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# Synthesis of Novel Diaziridinyl Quinone Isoxazole Hybrids and Evaluation of Their Anti-Cancer Activity as Potential Tubulin-Targeting Agents

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**ABSTRACT**

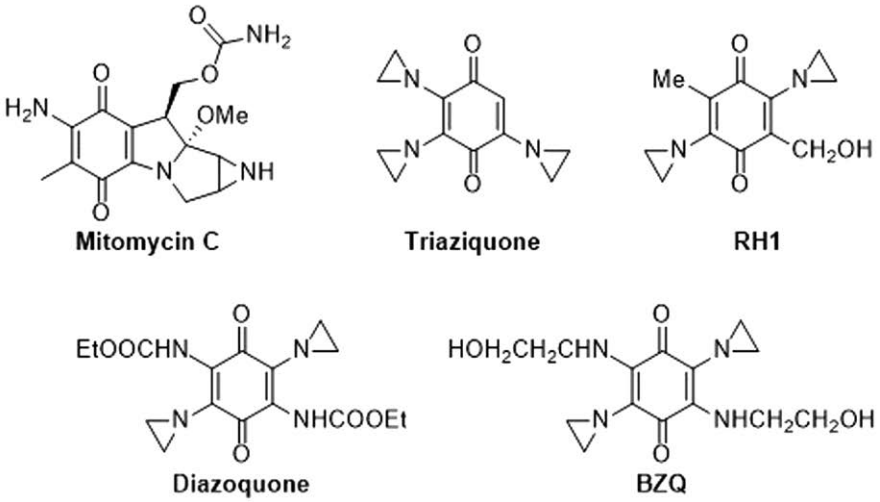
Two series of diaziridinyl quinone isoxazole derivatives were prepared and evaluated for their cytotoxic activity against MCF7, HeLa, BT549, A549 and HEK293 cell lines and interaction with tubulin. Compounds (**6a–m** ) showed promising activity against all the 5 human cancer cell lines. Compounds **6a**, **6e** and **6 m** were potent [$IC_{50}$ ranging between 2.21 µg to 2.87 µg] on ER-positive MCF7 cell line similar to the commercially available drug molecule Doxorubicin. The results from docking models are in consistent with the experimental values which demonstrated the favourable binding modes of compounds **6a–m** to the interface of α- and β-tubulin dimer.



## Introduction

Mitomycin C and other mitomycins are isolated from Streptomyces caespitosus[1]. These mitomycin derivatives are the most important clinically relevant compounds of all the aziridinyl quinone derivatives. Mitomycin C compound has 2 potentially active constituents, quinone and unusual aziridine ring system [2]. The aziridine substituted benzoquinones such as Mitomycin C, Triaziquone (TZQ), RH1 and tirapazamine (TPZ), are 4 of the principal aziridinyl quinone (▶ **Fig. 1**) class of hypoxia-specific cytotoxins that are being developed for clinical use [3–5]. These agents are composed of aziridinyl moieties on a quinone structure, and they are converted by reductive metabolism in to a bifunctional alkylating species that can cross-link major groove DNA by interacting predominantly at guanine-N7 [6]. In case of di-aziridinyl substituted quinones such as TPZ and CI-1010, the highly cytotoxic bifunctional alkylating agent can cross-link DNA in cells, resulting in complex cellular mechanisms that lead to cell death by apoptosis or necrosis [7, 8]. These bioreductive drugs have been developed to exploit the oxygen deficiency in the hypoxic fraction of solid tumors on the premise that hypoxic cells should show a greater propensity for reductive metabolism than well-oxygenated cells [9–13].

The tumor tissue has a lower oxidative reduction (redox) potential relative to most normal tissues, which could increase the reductive activation of these quinone derivatives in tumors [8]. Therefore, the selectivity of bioreductive drugs is governed not only by differences in oxygen tension between tumor and normal tissue, but also by levels of enzymes catalyzing bioreductive activation such as DT-diaphorase [9, 10]. In many cases, the biological activity of quinones is attributed to their ability to accept electrons to form the corresponding radical anion or dianion species. A quinone




▶ **Fig. 1** Structures of the drugs containing Aziridinyl quinones.

moiety substituted with an aziridine has been shown as a potent alkylating agent due to bioreduction either by one-electron reducing enzymes (e. g., NADPH cytochrome P450 reductase, cytochrome b5 reductase) or by 2-electron reducing enzyme ((NADP) H oxidoreductase, NQO1) to form the corresponding aziridinyl hydroquinones [12–14]. The hydroquinone in the corresponding aziridinyl hydroquinone effectively changes the pK of the aziridine ring such that it is protonated and become activated towards the nucleophilic attack under physiological pH.

