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Research Article

Evaluation of Extracts from *Figonia critica* and *Organotin* Derivatives as Potential Inhibitors of Multidrug Resistance ABCB1 Transporter

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Abstract

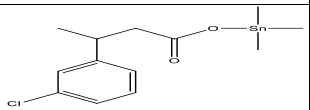
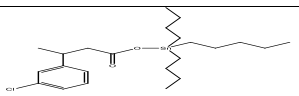
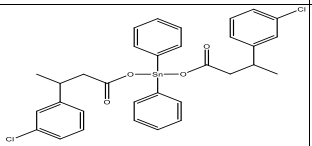
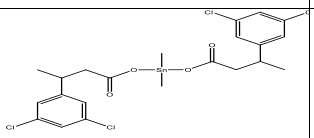
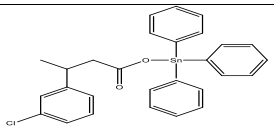
Human P-glycoprotein (P-gp) belongs to the ABC transporter family. Physiologically, ABCB1 is renowned for exporting a wide variety of toxins and drugs outside the cell, thereby providing a shielding effect to the cells. Overexpression of ABCB1 mediates cancer cell resistance to cytotoxic drugs in cancer patients. In the current study, *Organotin derivatives* and phytochemicals from *Figonia critica* (flavonoids, alkaloids) were screened as potential inhibitors of ABCB1 in a cell proliferation assay. Additionally, *Organotin* derivatives were docked into the human-mouse chimera P-gp structure (PDB ID;6FN4) in order to predict their interactions with ABCB1. The common interacting residues in the binding cavity were found to be Gln990, Phe303, Met986, Phe335, and Met966. In conclusion, the three Organotin compounds SB3, SB4, and SB1 showed good potential to be developed into anticancer agents due to their strong cytotoxicity against CCRF-CEM VCR_{1000} by demonstrating IC50 values of $16.62\pm0.05\,\mu M$, $16.88\pm0.21\,\mu M$, and $18.93\pm0.007\,\mu M$, respectively. In addition, the IC_{50} values were also calculated for flavonoids and alkaloids as 22.07±0.05µg/mL and 18.07±0.08 µg/mL, respectively. To our knowledge, this is the first report demonstrating both synthetic *Organotin* derivatives and natural phytochemicals from ***Fagonia critica*** as dual sources of potential ABCB1 inhibitors, offering new directions for overcoming multidrug resistance in cancer therapy.

Keywords: P-gp, MDR1, ABCB1, TMD, NBD, CCRF-CEM VCR_{1000}.

1. Introduction

ATP-binding cassette (ABC) transporters comprise one of the largest classes of transmembrane proteins, which are involved in the transportation of a wide variety of substances into and out of the cells by using the mechanism of ATP binding and hydrolysis (Elbahnsi et al. 2024).In humans, there are 49 ABC transporters, three of which are multidrug resistance transporters, including ABCB1, ABCC1, and ABCG2. ABCB1 (MDR1, P-glycoprotein or P-gp), widely known as a multidrug resistance drug efflux transporter, is a full-length transporter (2TMD and 2 NBD) localized in the plasma membrane (Choudhuri and Klaassen 2006). ABCB1 is also expressed in many normal cells and tissues, including the kidneys, liver, brain, intestine, and placenta, serving a key role in drug-drug interactions (DDI) (Aszalos 2007; Borst and Elferink 2002). Pathophysiological P-gp gained much more attention due to its crucial role in affecting the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of a drug (Gameiro et al. 2017).

Table 1: Structure and IC_{50} values of *Organotin derivatives* calculated from the cell proliferation assay.

