CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com *Research Article*

SYNTHESIS, CHARACTERIZATION, MOLECULAR DOCKING STUDIES OF SUBSTITUTED 2-AMINO 3-CHLORO NAPHTHOQUINONE DERIVATIVES

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Article Received: May 2022 **Accepted:** May 2022 **Published:** June 2022

Abstract:

The present work deals with designing of some novel substituted 2 -amino 3- chloro naphthalene – 1,4 dione derivatives on the basis reaction between 2,3- dichloronaphthalene -1,4-dione and various secondary amine like 5-amino uracil (L1), 5-amino 1,3-dimethyl uracil (L2), 2-amino-chromen (L3), 2-amino- furan (L4) and isonicotinohydrazide (L5) in presence of ethanol. The synthesized compounds were characterized by IR, 1H NMR, 13C NMR, Mass spectrometry and elementary analysis. Preliminary QSAR study was carried out for the synthesized compounds. All the compounds obeyed the Lipnski rule of 5. Solubility characters of synthesized compounds were carried out by using various solvents. The tested compounds are freely soluble in DMSO, soluble in chloroform and methanol, slightly soluble in acetone, ethyl acetate and ethanol and insoluble in water. The structures of the synthesized compounds were studied using Autodock software against Topoisomerease II as a target enzymes. The docking results showed that Compounds L3, L2, L4, and L1 were found to have significant binding score against target enzyme Topoisomerase compared to standard drug Etaposide.

Keywords: *Molecular Docking, Naphthoquinone, Synthesis, Characterization*

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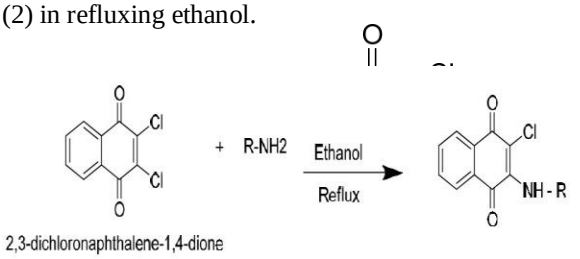
Please cite this article in press S S D Bhavani Raja et al, ***Synthesis, Characterization, Molecular Docking Studies Of Substituted 2-Amino 3-Chloro Naphthoquinone Derivatives.,*** *Indo Am. J. P. Sci, 2022; 09(6).*

INTRODUCTION:

Quinones are widespread in nature [1,2] (in plants, fungi, bacteria etc.), and many synthetic or natural quinones possess various pharmacological properties including anticancer [3–5], antibacterial [6], antifungal [6], anti-inflammatory [7], antimycobacterial [8], and molluscicidal [9] activities. Moreover, substituents such as halogen, amino, thio groups of the synthetic quinone derivatives can increase their pharmacological activities, such as antibacterial, cytotoxic, and antiproliferative [3,10,11]. Quinonoid systems' pharmacological specialties are related to their capacity to produce free radicals or semiquinones in redox reactions [11–13]. The main aim of this proposed research work is to establish the design and synthesize a novel class of substituted 2- amino 3-chloronaphthalene-1,4-dione derivatives and characterization by using IR, 1H NMR, 13C NMR and mass spectroscopy. Objective of The project describes the general methods of synthesis, purification and identification of some novel class of substituted 2- amino 3-chloronaphthalene- 1,4-dione derivatives by chromatographic & spectral methods.

SCHEME OF THE WORK

A series of 2-amino-3-chloro-1,4-naphthalene – 1,4-dione were synthesized via the nucleophilic substitution reaction of 2,3- dichloro-1,4-naphthoquinone (1) by appropriate secondary amines (2) in refluxing ethanol.



