International Journal of Chemical, Materials and Biomolecular Sciences
ISSN: 2415-6620
Vol:9, No:3, 2015

Comparative *in silico* and *in vitro* Study of N-(1-Methyl-2-Oxo-2-N-Methyl Anilino-Ethyl) Benzene Sulfonamide and Its Analogues as an Anticancer Agent

Pamita Awasthi, Kirna, Shilpa Dogra, Manu Vatsal, Ritu Barthwal

***Abstract*—**Doxorubicin, also known as Adriamycin, is an anthracycline class of drug used in cancer chemotherapy. It is used in the treatment of non-Hodgkin's lymphoma, multiple myeloma, acute leukemia, breast cancer, lung cancer, endometrium cancer and ovary cancers. It functions via intercalating DNA and ultimately killing cancer cells. The major side effects of doxorubicin are hair loss, myelosuppression, nausea & vomiting, oesophagitis, diarrhea, heart damage and liver dysfunction. The minor modifications in the structure of compound exhibit large variation in the biological activity, has prompted us to carry out the synthesis of sulfonamide derivatives. Sulfonamide is an important feature with broad spectrum of biological activity such as antiviral, antifungal, diuretics, anti-inflammatory, antibacterial and anticancer activities. Structure of the synthesized compound N-(1-methyl-2-oxo-2-N-methyl anilino-ethyl)benzene sulfonamide confirmed by proton nuclear magnetic resonance (^{1}H NMR),^{13}C NMR, Mass and FTIR spectroscopic tools to assure the position of all protons and hence stereochemistry of the molecule. Further we have reported the binding potential of synthesized sulfonamide analogues in comparison to doxorubicin drug using Auto Dock 4.2 software. Computational binding energy (B.E.) and inhibitory constant (Ki) has been evaluated for the synthesized compound in comparison of doxorubicin against Poly (dA-dT).Poly (dA-dT) and Poly (dG-dC).Poly (dG-dC) sequences. The in vitro cytotoxic study against human breast cancer cell lines confirms the better anticancer activity of the synthesized compound over currently in use anticancer drug doxorubicin. The IC_{50} value of the synthesized compound is 7.12 μM whereas for doxorubicin is 7.2 μM.

***Keywords*—**Anticancer, Auto Dock, Doxorubicin, Sulfonamide.

I. Introduction

DOXORUBICIN (7S, 9S)-7-[(2R, 4S, 5S, 6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6, 9, 11-trihydroxy 9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5, 12-dione known as Adriamycin Fig.1 have long been used as an effective anticancer drug against abroad spectrum of cancers. The exact mechanism of action is still unclear. In literature number of mechanisms is proposed like- inhibition of DNA biosynthesis by intercalation, poisoning of topoisomerase II and DNA double strand breaks etc. [1]-[4]. Evidences show necrotic mode of death induced by the anthracycline drugs (doxorubicin) in tumor cells. Major side effects associated are cardiac toxicity [5], [6].

The high affinity of Adriamycin for DNA appears to be the major driving force for the drug to enter cells [7], [8]. There is also much controversy regarding the role of apoptosis and necrosis in the cytotoxic action of anthracyclines. In this research paper we report the comparative in-silico and in-vitro studies of doxorubicin and sulfonamide analogues Figs. 2 (a)-(f).

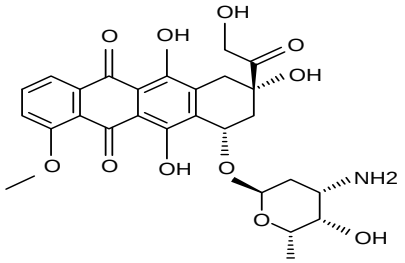

Fig. 1 Molecular structure of doxorubicin

II. Materials And Methods

A. Cytotoxic Studies

The growth inhibitory effect of synthesized sulfonamide analogous on human breast cancer cell line was measured by MTT assay [9]. The cells were seeded in cell culture medium to get 10^5 cells/ml, thereafter, 100μl of cell suspension per well was seeded in tissue culture plate. The assay was performed on 96 well plates in which cells were treated with three concentrations of compounds which were incubated for 24 h in CO_2 incubator. 20μl of freshly prepared MTT solution, 5 mg/ml in PBS (Phosphate buffer saline) was added to each well. The culture plates were stirred at 150 rpm for 5 min for mixing the MTT in to media. The culture plates were incubated again for 4 h to allow metabolization of MTT. The MTT formazan crystals were resuspended in 100μl of DMSO and plates were stirred for 20-25 min to dissolve the crystals of formazan. The absorbance was measured at 570 nm.