Tubulin polymerization is an essential for mitosis, chromosome segregation, intracellular transport and cell division [15]. Microtubule dynamics are regulated by guanosine triphosphate (GTP) and the tubulin binding effectors protein [16–19]. Thus, small molecules that target microtubule are widely used to study the microtubule cytoskeleton [20] and towards the development of anti-tubulin drugs molecule that blocks the tubulin mediated defective cell growth by interfering the tubulin polymerization process. In recent years, many such anti-cancer molecules are shown to inhibit cancer cell growth by inhibiting microtubule polymerization [21–23]. Thus, drugs like paclitaxel, colchicine, vinca alkaloids, etc stabilize or inhibit the microtubule dynamics by blocking its polymerization site [23–26] and consequently, block the mitosis progression in defective cell.

Pharmacomodulation of biologically active compounds through conjunctive approaches to produce a single hybrid molecule has become an area of active research in different fields of medicinal chemistry [27]. In this connection, Braga and da Silva Júnior have reported the synthesis of hybrid molecules having 2 redox centre which showed promising anti-tumour activity [28]. In the last few years, a series of bis-type aziridinyl-quinone-isoxazole bioreductive compounds have been developed in our laboratory and their anticancer activities evaluated [29]. These diaziridinyl-quinone-isoxazole compounds have 5-substituted thiophene moiety at the 5-position of isoxazole ring. Cytotoxicity to various tumor cells varies with the substituted pattern at the thiophene ring, but DNA alkylation reactivity is related to the presence of aziridinyl groups in each structure [30, 31]. The aziridinyl moiety within the analogues served as an important alkylation group [30], but the cytotoxic effects of the synthetic analogues towards carcinoma cells might not solely be due to the aziridinyl moiety, as the quinone structure is common in numerous natural products that are associated with antitumor activities [32]. The cytotoxic mechanisms induced by quinones include redox cycling and the production of superoxide and other reactive oxygen radicals, reactions with thiols and amines, drug-uptake and DNA alkylation [33, 34]. Among quinone, various substituted napthaquionones were synthesized and studied extensively for their anticancer activity [35]. In our continuation endeavour to prepare novel hybrid molecules consisting variety of natural products [36], we have developed interest in the synthesis of diaziridinyl quinone isoxazole derivatives for evaluation of their cytotoxic activities. The efficient 5 step synthesis developed herein provides speedy creation of a series of diaziridinyl quinone isoxazole analogues. Molecular docking studies of these diaziridinyl quinone isoxazole derivatives will predict the molecular interaction of the synthesized compounds with tubulin dimer and to obtain the best orientation of compounds.

## Results and Discussion

### Chemistry

We have synthesized 2 series of diaziridinyl quinone isoxazole derivatives **6a–m** from corresponding dihydroxy benzaldehyde by the route as describe in ▶ **Fig. 2** and evaluated their cytotoxic activity. To start with 2,5-dimethoxy-benzaldehyde (**2a**) was prepared by treating with commercially available 2,5-dihydroxy benzaldehyde (**1a**) and 2,5-dimethoxy-4-methylbenzaldehyde (**2b**) was prepared by treating with commercially available 2,5-dihydroxy-4-methylbenzaldehyde (**1b**) with MeI/$K_2CO_3$ in acetone for 18 h at room temperature. Then the compound **2a** and **2b** were reacted with $NH_2OH$. HCl to produce the oxime derivatives **3a** and **3b**. Subsequent one

pot reaction of oxime derivatives (**3a**, **3b**) with phenyl acetylene in the presence of $NaOCl/Et_3N$ in DCM as solvent gave the isoxazole compounds **4a** and **4 g**. These compounds **4a** was oxidized with cerium ammonium nitrate (CAN) to give the quinone isoxazole hybrids **5a**[37]. The compound **5a** was characterized by $^1$HNMR, $^{13}$CNMR and HRMS. The characteristic isoxazole proton at 7.14 δ and quinone proton at 6.88 δ in $^1$HNMR confirms the oxidation of compound **4a** to generate compound **5a**. IR spectra of the compound **5a** showed the carbonyl peak at 1655 $cm^{-1}$. The 2 carbonyl peaks at 186.7 & 185.1 δ in $^{13}$CNMR spectra validate the benzoquinone moiety. All the benzoquinones are characteristic yellowish colored solids. The quinone compound **5a** was treated with freshly prepared aziridine [38] in the presence of $Cu(OAc)_2$ in methanol at room temperature to obtain the target diaziridinyl quinone isoxazole hybrid **6a**. The characteristic aziridinyl protons at δ 2.35 and 2.29 confirms the aziridine insertion in the compound **5a** to generate compound **6a**. After successfully establishing the method to prepare compound **6a**, various substituted diaziridinyl quinone isoxazole derivatives (**6b–m**) were prepared by following the same protocol (▶ **Fig. 2**). All the substrates reacted well under these conditions and did not produce any by-products. All the diaziridinyl quinone isoxazole hybrids were fully characterized by spectral data and obtained in good yields (▶ **Table 1**).