S. No	Code	IUPAC Name	Structure & IUPAC Name	Ic50 Value/ µM
1	SB1	Trimethyl tin chloride		18.93±0.007
2	SB2	Tributyl tin chloride		54.95±4.41
3	SB3	Diphenyl tin dichloride		16.62±0.05
4	SB4	Dimethyl tin dichloride		16.88±0.21
5	SB5	Triphenyl tin chloride		39.29±0.16

Multidrug resistance is progressively an essential issue in the treatment of the majority of diseases (Singh et al. 2020). A great advancement has been made in the past several years in order to study the role of MDR transporters (Sarkadi *et al.*, 2006). ABCB1 has been found to develop resistance against the advanced chemotherapies (Longley and Johnston, 2005; Tiwari *et al.*, 2011; Binkhathlan and Lavasanifar, 2013) as well as being involved in drug-drug interactions and drug disposition. Later on, it has been studied that the cell membrane of cancer cells also expresses P-gp, which decreases the availability of drugs to the cells, thereby preventing these cells from experiencing chemotoxic effects (Gottesman, 2002). While physiologically having a protective role in the body, P-gp, in its overexpression form during cancer, repeatedly exports the anticancer drugs, thereby affecting the chemotherapy (Jain and Jain 2016). In addition, it is also exporting anti-histamines, antibiotics, human immunodeficiency virus (HIV) protease inhibitors, anti-epileptics, immunosuppressive agents, cardiac glycosides, cholesterol-lowering statins, anti-hypertensives, and calcium channel blockers, therefore, creating a hurdle in the treatment of related diseases due to the phenomenon of multidrug resistance. By expelling antineoplastic drugs from tumor cells, ABC transporter proteins prevent drug accumulation in tumor tissue and demonstrate low activity due to decreased drug accumulation at the target site.

Significant modification can occur in the membrane during the absorption and excretion of molecules into the cell. It is responsible for reducing the accumulation of drugs in cells and also mediates the production of anticancer drug multidrug resistance (Mesci et al. 2019). Commonly, ABCB1 is known for its function by blocking tumor cells to avoid chemotherapeutic agent efflux responses to treatment. The presence of an inhibitor for ABCB1 alters the bioavailability of a drug in the intestine and has an impact on the clinical safety of the selected drug (Aszalos 2007).

