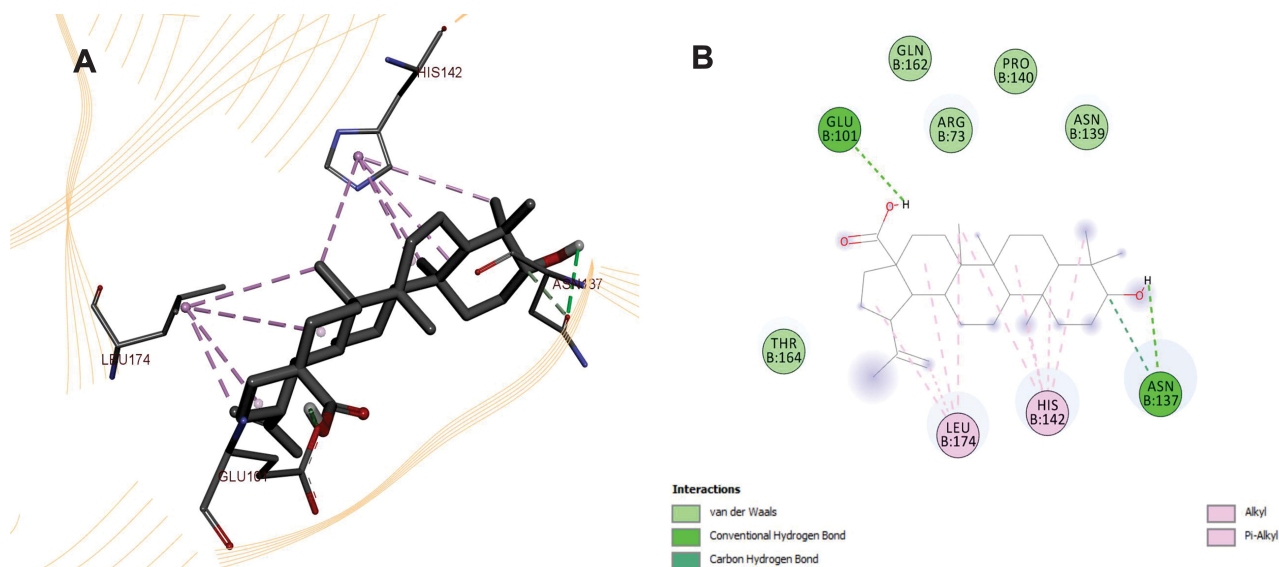
## Discussion

Cisplatin is being used extensively to treat cervical cancer. However, drug resistance and cytotoxicity are its two major limitations. Research is ongoing to overcome its toxicity and to ameliorate cisplatin sensitivity. The identification of chemosensitizers for cancer chemotherapy is an area of intensive investigation. Herbal remedies are emerging not only as chemosensitizers, but also as popular agents for cancer patients dealing with the side effects of chemotherapy.<sup>33</sup> The aim of this investigation was the search for ideal combination/s of cisplatin with phytochemicals with the potential of working

synergistically against cervical cancer cells. A synergistic effect is a combined effect which is numerically better than that obtained by either of single drugs.<sup>34</sup> Due to their lower IC<sub>50</sub> values, which were calculated in first part of this investigation, WDL and BA were selected for combination with cisplatin. The combination was taken as a function of concentration and sequence of drug addition. To the best of our knowledge the possible synergistic effect of WDL in combination with cisplatin is not investigated previously. Furthermore, in this investigation, it was established for the 1st time that WDL is cytotoxic against cervical cancer as determined in



**Figure 6** (A) 3D and (B) 2D poses of BA with Protein kinase B (Akt) PDB ID: 1GZK.



**Figure 7** (A) 3D and (B) 2D poses of BA with Nuclear factor kappa-light-chain-enhancer Protein of activated B cells (NF-kB) PDB ID: 2RAM.