## Biology

### Cell lines and cell culture

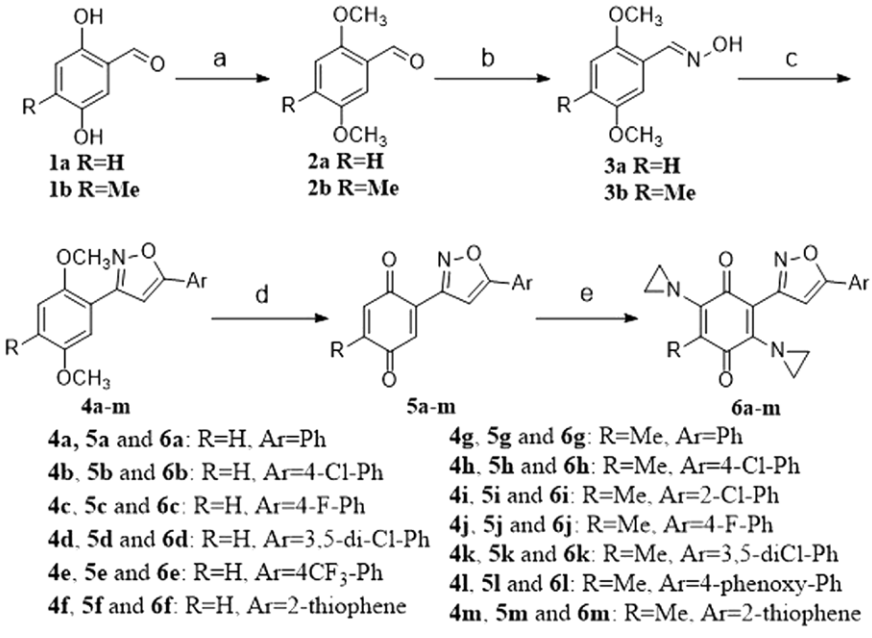
The cell lines MCF7 (human breast adenocarcinoma), HeLa (human cervical cancer), BT549 (human ductal adenocarcinoma), A549 (human lung carcinoma) and HEK 293 (human embryonic kidney) cell lines were obtained from the National Centre for Cellular Sciences (NCCS), Pune, India. Cells were cultured in DMEM media, supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 1 mM $NaHCO_3$, 2 mM glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. All cell lines were maintained in culture at 37 °C in an atmosphere of 5 % $CO_2$.

### Test concentrations

Initially, stock solutions of each test substances were prepared in 100 % Dimethyl Sulfoxide (DMSO, Sigma Chemical Co., St. Louis, MO) with a final concentration of 8 mg/ml. Exactly 25 µl of stock was diluted to 1 ml in culture medium to obtain experimental stock concentration of 200 µg/ml. This solution was further serially diluted with media to generate a dilution series of 1 µg to 100 µg/ml. Exactly 100 µl of each diluent was added to 100 µl of cell suspension (total assay volume of 200 µl) and incubated for 24 h at 37 °C in 5 % $CO_2$. Respected volume of DMSO used as a control.

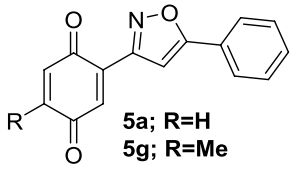
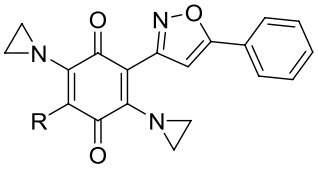
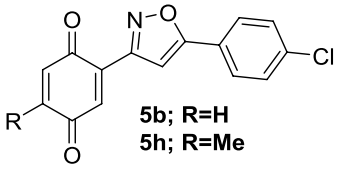
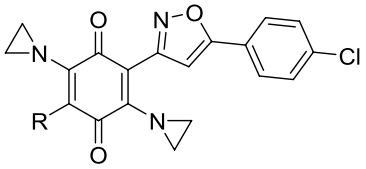
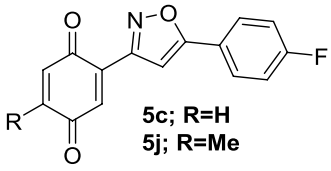
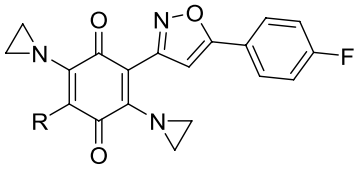
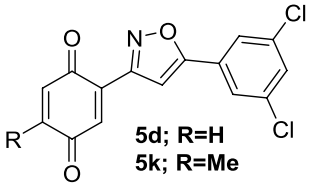
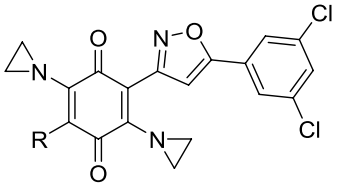
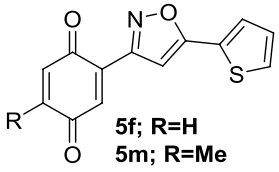
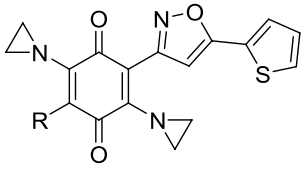
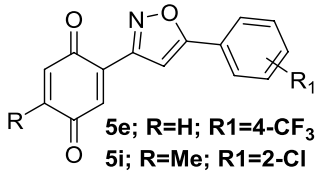
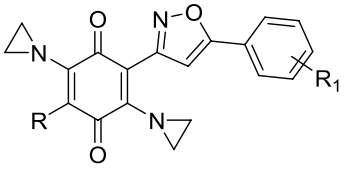
### Cytotoxicity