B. Docking Studies

The structure of doxorubicin is built using MOE software tool and optimized with MMF94x force field. Auto Dock Tools (ADT) (version 1.4.5) was used for DNA and ligands preparation. Further analogs were screened based upon their scoring function. In Auto Dock the implemented scoring

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function is defined as an empirical binding free energy function. For DNA sequences, all hydrogen, including non-polar, kollman charges and solvation parameters were added to all atoms. After adding charges, the non-polar hydrogen atoms were merged. Auto grid was used to generate the grid maps. The docking area was defined using Auto grid 40 x 40 x 40. 3D affinity grid centered on the binding site of receptor and a 0.375 Å grid point space was identified. Gasteiger charges were assigned and then nonpolar hydrogen atoms were merged for doxorubicin. Lamarckian genetic algorithm (LGA) was employed for ligand conformational searching because it has enhanced performance relative to simulated annealing or the simple genetic algorithm. For each complex, we used the default docking parameters with the exception of the following: initial population of 150 randomly placed individuals, maximum number of 2.5 x 10^6 energy evaluations and maximum number of 2.7 x 10^4 generations. The mutation rate and cross over rate were set to 0.02 and 0.80, respectively. Fifty independent docking runs were carried out for the ligand using these parameters for rapid screening. The best docked position was determined by comparing docking poses and taking total energy value into consideration. Among several similar docking poses, the more energetically favorable conformation was selected. The docking results were clustered on the basis of root-mean-square deviation between the Cartesian coordinates of the atoms using 2.0 cut off and ranked on the basis of the binding free energy ΔG and inhibitory constant K*i* [10].

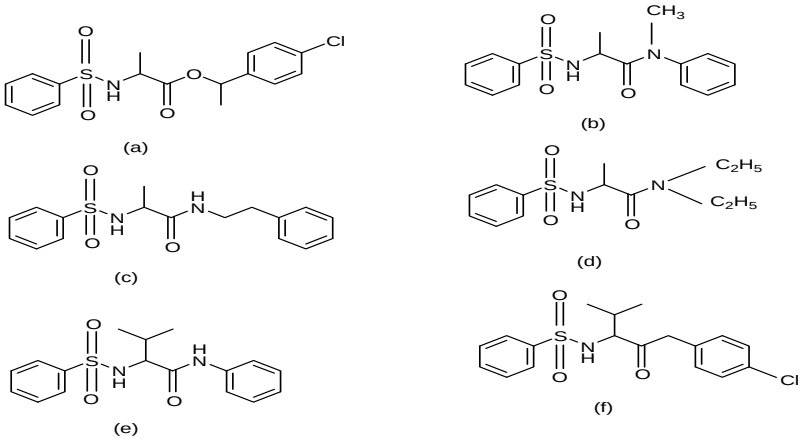

Fig. 2 (a)-(f) Structures of the benzene sulfonamides analogues

III. Results and Discussion

A. Cytotoxic Studies of Synthesized Analogues on Human Breast Cancer Cell Line

The IC_{50} values of synthesized compounds are calculated and results are compiled in Table I. The compound (a) shows better (6.21 μM) activity over other complexes against the Human Breast cancer cell lines. While known anticancer drug doxorubicin is showing 7.2μM IC_{50}value. These preliminary results are good and compounds may consider as potential anticancer agents.

B. Docking Studies

Compound (a) and (b) came out be better anticancer agents as per cytotoxic study, hence they are further chosen for docking study. Auto dock program has been applied to study the binding affinity of doxorubicin and sulfonamide compounds towards the two alternating DNA polymers. The ligand has been made flexible to attain different confirmations to predict the best fit orientation. 50 structures are saved in the database for each complex out of which lowest energy confirmation of ligand is chosen for analysis. The energy is estimated as free energy of binding of various confirmations of doxorubicin and (a-f) compounds bound to receptor DNA. The results of energy calculations and inhibitory constants are tabulated in Table I.