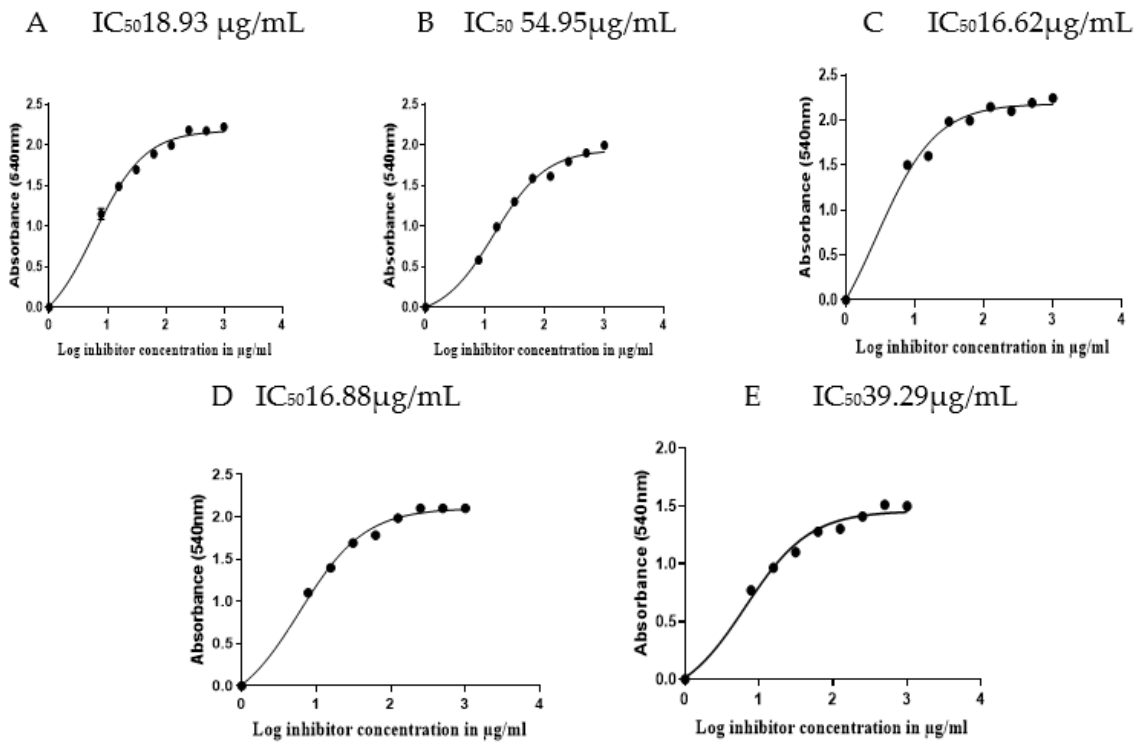


Figure 1: Cytotoxicity assay for SB1 to SB5 (A-E) is shown. The graph was generated using the X-axis log inhibitor concentration while absorbing on the Y-axis. The circle shows data points. In GraphPad Prism 7, the curve was developed using non-linear regression analysis. The curve is the average of a duplicate of two separate experiments. A variety of SB1 to SB5 concentrations (0.7µg / ml-50µg / ml) were used. The results were calculated using ELISA. IC_{50} values have been taken as occupancy values of 50 percent and interpreted as mean ±SD.

One of the well-employed strategies in MDR reversal, the property of P-gp is its modulation using low molecular weight compounds. Therefore, much effort has been devoted to the development of inhibitors, both natural products and synthetic, of P-gp to discover new compounds for cancer treatment. Inhibition of P-gp-mediated drug transport has also been employed in numerous studies (Elmeliegy et al. 2020). In this scenario, class IV of organotins has already been reported as antitumor, anti-inflammatory, antifungal, and antimicrobial agents based on the observation of synergistic effects following the binding of their respective ligands, resulting in the enhancement of their biological activities. The report provides a basis for studies of the antitumor activities of organotin (IV) compounds and highlights the potential applications of these compounds as anticancer metallodrugs with low toxicity and few side effects. Organotin (IV) compounds have wide applications in industrial and agricultural fields owing to their ability to act as poly (vinyl chloride) stabilizers and catalytic agents, as well as their medicinal properties.

P-glycoprotein suppression by plant constituents is an advanced method for overcoming resistance mechanisms within the therapy. Subsequently, several attempts are being made to identify biologically active compounds, obtained from herbal sources that inhibit P-gp, overturn the phenotype of the MDR, and sensitize tumor cells without adverse toxic potential to cancer therapy. Thus, researchers, along with their structures, identify the role of action of most of the already reported phytochemicals. This provides information to medical pharmacists and scientists

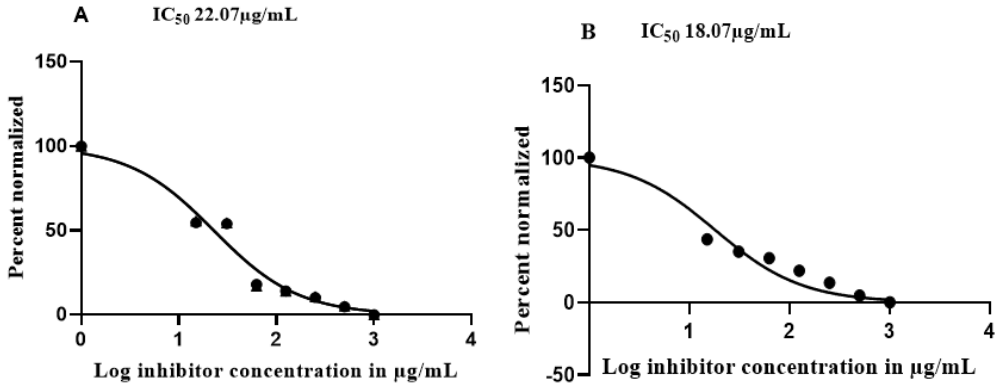


Figure 2: Cytotoxicity assay. A) Flavonoids B) Alkaloids: The graph was generated using the X-axis log inhibitor concentration while absorbing on the Y-axis. The circle shows data points. In GraphPad Prism 7, the curve was developed using non-linear regression analysis. The curve is the average of a duplicate of two separate experiments. A variety of flavonoid and alkaloid concentrations (0.7µg / ml-50µg / ml) were used. The results were calculated using ELISA. IC_{50} values have been taken as occupancy values of 50 percent and interpreted as mean ±SD.

in drug development and manufacturing to synthesize new scaffolds with the best efficiency in the long run. The current study was designed to screen organotin derivatives as inhibitors for P-gp, and the most active organotin compounds in the MTT assay have been docked in a homology model of human P-gp built on the template of mouse P-gp (Li et al. 2014). and the recently published cryo electron microscopy (CEM) structure of human P-gp (Alam et al. 2018) in order to map the interactions of these compounds with residues of human P-gp.