Cytotoxicity was measured using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay, according to the method of Mossman (1983). Briefly, the cells ($3 \times 10^3$) were seeded in each well containing 0.1 ml of medium in 96 well plates. After overnight incubation at 37 °C in 5 % $CO_2$, the cells were treated with 100 µl of different test concentrations of test compounds at identical conditions with 5 replicates each. The final test concentrations were equivalent to 1 – 100 µg/ml or 1 – 100 ppm. The cell viability was assessed after 24 h, by adding 10 µl of MTT (5 mg/ml) per well. The plates were incubated at 37 °C for additional 3 h. The medium was discarded and the formazan blue, which formed in the cells, was dissolved with 100 µl of DMSO. The rate of color formation was




▶ **Fig. 2** The synthesis route of target compounds **6a–m.** ***Reagents and conditions***: (a) MeI, $K_2CO_3$, acetone, RT, 18 h (**2a**–85 %; **2b**–80 %). (b) $NH_2OH.HCl$ NaOAc in MeOH, RT, 18 h (**3a**–95 %; **3b**–92 %) (c) corresponding alkyne, NaOCl (9–12 % in $H_2O$), TEA, DCM, RT, 20 h, 65–85 %. (d) Ceric ammonium nitrate, $CH_3CN/H_2O$ (8:2), RT, 1 h, 70–85 %. (e) Aziridine, $Cu(OAc)_2$, MeOH, RT, 1 h, 52–85 %.

▶ **Table 1** List of Synthesized Quinone isoxazoles and Diaziridinyl quinone isoxazoles.

| Quinone isoxazoles | Diaziridinyl-quinone-Isoxazoles | Yield (%) |
|---|---|---|
|  **5a; R=H** **5g; R=Me** |  | **6a; R=H (70%)** **6g; R=Me (76%)** |
|  **5b; R=H** **5h; R=Me** |  | **6b; R=H (62%)** **6h; R=Me (57%)** |
|  **5c; R=H** **5j; R=Me** |  | **6c; R=H (63%)** **6j; R=Me (62%)** |
|  **5d; R=H** **5k; R=Me** |  | **6d; R=H (54%)** **6k; R=Me (58%)** |
|  **5f; R=H** **5m; R=Me** |  | **6f; R=H (68%)** **6m; R=Me (57%)** |
|  **5e; R=H; R1=4-$CF_3$** **5i; R=Me; R1=2-Cl** **5l; R=Me; R1=4-OPh** |  | **6e; R=H; R1=4-$CF_3$ (67%)** **6i; R=Me; R1=2-Cl (52%)** **6l; R=Me; R1=4-OPh (81%)** |

measured at 570 nm in a spectrophotometer (Spectra MAX Plus; Molecular Devices; supported by SOFT max PRO-5.4). The percent inhibition of cell viability was determined with reference to the control values (without test compound). The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The $IC_{50}$ (inhibition of cell viability) concentrations were calculated using the respective regression equation.

The compounds (**5a–m** and **6a–m**) were screened for cytotoxicity against 5 human cancer cell lines, namely human breast adenocarcinoma cells (MCF7-ER-positive), human cervical cancer cells (HeLa), human ductal adenocarcinoma cells (BT549), human lung carcinoma cells (A549) and one normal human embryonic kidney cells (HEK293) by using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, according to the method of Mossman [39] (1983). $IC_{50}$ values of the test compounds for 24 hrs on each cell line were calculated and presented in ▶ **Table 2**.

It is an evident from the results that most of the prepared compounds have shown significant cytotoxic activity on all the cancer cell lines tested in a concentration dependent manner (▶ **Table 2**) but less active on normal cell line (HEK 293). This data indicated that diaziridinyl quinone isoxazole derivatives may selectively inhibit the proliferation of cancer cells which make it as a potential anticancer agents. Compare to aziridine containing derivatives (**6a–m**), non aziridine derivatives (**5a–m**) were shown less cytotoxic activity against all cell lines. Most of the diaziridinyl quinone isoxazole derivatives (**6a–m)** were exhibited cytotoxic properties against all

▶ **Table 2** *In vitro* cytotoxic evaluation of diaziridinyl quinone-isoxazole derivatives against MCF7, HeLa, BT549, A549 and HEK 293 cell lines by MTT assay.