2,3-dichloronaphthalene-1,4-dione

General procedure for the synthesis of title compoundsA mixture of appropriate secondary amine (5 mmol) and 2,3- dichloronaphthalene -1,4-dione (5 mmol) in absolute ethanol (20 mL) was stirred under reflux with stirring until completion of the reaction as monitored by TLC, Pet. Ether and ethyl acetate (5:5) used as solvent system and then concentrated under reduced pressure. Water (20 mL) was added and extracted with Ethyl acetate (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified using silica gel column chromatography to afford the pure product.

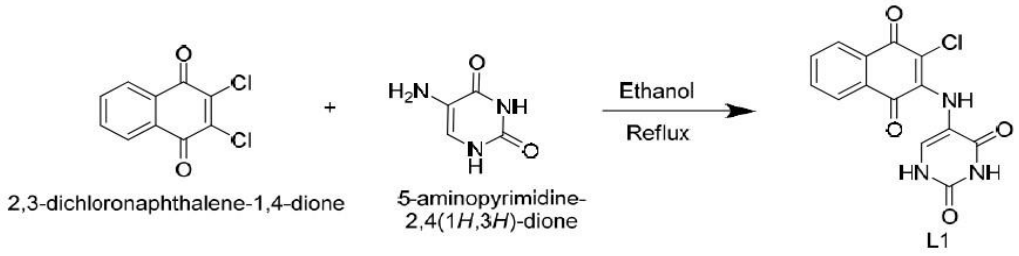
MATERIALS AND METHODS:

All the chemicals were purchased from sigma Aldrich U.S.A. Analytical TLC was performed on Precoated sheets of silica gel G/UV-254 of 0.2mm thickness (Macherey-Nagel, Germany) using analytical grade solvent and visualized with iodine spray (10% w/w I2 in silica gel) or UV light. We also used bioinformatics tools, biological databases like PDB (Protein Data Bank) and software's like Autodock and ACD ChemSketch. The PDB (Protein Data Bank) is the single worldwide archive of Structural data of Biological macromolecules, established in Brookhaven National Laboratories (BNL). It contains Structural information of the macromolecules determined by X-ray crystallographic, NMR methods etc. Auto Dock is an automated docking tool. It is designed to predict how small molecules, such as substrates, bind to a receptor of known 3D structures. Argus lab also one of the automated docking tool.

Equipment and analytical instrument

Melting point was determined in capillary tubes and is uncorrected. IR spectra were taken as KBr pellets for solids on Perkin Elmer Spectrum FT-IR. 1H NMR (400MHz) and 13C NMR (100 MHz) spectra were recorded in DMSO-d6 solution with TMS as an internal standard on Bruker instrument. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Coupling constant (J) is given in hertz. Mass spectra were recorded on a thermo Finnigan LCQ Advantage MAX 6000 ESI spectrometer.

Synthesis of 5-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl) amino) pyrimidine -2,4(1H,3H)-dione (L1)