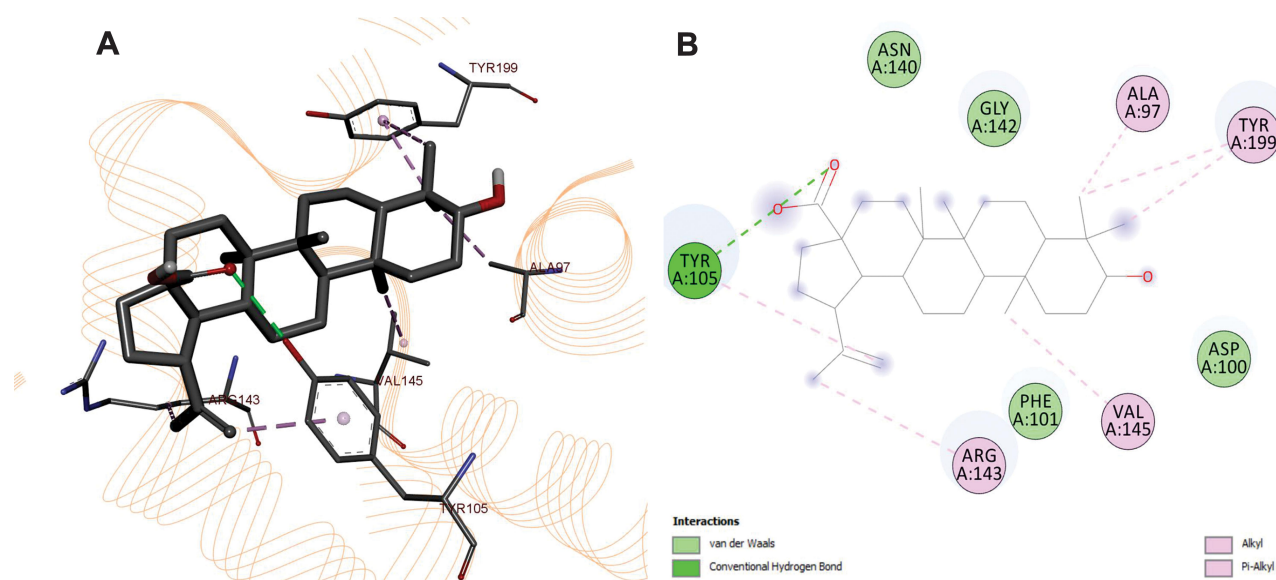
HeLa cell line. The binary combination of cisplatin with WDL in all three sequences of administration resulted in synergistic

**Table 3** Binding Affinities (Kcal/Mol) of WDL and BA with Target Proteins

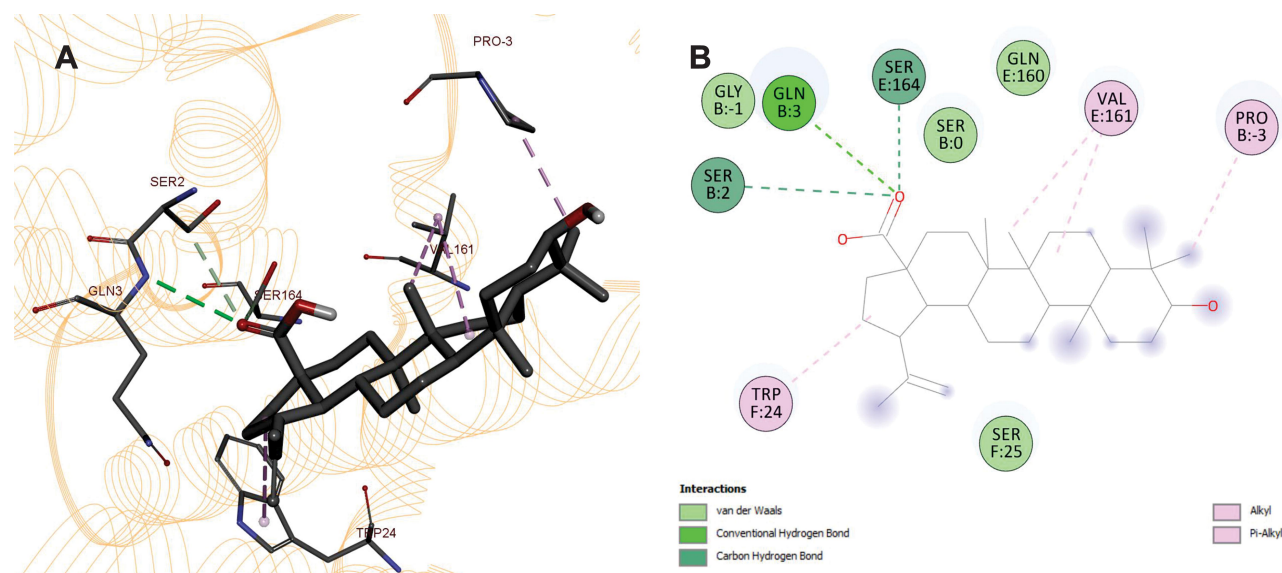
Protein	Binding Affinity (Kcal/Mol)	
	BA	WDL
Akt	-5.5	-7.6
BCL-2	-7.8	-7.4
BCL-XL	-8.0	-7.4
NF-kB	-6.4	-7.7

(0/4 and 4/0 administration) or nearly synergistic (0/0) outcome (Table 2; Supplementary Table for more explanation). Among the three administered combinations of cisplatin with BA, bolus (0/0) administration was found out to be synergistic while 0/4 and 4/0 sequence was antagonistic, highlighting that both compounds if administered simultaneously (co-treatment; 0/0) can result in better outcome through sensitization of HeLa cells towards cisplatin (Table 2).