| Compd | §$Ic_{50}$ ± SD (µg/ml) | | | | |
|---|---|---|---|---|---|
| | MCF7 | HeLa | BT549 | A549 | HEK 293 |
| **6a** | 2.21 ± 0.42 | 15.12 ± 1.09 | 13.46 ± 1.21 | 62.01 ± 5.19 | 39.52 ± 1.63 |
| **6b** | 3.12 ± 0.89 | 8.18 ± 2.22 | 18.88 ± 3.21 | 28.33 ± 4.10 | 42.32 ± 3.01 |
| **6c** | 16.02 ± 2.31 | 41.22 ± 5.53 | 55.50 ± 5.01 | 33.7 ± 2.09 | NA |
| **6d** | 18.43 ± 3.63 | 21.98 ± 1.98 | 51.99 ± 2.89 | 42.31 ± 5.02 | NA |
| **6e** | 2.32 ± 1.11 | 11.01 ± 2.12 | 16.81 ± 1.79 | 8.03 ± 1.67 | 56.01 ± 4.19 |
| **6f** | 18.16 ± 2.67 | 56.26 ± 4.62 | 69.58 ± 6.6 | 64.60 ± 5.21 | NA |
| **6g** | 12.12 ± 1.76 | 21.96 ± 0.98 | 38.45 ± 2.60 | 45.17 ± 3.54 | 36.44 ± 2.89 |
| **6h** | 14.16 ± 1.90 | 35.66 ± 4.07 | 47.03 ± 5.33 | 29.07 ± 1.98 | NA |
| **6i** | 10.34 ± 2.01 | 26.57 ± 3.03 | 56.71 ± 4.98 | 39.60 ± 4.64 | 41.14 ± 4.09 |
| **6j** | 6.35 ± 0.91 | 11.35 ± 1.19 | 19.01 ± 2.56 | 15.8 ± 2.37 | 39.60 ± 4.64 |
| **6k** | 21.14 ± 4.23 | 32.07 ± 2.78 | 50.30 ± 4.04 | 35.19 ± 4.04 | NA |
| **6l** | 21.93 ± 3.33 | 25.65 ± 4.12 | 38.81 ± 3.03 | 19.2 ± 2.49 | NA |
| **6m** | 2.87 ± 0.86 | 13.81 ± 1.45 | 22.20 ± 1.01 | 13.09 ± 3.41 | 24.36 ± 4.19 |
| **5a** | 51.02 ± 7.1 | 83.66 ± 7.48 | NA | 49.34 ± 2.98 | NA |
| **5b** | 88.99 ± 6.06 | NA | 94.09 ± 5.03 | NA | 59.02 ± 4.56 |
| **5c** | NA | NA | 98.77 ± 4.06 | NA | 86.36 ± 7.77 |
| **5d** | NA | NA | NA | NA | NA |
| **5e** | NA | NA | NA | 56.69 ± 2.9 | NA |
| **5f** | 38.34 ± 4.40 | NA | NA | 87.65 ± 6.01 | NA |
| **5g** | 33.11 ± 2.85 | 45.84 ± 2.79 | 55.62 ± 2.75 | 36.06 ± 4.01 | NA |
| **5h** | 27.90 ± 3.98 | 91.26 ± 7.81 | NA | NA | 72.80 ± 5.06 |
| **5i** | 48.83 ± 3.09 | NA | 98.71 ± 8.03 | NA | NA |
| **5j** | 26.71 ± 1.54 | NA | NA | NA | NA |
| **5k** | NA | NA | NA | NA | NA |
| **5l** | 85.17 ± 7.10 | NA | NA | 27. 46 ± 3.18 | NA |
| **5m** | 50.43 ± 3.82 | NA | 99.62 ± 8.51 | 43.51 ± 1.87 | 76.75 ± 6.32 |
| [a]**Doxorubicin** | 2.03 ± 0.22 | 2.23 ± 0.81 | 2.16 ± 0.31 | 2.64 ± 0.67 | 1.13 ± 0.13 |

Exponentially growing cells were treated with different concentrations of diaziridinyl quinone-isoxazole derivatives for 24 h and cell growth inhibition was analyzed through MTT assay.; §$IC_{50}$is defined as the concentration, which results in a 50 % decrease in cell number as compared with that of the control cultures in the absence of an test compound and were calculated using the respective regression analysis. The values represent the mean ± SE of 3 individual observations.; [a]Doxorubicin was employed as positive control. NA indicates that the derivatives are not active at 100 µg/ml concentration.

cancer cell lines (MCF7, HeLa, BT549 and A549) at below 100 µg/ml concentration. It clearly indicating that aziridine moiety is important for the cytotoxic property of the diaziridinyl quinone isoxazole derivatives. (▶ **Table 3**)