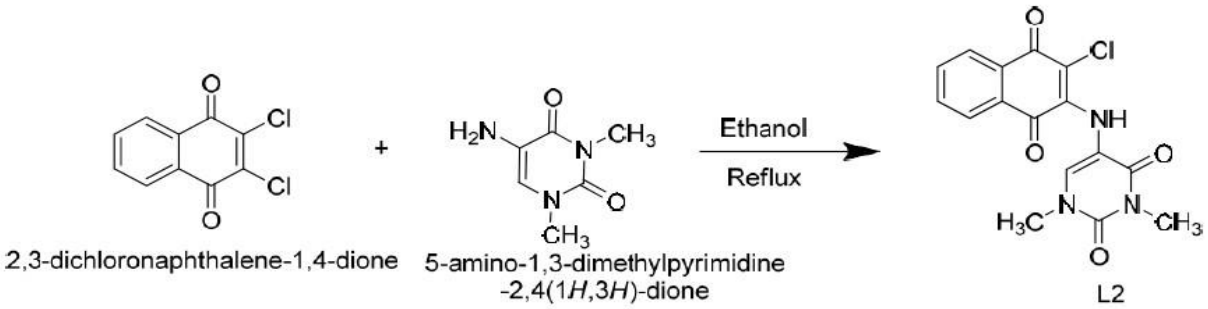
2,3-dichloronaphthalene-1,4-dione

5-aminopyrimidine-2,4(1*H*,3*H*)-dione

L1

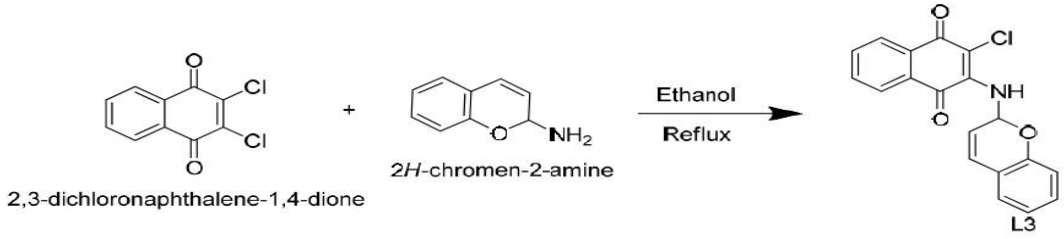
A mixture of 5-aminopyrimidine-2,4dione (5-amino uracil) (5 mmol) and 2,3- dichloronaphthalene -1,4-dione (5 mmol) in absolute ethanol (20 mL) was stirred under reflux with stirring until completion of the reaction as monitored by TLC, Pet. Ether andethyl acetate (5:5) used as solvent system and then concentrated under reduced pressure. Water (20 mL) was added and extracted with Ethyl acetate (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified using silica gel column chromatography to afford the pure product.

Synthesisof5-((3-chl



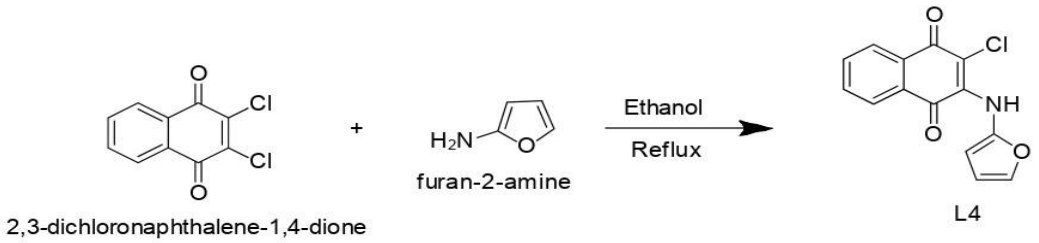
oro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-1,imethylpyrimidine-2,4(1H,3H)-dione (L2)A mixture of 5-amino 1,3-dimethylpyrimidine-2,4dione (5-amino 1,3-dimethyl uracil) (5 mmol) and 2,3- dichloronaphthalene -1,4-dione (5 mmol) in absolute ethanol (20 mL) was stirred under reflux with stirring until completion of the reaction as monitored by TLC, Pet. Ether and ethyl acetate (5:5) used as solvent system and then concentrated under reduced pressure. Water (20 mL) was added and extracted withEthyl acetate (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified using silica gel columnchromatography to afford the pure product.

Synthesis of 2-((2H-chromen-2-yl) amino)-3-chloronaphthalene-1,4-dione



A mixture of 2-amino-chromen (5 mmol) and 2,3- dichloronaphthalene -1,4- dione (5 mmol) in absolute ethanol (20 mL) was stirred under reflux with stirring until completion of the reaction as monitored by TLC, Pet. Ether and ethyl acetate (5:5) used as solvent system and then concentrated under reduced pressure. Water (20 mL) was added and extracted with Ethyl acetate (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified using silica gel column chromatography to afford the pure product.