Cisplatin mediated anticancer effect is interlinked to several other associated signaling pathways and so is the development of resistance. Inactivation of cisplatin-



**Figure 8** (A) 3D and (B) 2D poses of BA with B-cell lymphoma 2 Protein (Bcl-2) PDB ID: 4LXD.

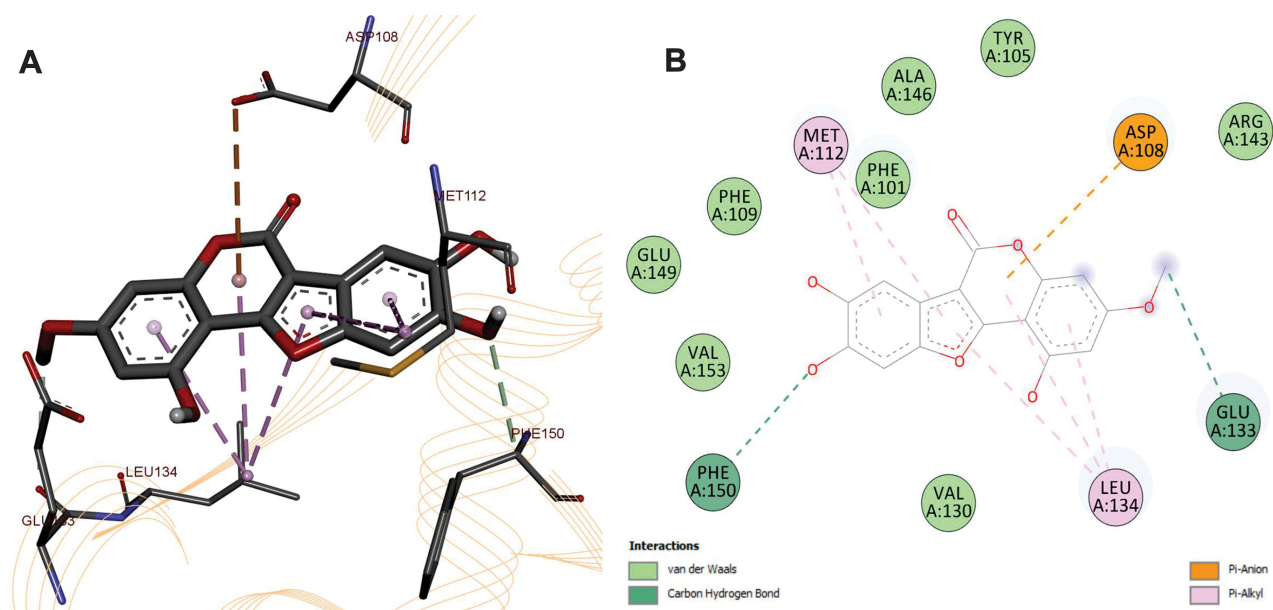


**Figure 9** (A) 3D and (B) 2D poses of BA with B-cell lymphoma-extra large Protein (Bcl-xL) PDB ID: 4TUH.

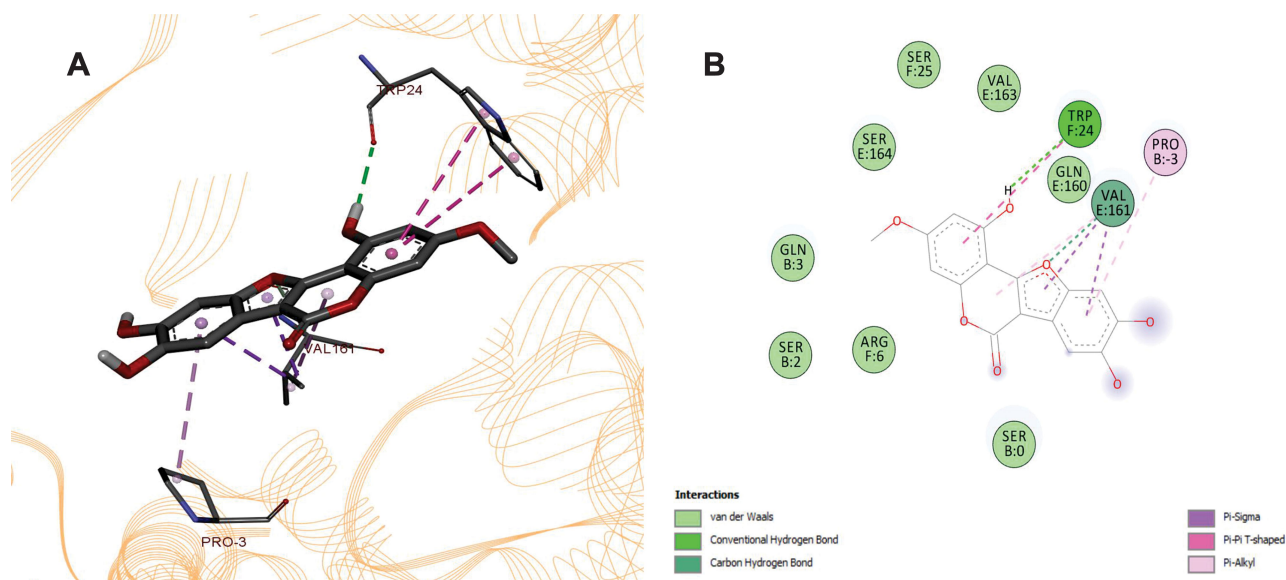
induced apoptosis is a considerable factor in this regard which can contribute towards resistance. Cisplatin-induced apoptosis works not only through DNA damage cascade but also through triggering of extrinsic death receptor or that of intrinsic mitochondrial pathway. Key elements involved here are nuclear factor- $\kappa$ B (NF- $\kappa$ B), MAPK pathways, *Bcl-2* family and *Bcl-xL* family proteins (Figure 12A–C). Dysfunction of these proteins and pathways can lead to the development of resistance to cisplatin-induced apoptosis.