Although *Organotin derivatives* such as SB3, SB4, and SB1 have shown promising cytotoxicity against P-gp overexpressing cancer cells, their therapeutic viability hinges on safety in non-target cells and minimal environmental harm. Several recent studies demonstrate that selectivity is achievable. For example, (Gómez et al. 2023) found that organotin compounds with particular substituents had significantly lower cytotoxicity in COS-7 normal cells compared to cancer cell lines, yielding favorable selectivity indices. Likewise, a glutamine-conjugated organotin study (2025) showed potent anti-tumor activity in colon carcinoma models with minimal toxicity in non-tumor tissue and selective accumulation in the target organ. On the other hand, reviews of cytotoxic organotin compounds (e.g. ***Organotin Dithiocarbamate Compounds: A Review***, 2023) caution that increasing alkyl or aryl substitution and higher lipophilicity often correlate with greater toxicity in both cancerous and non-cancerous cells. Indeed, some organotins also manifest known environmental and systemic toxicities (renal, endocrine, neural), as suggested by work on mammalian organotin toxicity (e.g. "Organotin Compounds Toxicity: Focus on Kidney"). To improve safety, future development of SB3, SB4, and SB1 should include testing on normal cell lines for selectivity indices, delivery systems to reduce off-target exposure, in vivo toxicity studies, and monitoring of environmental persistence.

2. Materials & Methods

2.1 Synthetic Compounds

2.1.1 Synthetic Compound Selection

Synthetic *Organotin* derivatives were a kind gift from Assistant Prof. Dr Muhammad Niaz, Department of Chemistry, Abdul Wali Khan University, Mardan.