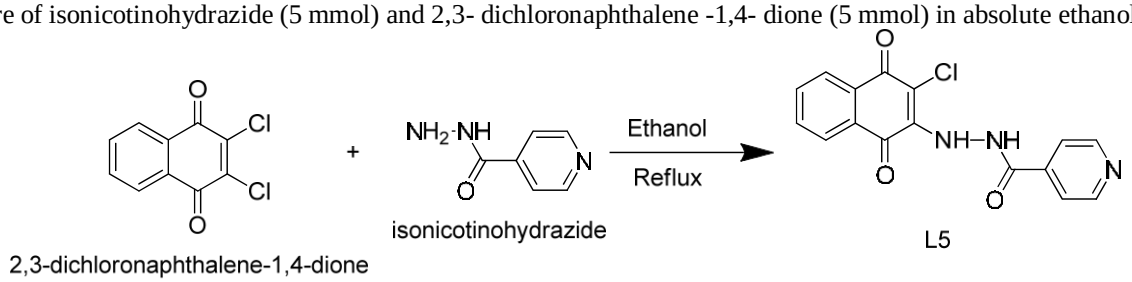
Synthesis of 2-chloro-3-(furan-2-ylamino) naphthalene-1,4-dione



A mixture of 2-amino-furan (5 mmol) and 2,3- dichloronaphthalene -1,4-dione(5 mmol) in absolute ethanol (20 mL) was stirred under reflux with stirring untilcompletion of the reaction as monitored by TLC, Pet. Ether and ethyl acetate (5:5) used as solvent system and then concentrated under reduced pressure. Water (20 mL) was added and extracted with Ethyl acetate (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified using silica gel column chromatography to afford the pure product.

Synthesis of N'-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl) isonicotino hydrazide

A mixture of isonicotinohydrazide (5 mmol) and 2,3- dichloronaphthalene -1,4- dione (5 mmol) in absolute ethanol



2,3-dichloronaphthalene-1,4-dione isonicotinohydrazide L5

(20 mL) was stirred under reflux with stirring until completion of the reaction as monitored by TLC, Pet. Ether and ethyl acetate (5:5) used as solvent system and then concentrated under reduced pressure. Water (20 mL) was added and extracted with Ethyl acetate (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified using silica gel column chromatography to afford the pure product.

RESULTS

Synthetic methodology

A series of novel class of 2 amino 3 chloro napthelene – 1,4, dione derivatives were synthesized by using 2,3-dichloronaphthalene -1,4-dione and various secondary amine like 5-amino uracil (L1), 5-amino 1,3-dimethyl uracil (L2), 2-amino-chromen (L3), 2-amino-furan (L4) and isonicotinohydrazide (L5) in presence of ethanol.

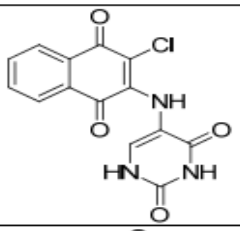
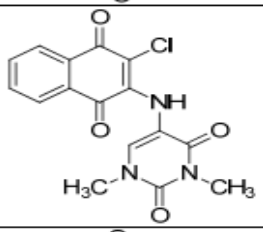
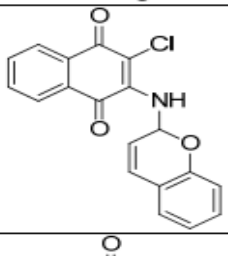
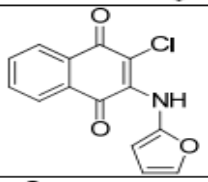
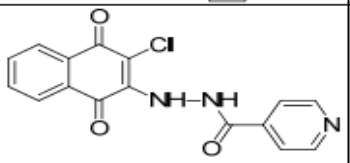
Characterization of synthesized compounds

The purity of synthesized compounds was confirmed by melting point and TLC using Ethyl Acetate: Petroleum Ether (50:50) as solvent system.

The structures of synthesized compounds were confirmed by FT-IR, 1H-NMR, 13C-NMR and Mass spectral analysis the result was correlated with the expected structure.

Preliminary QSAR study:

The physic chemical character of title compounds was evaluated and given in the table 1.