<sup>35</sup> Cisplatin resistant cervical cancer cells have been found to overexpress the anti-apoptotic *Bcl-2* family proteins (Figure 12C).<sup>36</sup> BA is

reported to suppress the expression of *Bcl-2* transcripts (anti-apoptosis proteins of *Bcl-2* family) in HeLa cells.<sup>12</sup> Binding interactions of BA revealed more strong binding (Figures 8A–B and 9A–B) as compared to WDL with *Bcl-2* and *Bcl-xL* (Figures 10A–B and 11A–B). In our study, the synergism observed at bolus (0/0) administration of BA with cisplatin might have resulted from the activation of the intrinsic mitochondrial apoptosis pathway, involving inhibition of over activated NF- $\kappa$ B and *Bcl-2* entities (as explained in Figure 12A and C), resulting in the enhancement of BA-induced apoptosis of cervical cancer cells (HeLa).





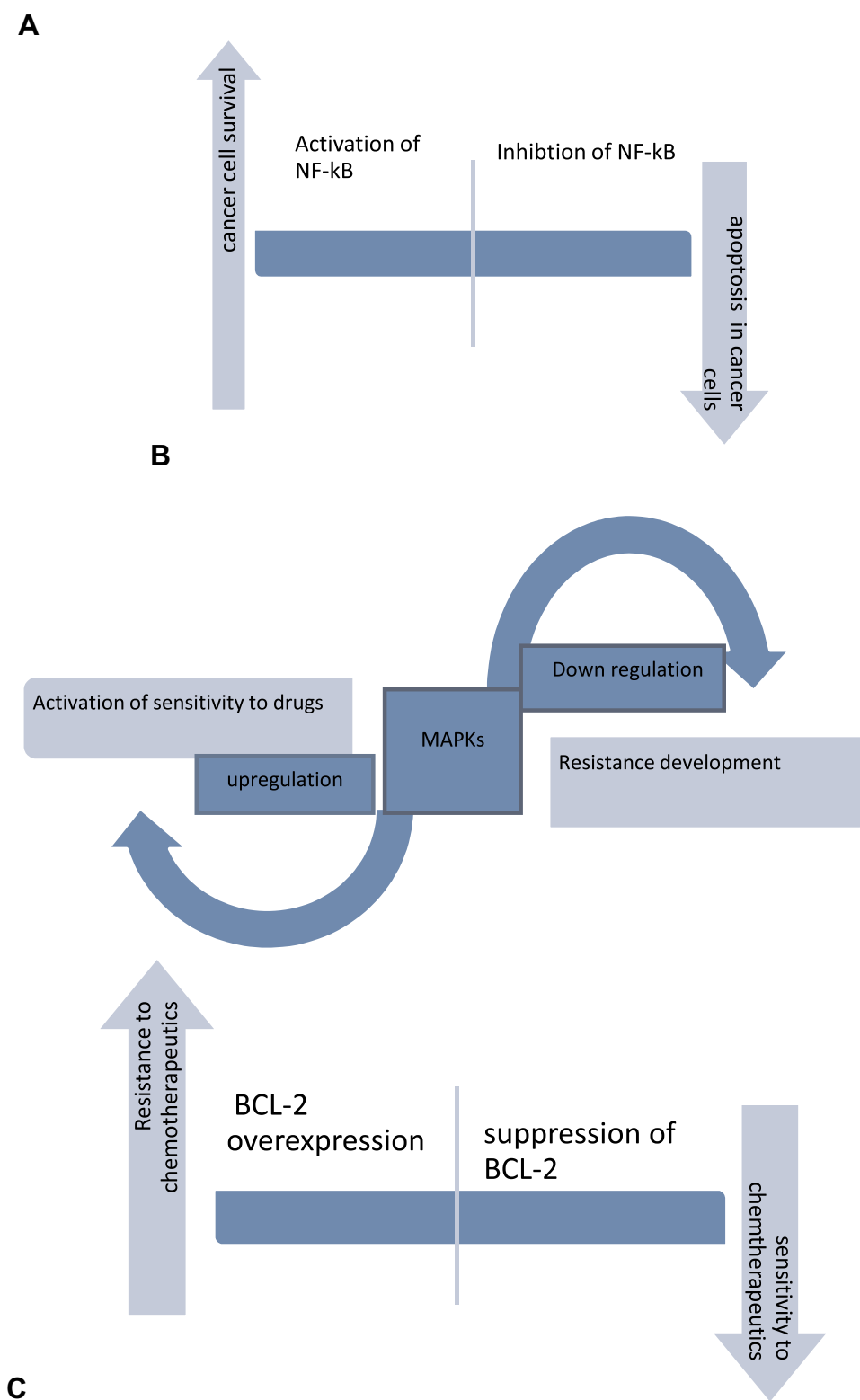
**Figure 10** (A) 3D and (B) 2D poses of WDL with B-cell lymphoma 2 Protein (Bcl-2) PDB ID: 4LXD.