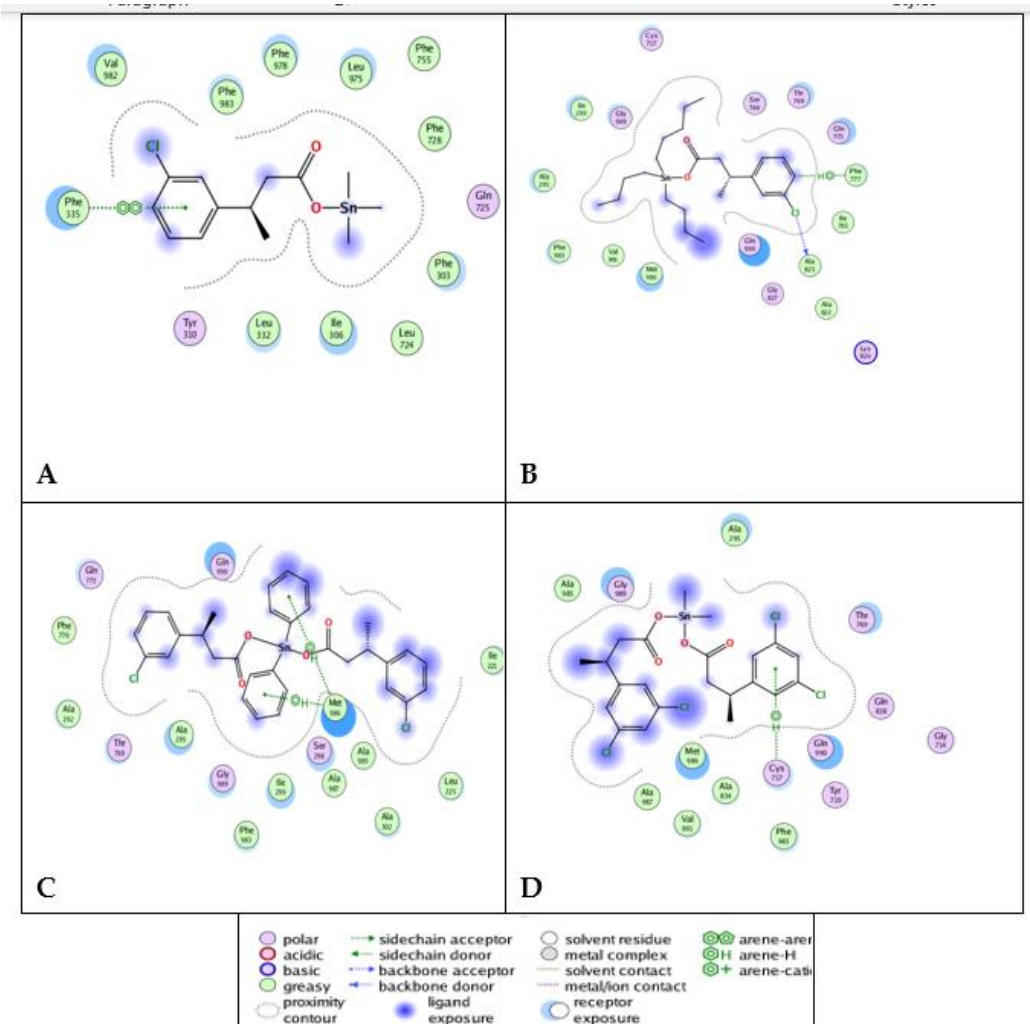


Figure 3: Docking pictures of the interaction of SB1, SB2, SB3, SB4, and SB5 with P-gp.

2.1.2 Stock Preparation

All compounds were recognized, weighted, and subjected to a stock solution (1mM in DMSO) and stored at -20 °C. From the stock solution, a concentration range of (1.5-50 µl) was prepared to screen compounds for the MTT Assay.

2.2. Phytochemical Extraction and Quantification

2.2.1 Plant Collection

Medicinal plant *Fagonia Critica* was collected from Attock, Punjab, Pakistan, during October 2019. Before conducting the study, the plant was identified by the Department of Botany, Abdul Wali Khan University, Mardan, Pakistan.

2.2.2 Sample Preparation

The sample was dried at room temperature. Using an electric blender, the dried sample was ground to a fine powder. For further analysis, samples were preserved at 4 ° C. Powder form of samples was extracted at 1:10 for 24 hours at 37 ° C with the aid of various organic solvents on the basis of polarity.

2.2.3 Flavonoids Extraction and Determination

Determinations of flavonoids were carried out according to Barros et al. 2008. Flavonoids were extracted by dissolving 5 g of sample in 50 mL of 80% aqueous ethanol (at a ratio of 1g: 10mL). The mixture was left for 24 hours at 37°C in a shaker incubator. After shaking, the extract was filtered. The pellet was removed while the filtrate containing flavonoids was stored at 4ºC. The flavonoid sample (250 µl) was mixed with 1.25 mL of distilled water and 75 µl of 5% NaNO2 solution. Then 150 µl of a 10% AlCl3.H2O was added after 5 minutes, while 500 µl of 1 M NaOH and 275 µl

Table 2. Log p values and docking scores of Organotin derivatives

S.NO	Compound Code	Log p	Docking score
1	SB1	4.0882	**-6.7732**
2	SB2	7.6802	**-8.3351**
3	SB3	9.9302	**-9.6866**
4	SB4	8.2486	**-7.4710**
5	**SB5**	**6.1100**	**-6.5507**

of distilled water were added to the mixture after 6 minutes. The blank containing 80% aqueous ethanol was also prepared in the same way. The solution was shaken well, and absorbance was measured at 415 nm using a spectrophotometer (Modle UV5100B). All absorbance was measured in triplicate. Different concentrations of quercetin (7.81µg/ml - 1000µg/ml) were used as standard (Barros et al. 2008).

2.2.4 Alkaloids Extraction and Determination

Alkaloids were extracted (using acid-base shifting) by the modified process of Edeoga *et al*. 2007. 10g of sample was added with 100 mL of ethanol, and the solution was incubated in a shaking incubator for 24 hr at 37°C. The solution was filtered through Whatman No. 1 filter paper, and the resulting extract was collected and stored at 4 °C until further use. The extract was then re-dissolved by the addition of 1%HCl in ethanol. Then the mixture was put in the refrigerator for 3 days. The solution was then filtered, and the pH (8-10) was maintained, and chloroform was collected using a separate funnel. The chloroform layer was preserved, and the ethanol layer was then removed, whereas the solution was heated for evaporation in a hot water bath.