Com code	Structure	Molecular Formula	Mol weight	Melting point	Appearance
L1		$C_{14}H_8ClN_3O_4$	317.68	515 – 518	Dark Orange
L2		$C_{16}H_{12}ClN_3O_4$	345.74	525 – 527	Dark Orange
L3		$C_{19}H_{12}ClNO_3$	337.76	451 - 453	Orange solid
L4		$C_{14}H_8ClNO_3$	273.67	410 -412	Green colour
L5		$C_{16}H_{10}ClN_3O_3$	327.72	560 – 562	Puff colour Colour

Solubility parameters of synthesized compounds were tested, using various organic solvents and the result was tabulated in Table 1.

Table.2. Solubility data of synthesized compounds

Compound code	Water	Acetone	Chloroform	DMSO	Ethanol	Methanol	Ethyle Acetate
L1	-	+	++	+++	+	++	++
L2	-	+	++	+++	+	++	++
L3	-	+	++	+++	+	++	++
L4	-	+	++	+++	+	++	++
L5	-	+	++	+++	++	++	++

+++ = Freely soluble; ++ = Soluble; + = Slightly soluble; - = Insoluble

The synthesized compounds were subject to Preliminary QSAR studies by using MOLINSPIRATION software and the results are given in the Table 3. All the synthesized compounds obey Lipinski rule of 5.

Table 3. QSAR studies of synthesized compounds

Compound Code	Log P	TPSA	N atoms	n ON	N OHNH	N Violation	N rotb	Volume
L1	1.3	104.3	22	7	3	0	2	250.8
L2	1.82	86.79	24	7	1	0	2	283.97
L3	4.08	55.4	24	4	1	0	2	278.32
L4	3.34	55.4	19	4	1	0	2	217.5
L5	1.56	88.16	23	6	2	0	3	263.16

Docking study

The newly designed molecules are energy minimized and the resulting molecules are considered for docking analysis using Auto Dock 4.0.1. Auto Dock is employed to study the docking molecules within active site region of 3L4K and the H- bond interaction. Docked scores of newly designed molecules along with inhibition constant, Vdw. Desolvation Energy, ligand efficacy and electrostatic energy and hydrogen bonds are represented. Among the studied compounds, compound L3 have highest binding score (-10.66 K Cal/mol with one hydrogen bond) when compared with standard drugs such as Etaposide -9.56 K Cal/mol followed by compound L2 with score of -9.86 K Cal/mol with 2 hydrogen bond, compound L1 with score of -7.83 K Cal/mol with one hydrogen bond, compound L4 have score of –7.93 with one hydrogen bond and lastly compound L5 with score of -6.57 K Cal/mol. The docked molecule with Topoisomerase II was visualized in Chimara software and the docking pose of compounds showed in figure 1 – 6. Docking results show that all the designed molecules have similar orientations in the binding pocket of Topoisomerase II.

Table 4: Energy minimization table of Ligand and *Topoisomerase II* Interaction

Code	**Binding Energy**	**Inhibition Constant**	**Vdw. Desolvation Energy**	**Interm ol Energy**	**Ligand efficiency**	**Electrostat ic Energy**	**Total internal**
L1	-7.83	715.9	-8.92	-8.98	-0.38	-0.06	-0.34
L2	-9.86	9.36	-9.15	-9.16	-0.29	-0.01	-0.44
L3	-10.66	15.28	-11.2	-11.26	-0.44	-0.66	-0.89
L4	-7.98	17.73	-8.45	-8.56	-0.65	-0.98	-0.32
L5	-6.57	15.19	-7.75	-7.47	-0.29	0.1	-0.24
Standard	-9.56	24.87	-10.65	10.79	-0.24	-0.75	-0.64

Table 5: Hydrogen bond and hydrophobic interaction of ligand against target enzyme *Topoisomerase II*