It is an apparent from the results that diaziridinyl quinone isoxazole derivatives (**6a–m**) are exhibited very good cytotoxicity on breast cancer cell line (MCF7) than the cervical (HeLa) and lung cancer (A549) cell lines. Compounds **6a**, **6b**, **6e** and **6m** were found to be most active compounds among the diaziridinyl quinone isoxazole derivatives with $IC_{50}$ concentration < 3.5 µg/ml on ER-positive MCF7 cell line. Compare to triple negative breast cancer cells (BT549), ER-positive breast cancer cells (MCF7) are more sensitive to diaziridinyl quinone isoxazole derivatives. Also compounds **6b**, **6e**, **6j** and **6m** showed significant cytotoxicity against HeLa cell line. The compounds (**6g–m)** which has $CH_3$ group on quinone ring showed less cytotoxicity compared to compounds (**6a–f)** where there is no methyl group on quinone ring. Similarly, when there is $CF_3$ group on aryl ring of isoxazole **6e**, the cytotoxic activity was similar to compound **6a** against MCF7. Compound **6j** which has F on aryl ring show good cytotoxicity against all the 5 tested human cancer cell lines. Among the compounds (**6a–m**), compounds having halogen on aromatic ring of (**6b**, **6e**, **6j**) showed good cytotoxicity. Whereas electron donating groups on the compound (**6l**) having substituted aryl on the isoxazole ring showed less cytotoxicity. In particular, the compound **6m** having thiophene ring on isoxazole showed more cytotoxicity compared to phenoxy (**6l**) substituted ring. In general, compounds having thiophene and aryl with fluorine as substituent on isoxazole ring exhibited more cytotoxicity.

## Molecular docking studies

Molecular docking studies was performed to understand whether the synthesized compounds have any role for tubulin binding and regulation of tubulin polymerization process. The docking studies were performed for the binding activity of **5a–m** and **6a–m** compounds with tubulin dimer using Autodock vina [40]. The docking

▶ **Table 3** Selectivity index (SI) for the diaziridinyl quinone-isoxazole derivatives [Selectivity index, represented by the ratio of cytotoxicities between normal cell and different lines of cancer cells.]

| Compound | Selectivity index (SI) *Vs* HEK293 | | | |
|---|---|---|---|---|
| | MCF7 | HeLa | BT549 | A549 |
| **6a** | 17.88235 | 2.61376 | 2.93611 | 0.63732 |
| **6b** | 13.5641 | 5.17359 | 2.24153 | 1.49382 |
| **6c** | >6.2422 | >2.42601 | >1.8018 | >2.96736 |
| **6d** | >5.42594 | >4.54959 | >1.92345 | >2.36351 |
| **6e** | 24.14224 | 5.08719 | 3.33195 | 6.97509 |
| **6f** | >5.50661 | >1.77746 | >1.43719 | >1.54799 |
| **6g** | 3.0066 | 1.65938 | 0.94772 | 0.80673 |
| **6h** | >7.06215 | >2.80426 | >2.1263 | >3.43997 |
| **6i** | 3.97872 | 1.54836 | 0.72545 | 1.03889 |
| **6j** | 6.23622 | 3.48899 | 2.08311 | 2.50633 |
| **6k** | >4.73037 | >3.11818 | >1.98807 | >2.84172 |
| **6l** | >4.55996 | >3.89864 | >2.57666 | >5.20833 |
| **6m** | 8.4878 | 1.76394 | 1.0973 | 1.86096 |
| **5a** | >1.96002 | >1.19531 | >1 | >2.02675 |
| **5b** | 0.66322 | <0.5902 | 0.62727 | <0.5902 |
| **5c** | 0.8636 | 0.8636 | <0.87435 | 0.8636 |
| **5d** | 1 | 1 | 1 | 1 |
| **5e** | 1 | 1 | 1 | 1.76398 |
| **5f** | 2.60824 | 1 | 1 | 1.1409 |
| **5g** | 3.02024 | 2.1815 | 1.79791 | 2.77316 |
| **5h** | 2.60932 | 0.79772 | <0.728 | <0.728 |
| **5i** | >2.04792 | 1 | >1.01307 | 1 |
| **5j** | >3.74392 | 1 | 1 | 1 |
| **5k** | 1 | 1 | 1 | 1 |
| **5l** | >1.17412 | 1 | 1 | >3.64166 |
| **5m** | 1.52191 | 0.7675 | >0.77043 | 1.76396 |
| **Doxorubicin** | 0.55 | 0.461 | 0.523 | 0.428 |

▶ **Table 4** Ligand-tubulin Docking score obtained through Autodock vina [40].]