2.3 Cell Culture

CCRF-CEM VCR_{1000} (P-gp overexpressing cell line) was kindly provided by Prof Dr. Volker Gekeler (Gekeler et al. 1992). The Cell line was maintained in RPMI1640 media (Gibco, cat No: 11875093) that contained 10%FBS (cat No: 10082139) and Penstrep (Cat No: 15070063). The cells were then placed in CO2 incubator at 37°C. Automated Cell Counter Countless II (Invitrogen) was used to take the cell count. The overall method was performed under sterilized conditions in a Laminar flow hood.

2.3.1 Cytotoxicity Assay

Cytotoxicity assay was performed for 5 *Organotin derivatives* as well as phytochemicals using the MTT assay. CCRF-CEM VCR_{1000} cells were grown up and seeded in a 96-well plate at a concentration of 5000µL -10,000µL cells/well. Different concentration of samples (0.7µg/ml-50µg/ml) was added to all wells and then incubated at 37°C and 5% CO2 overnight. The next day, 20µl of MTT, at the concentration of 0.5mg /ml (5mg /ml) was applied to each well, incubated for 24hrs. Subsequently, 100µL of DMSO was applied to all wells and allowed to stand for 20 minutes to dissolve the blue color. The absorbance was taken at 540nm on the ELISA (model no: Biotic ELx800).

2.4 *In silico* Study

An in silico study was conducted using human-mouse chimera P-gp structure (PDB ID; 6FN4) (Alam et al. 2018) for molecular docking analysis of 5 organotin compounds.

2.4.1 Molecular Docking

Molecular docking was performed using MOE 2016. The P-glycoprotein crystal structure (PDB ID: 6FN4) was downloaded from the Protein Data Bank and prepared in MOE. Non-relevant chains and heteroatoms were removed, and crystallographic waters were deleted except those that mediate binding (retained where justified). Missing side chains and loop segments were modeled using MOE's loop modeling routine, and the best model (lowest energy and favorable geometry) was selected. Protonation states of

Table 3 Log p values and docking scores of Zosuquidar

S.NO	Compound Name	Log p	Docking score
1	Zosuquidar	4.96	-8.3042

ionizable residues and histidine tautomers were assigned with Protonate3D at pH 7.4. The protein was energy-minimized with backbone restraints (10 kcal·mol^{-1}·Å^{-2}) using the AMBER99 force field to relieve steric clashes. Ligand structures (*organotin derivatives*) were drawn in Chem Draw 8.0, imported into MOE, and converted to 3D. Protonation states and low-energy tautomers were enumerated for pH 7.4 ± 0.2, and up to three representative protomers/tautomers per compound were retained. Docking used a binding box centered on the centroid of residues Gln990, Phe303, Met986, Phe335, and Met966 (box side length 20 Å; increase to 24–28 Å for larger ligands) and the Triangle Matcher placement algorithm. For each ligand (each retained tautomer/protomer) 10 initial poses were generated and initially scored with London dG. The top 4 poses per ligand were refined (ligand minimization; limited side-chain sampling for residues within 4 Å where indicated) and rescored with GBVI/WSA dG. Top poses were selected based on rescored energies and inspection of molecular interactions (H-bond cutoff ≤ 3.5 Å, donor–acceptor angle ≥ 120°; aromatic contacts centroid ≤ 5.5 Å). Docking of the full library was performed in a single run; top hits were redocked in triplicate to confirm score stability and pose reproducibility (reporting mean ± SD). Interaction maps, clustering of poses, docking scores, and pose files were saved.

2.5 Statistical Analysis

For statistical analysis, data were analyzed by using MOE 2016 and GraphPad Prism 7. Using the software tool MOE 2016, docking of compounds with selected binding pockets was carried out. The docking score and logP were evaluated. IC_{50} values were determined by using GraphPad Prism 7. The Graph was plotted using the log inhibitor concentration on the X-axis, while absorbance on the Y-axis. The non-linear regression analysis data were used to match the sigmoidal dose-response curve, which was the mean of two separate experiments. Data of the IC_{50} values was defined as mean ± SE.A probability of 0.05 or less was deemed statistically significant ($p< 0.05$).