Compound code	No. of Hydrogen bonds formed	Amino acid involvedin H bond interaction	H bonddistance	Amino acid involved in vander waals interaction
L1	1	Pro A: ASN828:O	2.8	Asn 828, Ser 755, Asn 756, Gln 703, Gly 747, Gln 773.
L2	2	1. Pro A: TYR734:O . pro A: His735:ND1	2.54 2.92	His 736, Tyr 734, Lys 603, Ala 733, Asp 809.
L3	2	1.proB: DC8:O5' 2.pro:D:DG8: O5'	2.80 2.78	Asn 828, Ser 755, Asn 756, Gln 703, Lys 603, Ala 733, Asp 809.
L4	1	Pro A: GLN732	2.90	His 736, Tyr 734, Lys 603, Ala 733, Asp 809
L5	Nil	Nil	NIL	Asn 828, Ser 755, Asn 756, Gln 703, Lys 603, Ala 733, Asp 809.
Standard (Etoposide)	Nil	Nil	Nil	His 736, Tyr 734, Lys 603, Ala 733, Asp 809.

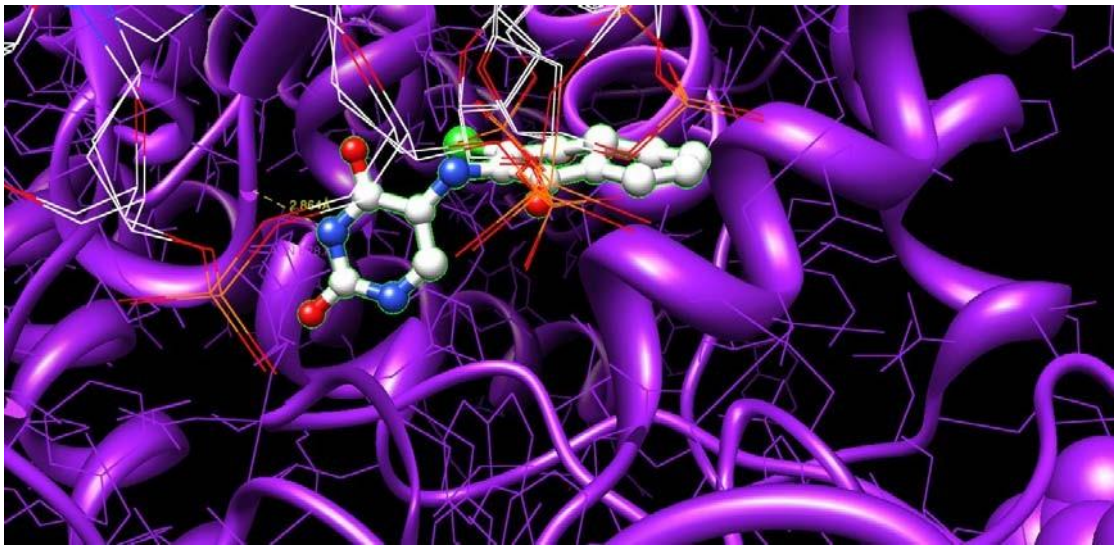


Figure 1: Docking pose of Compound L1 against Topoisomerase II as TargetEnzyme

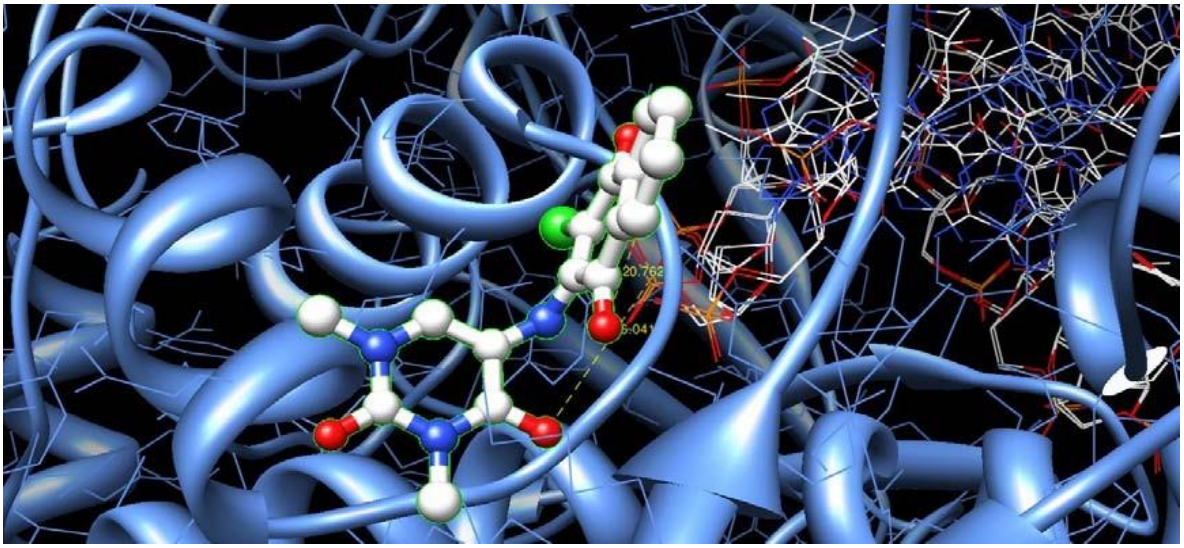