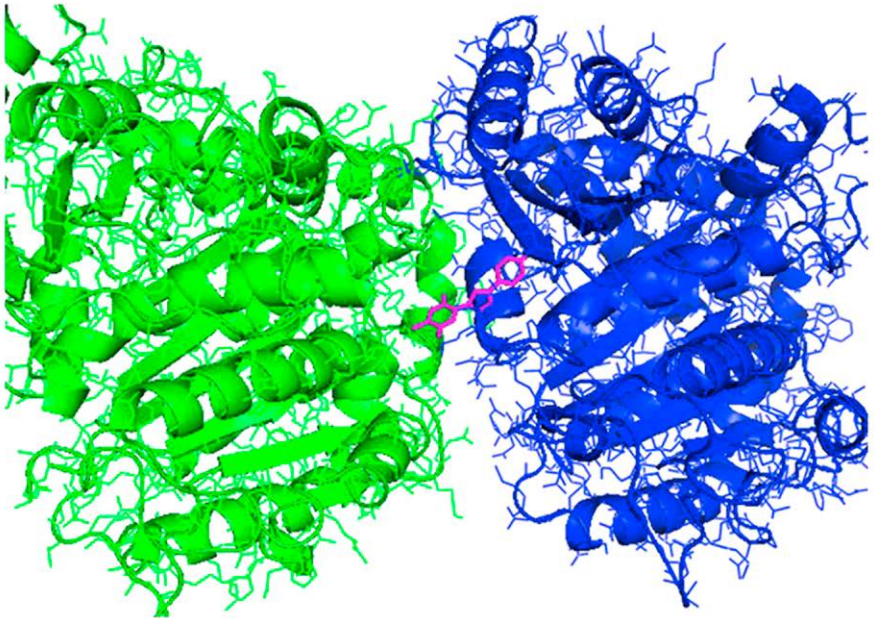
| Ligand | ΔG(Kcal/mol) | K ($10^6 M^{-1}$) | Binding side | Ligand | ΔG(Kcal/mol) | K($10^6 M^{-1}$) | Binding side |
|---|---|---|---|---|---|---|---|
| **5a** | −8.5 | 1.7 | GTP | **6a** | −8.7 | 2.4 | GTP |
| **5b** | −9.1 | 4.7 | GTP | **6b** | −8.7 | 2.4 | GTP |
| **5c** | −8.9 | 3.3 | GTP | **6c** | −8.5 | 1.7 | STK-C |
| **5d** | −8.7 | 2.4 | GTP | **6d** | −8.7 | 2.4 | STK |
| **5e** | −9.7 | 12.9 | GTP | **6e** | −9.5 | 9.2 | STK-C |
| **5f** | −8.1 | 0.8 | GTP | **6f** | −7.8 | 0.5 | GTP |
| **5g** | −8.6 | 2.0 | GTP | **6g** | −8.6 | 2.0 | GTP |
| **5h** | −8.7 | 2.4 | GTP | **6h** | −9.0 | 3.9 | STK-C |
| **5i** | −9.1 | 4.7 | GTP | **6i** | −9.2 | 5.5 | STK-C |
| **5j** | −9.1 | 4.7 | GTP | **6j** | −9.1 | 4.7 | STK-C |
| **5k** | −9.5 | 9.2 | STK | **6k** | −9.7 | 12.9 | STK-C |
| **5l** | −9.4 | 7.8 | STK | **6l** | −9.8 | 15.3 | STK-C |
| **5m** | −8.1 | $0.8 \times 10^6$ | GTP | **6m** | −8.2 | 1.0 | GTP |
| **Doxorubicin** | −9.9 | 18.1 | STK-C | | | | |
| **STK** | −10.2 | 30.0 | STK | | | | |
| **GTP** | −8.6 | | GTP | | | | |

results of the synthesized compounds with tubulin dimer are presented in ▶ **Table 4**. The globular protein tubulin dimer consist of 2 units of α and β tubulin monomer shown in green and blue color respectively (▶ **Fig. 3**). Both of these monomer units held to each other through their head to tail and tail to head side chain interaction. The tubulin polymerization occurs through combination of several such tubulin dimer through their head to tail interaction. Hence, development of molecules that regulates tubulin polymerization process is urgently needed. For docking calculation, we used the pdb structure of tubulin dimer (protein PDB ID: 1SA0) deposited in RCSB (Research Collaboratory for Structural Bioinformatics) protein data bank. The pdb files of **5a–m** and **6a–m** series are created and optimized to their minimum energy using chemBiodraw ultra12. The docking scores are summarized in ▶ **Table 4**. The purpose is to characterize the binding interface and how efficiently **5a–m** and **6a–m** series compounds can bind to tubulin dimer. Further, if molecule binds, then there is a possibility of regulating the tubulin polymerization process. From docking calculation, it is shown that these molecules bind to tubulin dimer with 2 type of binding sides, (a) interface of dimer, which overlap the STK [41] and colchicine binding side and (b) α unit of tubulin which overlap the GTP binding side, whose major part is on the α unit of tubulin monomer (▶ **Fig. 4**). It is reported that GTP bound tubulin somehow unable to participate in polymerization process. However, hydrolysis of GTP to GDP bound tubulin, initiate the polymerization in tubulin.