50 conformations of DOX, compound (a) and (b) bound to DNA were saved out of total of about 150 different conformations randomly searched for each alternating polymer AT as well as GC and best conformation of compound (a) are shown in Figs. 3, 4, 5 & 6. The minimum energy structure amongst these 50 conformations for binding of DOX, compound (a) and (b) with Poly(dG-dC).Poly(dG-dC), having ΔG = -4.43; -8.0 and -7.6 kcal mol^{-1} respectively. But with Poly(dA-dT).Poly(dA-dT) sequence value of ΔG for DOX, compound (a) and (b) comes out at litter higher scale. Unlikely value of K*i* is not uniform with both the polymer for DOX, compound (a) and (b). The free energy (ΔG) for binding of DOX, compound (a) and (b) with alternating AT polymer and GC polymer, is compiled in Table I. Interestingly, we have observed some common interactions of compound (a), (b) and DOX with Poly(dG-dC).Poly(dG-dC) and Poly(dA-dT).Poly(dA-dT). While in case of DOX with Poly(dG-dC).Poly(dG-dC), an additional interaction has been observed which might restrict the favorable interaction with the receptor and that could be the possible reason for better cytotoxic effect of compound (a) and (b) over DOX. Over all present study indicate the better binding behavior of compound (a).

IV. Conclusion

Compound (a) comes out to best among all sulfonamide

compounds synthesized in our laboratory and can be potential drug candidate. Interestingly both the in-silico and in-vitro study support each other. Detailed study on the present compound is going on and will be published subsequently.

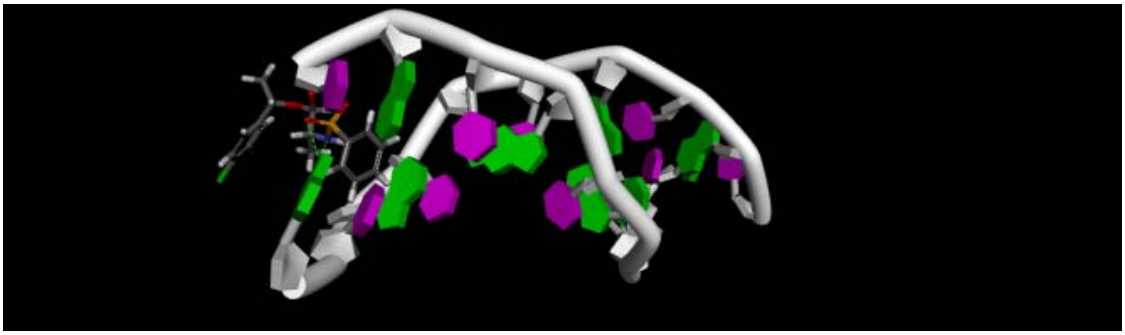

Fig. 3 Docked structure of compound (a) bound to Poly(dG-dC).Poly(dG-dC)

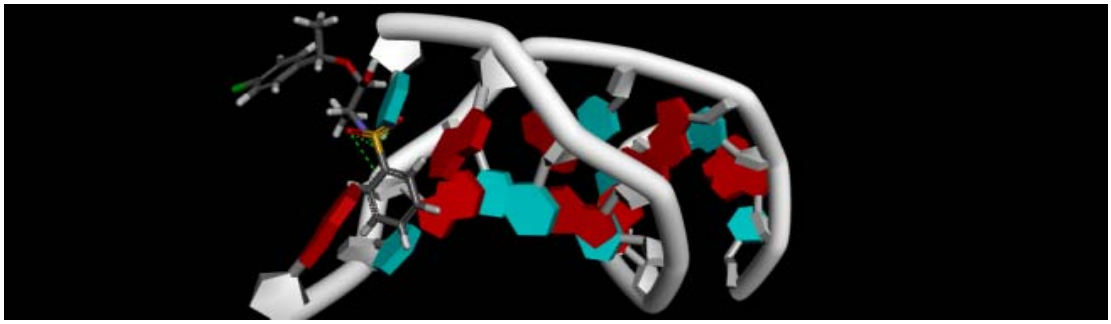

Fig. 4 Docked structure of compound (a) bound to Poly(dA-dT).Poly(dA-dT)