**Figure 11** (A) 3D and (B) 2D poses of WDL with B-cell lymphoma-extra large Protein (Bcl-xL) PDB ID: 4TUH.

The association of *MAPK* and cisplatin resistance has been recognized (Figure 12A). Cisplatin-induced activation of *SAPK/JNK* and *ERK* (three main types of MAPKs) in cisplatin-sensitive HeLa cells while inhibition of this activation of *SAPK/JNK* is reported in resistant cells. WDL has been reported to induce apoptosis through activation of *JNK* in prostate cancer cells.<sup>37</sup> WDL has also been previously reported to inhibit the activated *NF- $\kappa$ B* in multiple cancer types in *in vitro*

studies.<sup>38,39</sup> Another key factor involved in resistance is *Akt*. The activation of the *Akt* pathway has a role in the development of chemotherapeutic drug resistance and reduction of the apoptotic potential of chemotherapeutic agents in several cancer types. The inhibitors of the the *Akt* pathway can improve cisplatin-induced chemotherapy.<sup>40</sup> WDL is also reported as a potent inhibitor of the *Akt* signaling pathway<sup>41</sup>. This effect has been validated through our docking experiment through



**Figure 12** Proposed mechanisms of resistance in cervical cancer: (A) Activation of NF-kB (B) Down-regulation of MAPKs (C) Over expression of anti-apoptotic members of BCL-2 family.

AutoDock vina via PyRx. WDL formed stronger interactions with both *Akt* and *NF-kB* (Figures 4 and 5) as compared to BA. Thus, the increased inhibition of growth

of cervical cancer cells resulting from the addition of WDL may be through the regulation of the *Akt*, NF-kB, or *JNK* pathway.

One of the serious concerns in the cancer chemotherapy is further increase in the concentration of chemotherapeutic agent, thereby, increasing the cellular toxicity. Therefore, the sensitization of cisplatin-induced growth inhibition of HeLa cells by WDL and BA (at 0/0) may be a remedy for chemotherapeutic drug toxicity-induced damages. This combination is a less toxic option in the treatment of cervical cancer, especially in case of chemoresistance to cisplatin. Future studies are warranted to further investigate the effect of combination of WDL and cisplatin on key elements and pathways involved in cervical cancer resistance considering the urgent need to search for compensation of associated side-effects of current treatment options.

## Conclusion

In conclusion, the combination of WDL and cisplatin has the potential to work synergistically towards the inhibition of growth of cervical cancer cells in any sequence of application. To the best of our knowledge, there is no any previous report of such action of WDL either individually or in combination.

## Acknowledgments

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Disclosure

The authors report that they have no conflicts of interest for this work.

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