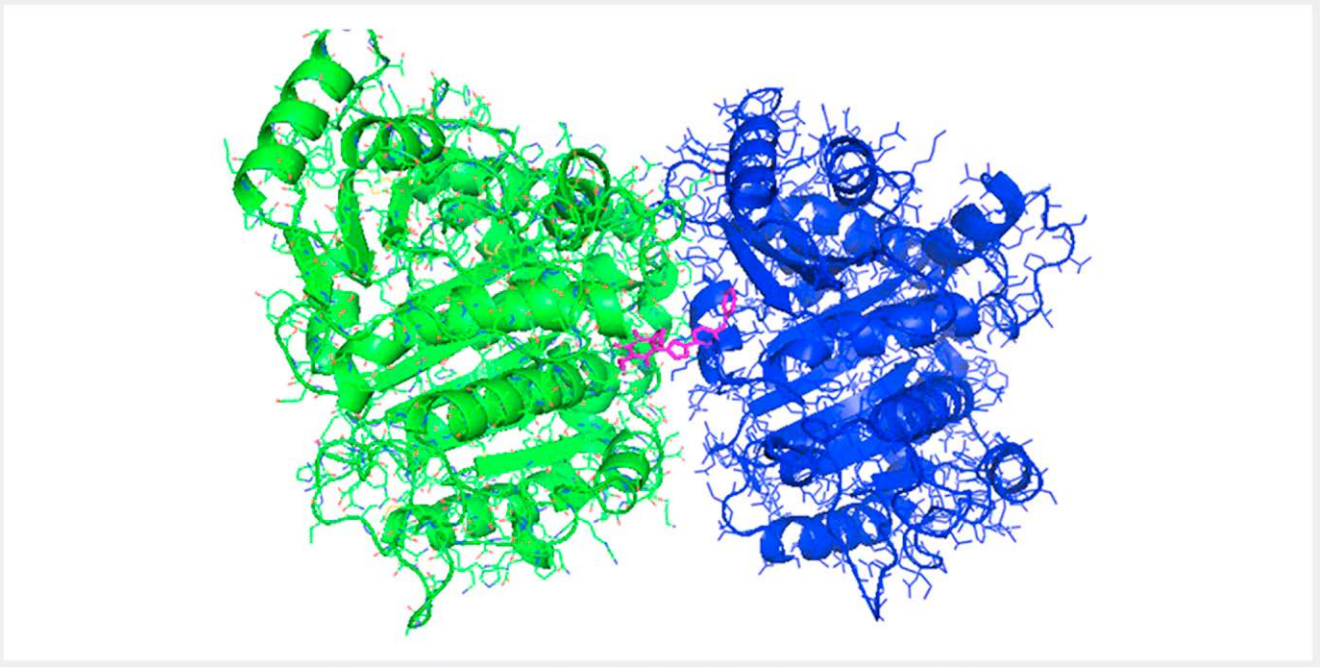
As the binding sides are situated between the interface of α and β monomer units, particularly to the GTP binding side, it is our hypothesis that these compounds have higher possibility of regulating the tubulin polymerization processes. The binding side which overlap GTP binding side is situated on the α tubulin monomer surrounded by the amino acids Gln11, Asp71, Ser140, Asn205 and Tyr224 of α tubulin and Lys254 of β tubulin provide the cavity for binding site. Similarly, for other binding side which overlap the GTP and colchicine binding side is situated on the interface of both α and β tubulin surrounded by amino acids Gln11, Asp71, Thr73 of α tubulin and Asn249, Lys254 of β tubulin that provide binding cavity for ligand. Based on the binding energy (score value) that obtained through Autodock vina, it is suggested that compound **5b, 5e, 5i, 5j** strongly bind to the GTP binding side, whereas **5k** and **5 l** strongly bind to the dimer interface of α and β tubulin which overlap the colchicine binding side. Similarly, compound **6e, 6 h, 6i, 6j, 6k, 6 l** strongly bind to the dimer interface of α and β tubulin, which overlap the colchicine binding side (▶ **Fig. 4**). So these molecules can be used to regulate the tubulin mediated biological activities.

## Conclusion

In summary, we have synthesized 2 series of diaziridinyl quinone isoxazole hybrids and were screened for cytotoxic activity against 5 human cancer cell lines. The order of sensitivity of human cancer and normal cell lines towards diaziridinyl quinone isoxazole derivatives are MCF7 > HeLa > A549 > BT549 > HEK293. Compounds **6a, 6b, 6e** and **6 m** were potent on ER-positive MCF cell line similar to the commercially available drug molecule, Doxorubicin. Based on docking study on tubulin crystal structure (PDB code: 1SA0), compound **6 l** exhibited the most potent affinity for tubulin. The above results indicates this new compounds might be useful in designing and developing new drugs for cancer treatment.



▶ **Fig. 3** Docked structure of tubulin dimer (PDB: 1SA0) with **5k** (magenta). The binding site present at the interface of alpha (green) and beta (blue) tubulin monomer.



▶ **Fig. 4** Docked structure of tubulin dimer (PDB: 1SA0) with **6l** (magenta). The binding side present at the interface of alpha (green) and beta (blue) tubulin monomer.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## Conflict of Interest

The authors declare that they have no conflict of interest.

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