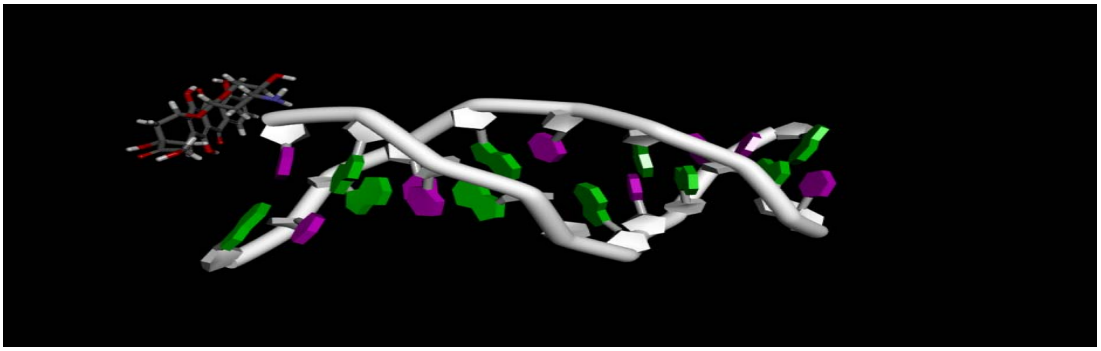

Fig. 5 Docked structure of DOX bound to Poly(dG-dC).Poly(dG-dC)

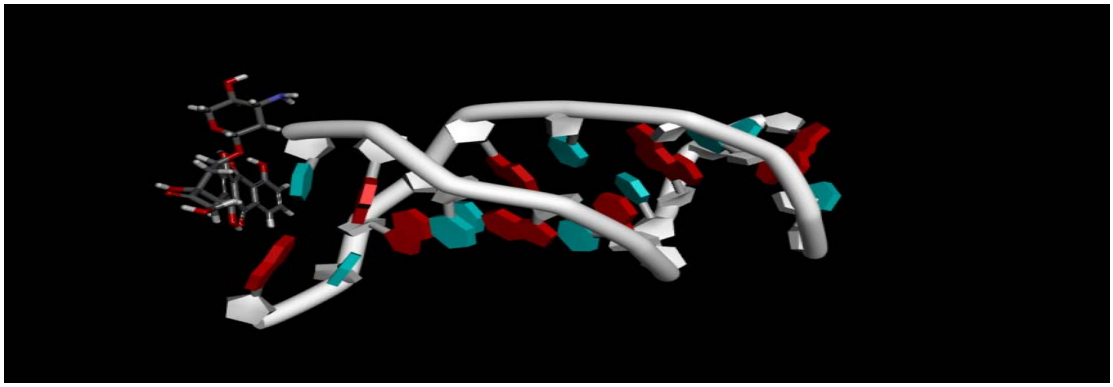

Fig. 6 Docked structure of DOX bound to Poly(dA-dT).Poly(dA-dT)

TABLE I
FREE ENERGY (ΔG) AND INHIBITORY CONSTANT (K*I*) OBTAINED FROM DOCKING STUDY OF DOXORUBICIN AND SULFONAMIDES ANALOGUES WITH POLY(DA-DT).POLY(DA-DT) AND POLY(DG-DC).POLY(DG-DC) AND IC_{50} VALUE OF DOXORUBICIN AND BENZENE SULFONAMIDES ANALOGUES

Complex	ΔG (kcal mol^{-1})	K*i*(μM)	Compound	IC_{50} value(μgm/ml)
DOX-Poly(dA-dT).Poly(dA-dT)	-5.914	6.54	(a)	6.21
DOX-Poly(dG-dC).Poly(dG-dC)	-4.43	570.16	(b)	7.21
(a)-Poly(dA-dT).Poly(dA-dT)	-4.65	388.63	(c)	8.39
(a)- Poly(dG-dC).Poly(dG-dC)	-8.0	1.36	(d)	28.09
(b)-Poly(dA-dT).Poly(dA-dT)	-7.86	1.73	(e)	8.82
(b)- Poly(dG-dC).Poly(dG-dC)	-7.6	2.67	(f)	13.19
			DOX	7.2

ACKNOWLEDGMENT

The authors would like to thank Director of National Institute of Technology, Hamirpur, India, for providing necessary laboratory facilities to carry out this work. The authors are grateful to Dr. R. Barthwal, Biotechnology Department, IIT, Roorkee for Bio assay.

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