3. Results

3.1. Cell Proliferation Inhibition by Organotin Derivatives

Cell proliferation assay was performed to determine the sensitivity of the CCRF-CEM VCR_{1000} cancer cell line towards the inhibitory activity of *Organotin derivatives*. For this purpose, different concentrations of 5 *Organotin* derivatives were used in the assay. Results revealed inhibitory activity for *Organotin* derivatives. All measurements were taken on ELISA at 540nm and are shown in Figure 1. Results revealed that these phytochemicals are killing 50% of cells at a high concentration, thus bearable physiologically. IC50 values were calculated for all of these compounds by Graphpad Prism 7, out of which three compounds, SB3 and SB4, and SB1 showed the lowest IC_{50} Values of 16.62±0.05μM, 16.88±0.21μM, and 18.93 ±0.007μM. In addition, SB5 and SB2 showed IC_{50} values of 39.29±0.16 μM and 54.95±4.4 μM, respectively, as shown in Table 1.

3.2. Cell Proliferation Inhibition Using Phytochemicals

In a second series of experiments, a Cell proliferation assay was performed with phytochemicals to determine the inhibitory activity of flavonoids and alkaloids on the CCRF-CEM VCR_{1000} cancer cell line (Figure 2). This cell line was incubated with different concentrations of flavonoids and alkaloids, followed by staining

Table 4 Comparison of docking results of organotin derivatives and zosuquidar in human-mouse chimeric P-gp.

Zosuquidar	**Glu946, Phe983, Tyr950, Tyr953, Leu879, Gln990, Val991, Glu875, Met986 Phe336, Phe303, Gln725, Trp232, Ala233, Ile306**
Interacting residues in human-mouse chimeric P-gp	**Gln990, Met986, Gly714, Phe303, Leu975, Tyr710, Ser766, Phe983, Thr769, Ala823, Phe335, Gln725, Val713, Cys717, Phe978, Phe777, Met966, Val982, Ala985, Phe343**
Common interacting residues	**Gln990, Phe303, Met986, Phe335,Met966**

with MTT reagent. To generate a dose-response curve, the log inhibitor concentration was plotted on the X-axis and absorbance on the Y-axis. From the dose response curve, the IC_{50} value for flavonoids was calculated to be 22.07±0.05µg/mL. In Figure 1A, data points are represented by circles whole curve shows the trend in inhibition. Cell proliferation assay was also carried out for alkaloids, and IC50 value was calculated from the dose response curve to be 18.07±0.08 µg/mL as shown in Figure 1B. All measurements were taken on ELISA at 540nm. Results revealed that these phytochemicals are killing 50% of cells at a high concentration thus bearable physiologically.

3.3. Molecular Docking Analysis of Organotin Derivatives

In the present study, molecular docking analysis was also performed. A set of organotin derivatives, SB1, SB2, SB3, SB4, and SB5, was docked using the human mouse-chimeric P-gp structure, reported recently by Alam et al. 2018. Their log P values and docking scores are shown in Table 2. For the molecular docking study, MOE (2016.08) software was used. Molecular docking results for SB1, SB2, SB3, SB4, and SB5 with P-gp are shown in Figure 3. It has been found that Phe335 and Gln990 interact directly. Whereas Ala823 and Ser766 directly interacted with the chlorine (Cl) atom of SB2. Gln990 and Phe777 make an arene-hydrogen interaction with the benzene ring of the SB2. Met986 is making an arene-hydrogen interaction with two benzene rings of the SB3. Phe303, Phe777, and Gln990 are common interacting residues in the docking results of SB4. Results show these residues, Gly306 and Glu233, are observed to interact directly in the case of SB5. Some residues, Gly306, Tyr151, etc., are present in the vicinity. Glu233 interacts directly with the SB5 chlorine (Cl) atom. Gly306 is directly connected with the benzene ring through arene-hydrogen (Figure 3 & Table 3, and 4). When docking results of all *Organotin derivatives* were compared, it was found that five residues were found to be common interacting residues in all compounds. The list of these common interacting residues shows Gln990, Phe303, Met986, Phe335, and Met966.

4. Discussion

ABCB1 is a multipurpose drug-efflux transporter that mediates the resistance of cancer cells to cytotoxic drugs. Human P-gp is an ATP-dependent transporter mediating the outflow by the hydrolysis process of ATP of different hydrophobic compounds. Multidrug resistance (MDR) is a major challenge that must be resolved in order to treat cancer effectively. ABCB1 plays a key role in ADMET properties of drugs, and its expression has been shown to attenuate the accumulation of cellular drugs and thus increase MDR across a variety of cancers. Overcoming MDR is one desired approach to improving the survival rate of patients. In this study, a series of organotin derivatives and phytochemicals were used to evaluate

the ABCB1 inhibition assay and molecular docking study using the human P-gp structure as a template. According to molecular docking, commonly interacting residues are Gln990, Phe303, Met986, Phe335, and Met966. Cytotoxicity assay results indicated that these compounds regulate high-potency cell proliferation by presenting IC_{50} values in the low micro-molar range, where SB3, SB4, and SB1 were the most active compounds, revealing IC_{50} values of 16.62±0.05μM, 16.88±0.21μM, and 18.93±0.007μM. Molecular docking was performed to establish possible binding interactions between a quassinoid and the human P-gp. Results indicate that quassinoids form hydrogen bonds with Gln 946 and Tyr 953 residues. Docking with verapamil showed interaction with Tyr 953 and Gln946. Moreover, organotin derivative also show interaction with aromatic residues. Our results are also consistent with these studies by showing the interaction of organotine derivative with aromatic residues Phe303 and Phe335. In addition, residues Gln990, Phe303, Met986, Phe335, and Met966 were also found interacting with other residues. The following conclusions can be drawn from this study: Firstly, phytochemicals extracted from *Fagonia critica*, such as flavonoids, alkaloids, and organotin derivatives, are found to be potent inhibitors for P-gp by showing IC_{50} values in the low micromolar range. Secondly, organotin derivatives are docked in human-mouse chimera P-gp, which shows that these scaffolds are strong interaction partners for P-gp, as indicated by docking results. These also interact in the same binding pocket as that of zouquidar reported by Alam et al. 2018. The cytotoxicity observed in the MTT assay can be interpreted as an indirect measure of P-gp inhibition. The CCRF-CEM VCR1000 cell line is a multidrug-resistant phenotype characterized by overexpression of P-gp, which actively effluxes chemotherapeutic agents and thereby reduces intracellular drug concentrations. Compounds that inhibit P-gp reverse this efflux function, restoring intracellular accumulation of cytotoxic molecules, which ultimately enhances cell death and is reflected as reduced cell viability in MTT assays.

In our study, the tested *Organotin derivatives* (SB3, SB4, SB1) exhibited potent cytotoxic effects with low IC_{50} values against CCRF-CEM VCR_{1000} cells. Since this cell line typically resists cytotoxicity through P-gp–mediated efflux, the observed activity suggests that these compounds interfere with P-gp function, either by direct binding to its drug-binding cavity (as supported by docking with residues Gln990, Phe303, Met986, Phe335, and Met966) or by impairing its ATPase activity. The validation of docking predictions with cell-based cytotoxicity provides strong evidence that the derivatives act not only as cytotoxic agents but also as functional P-gp inhibitors. Similarly, the flavonoid and alkaloid extracts demonstrated cytotoxic effects, which may arise from both their intrinsic pro-apoptotic properties and their ability to modulate P-gp activity, consistent with previous reports on phytochemicals acting as natural P-gp inhibitors. Thus, the MTT assay outcomes serve as a biological validation of our docking results, highlighting the therapeutic potential of both synthetic and natural compounds as dual-action anticancer agents: directly cytotoxic and capable of reversing multidrug resistance through P-gp inhibition. In the future, these low molecular weight compounds can be used to synthesize new drugs for cancer treatment, both from natural and synthetic sources. However, *in vivo* experiments are further required.

Conflict of interest

The authors have declared no conflict of interest.

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Consent Forms

NA

Authors' Contribution

SF and NR performed the literature search and manuscript preparation. ZP conceptualized the study and supervised it. BK, NF, SF & NR performed the bench work; NM & MA refined the manuscript for publication. The authors read and approved the final manuscript for publication.

Data Availability

All the relevant data of this manuscript are available from the authors.

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