Figure 2: Docking pose of Compound L2 against Topoisomerase II as TargetEnzyme

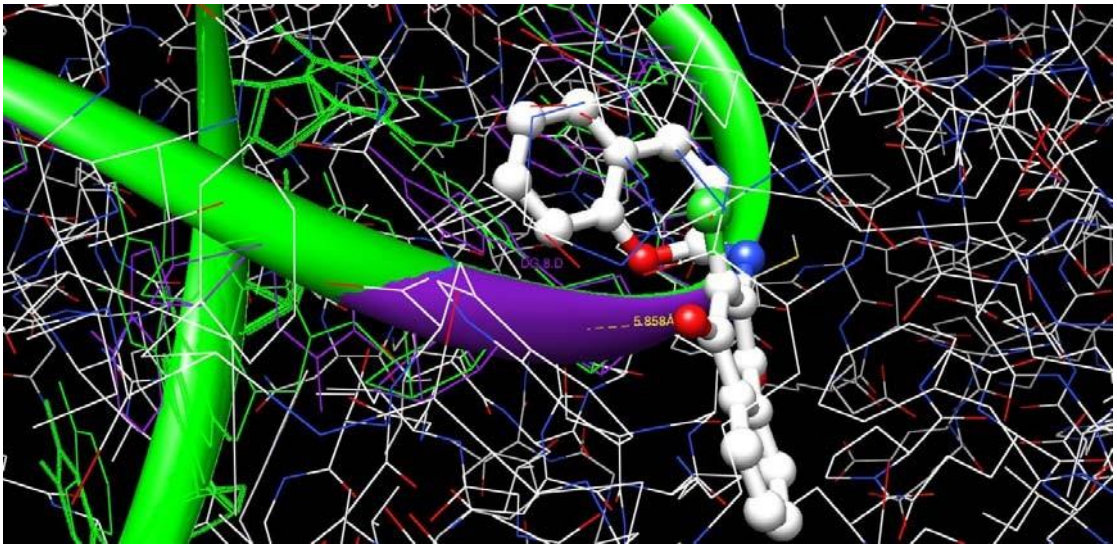


Figure 3: Docking pose of Compound L3 against Topoisomerase II as TargetEnzyme

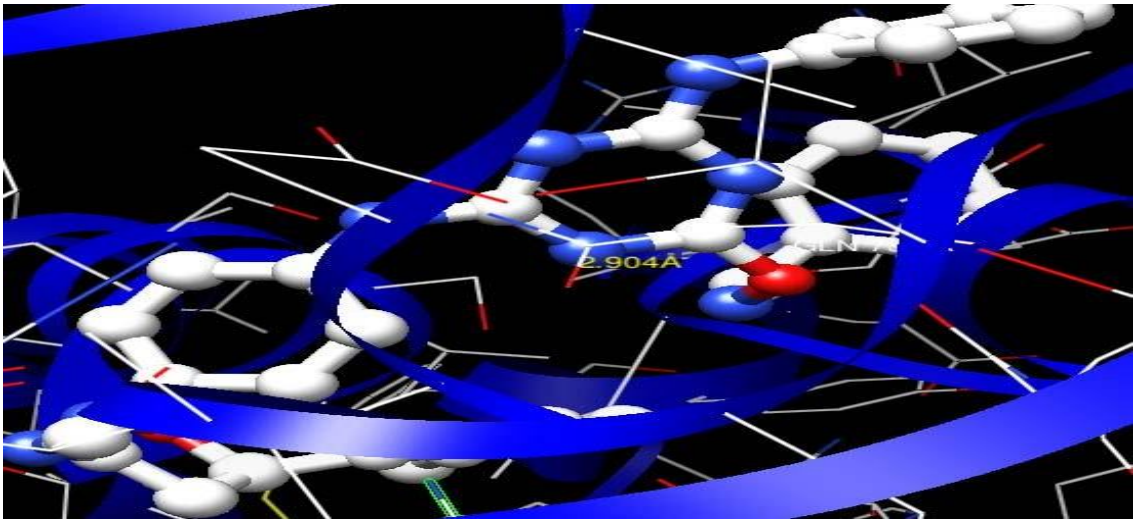


Figure 4: Docking pose of Compound L4 against Topoisomerase II as TargetEnzyme

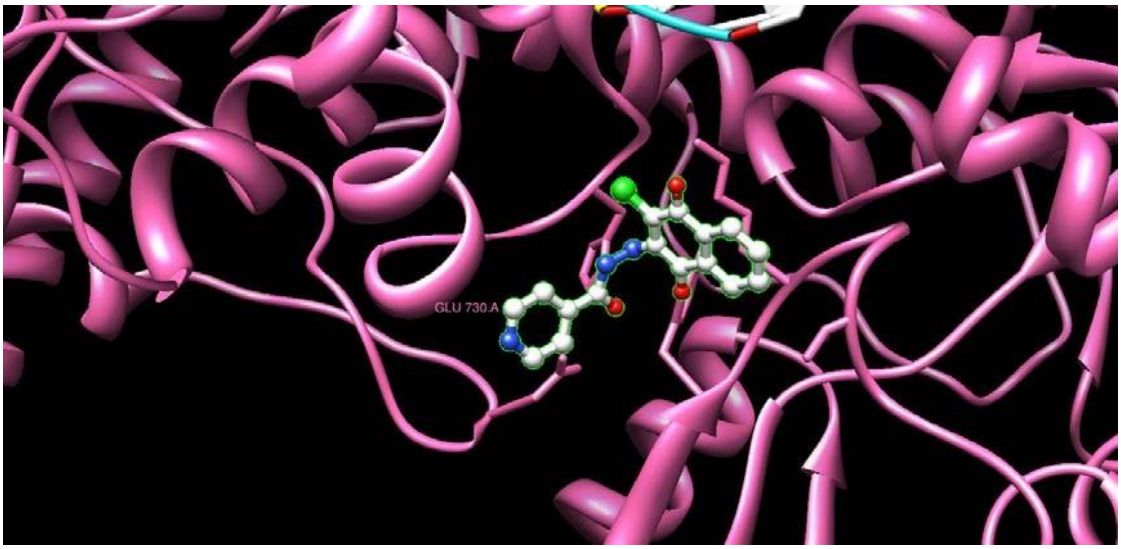


Figure 5: Docking pose of Compound L5 against Topoisomerase II as TargetEnzyme

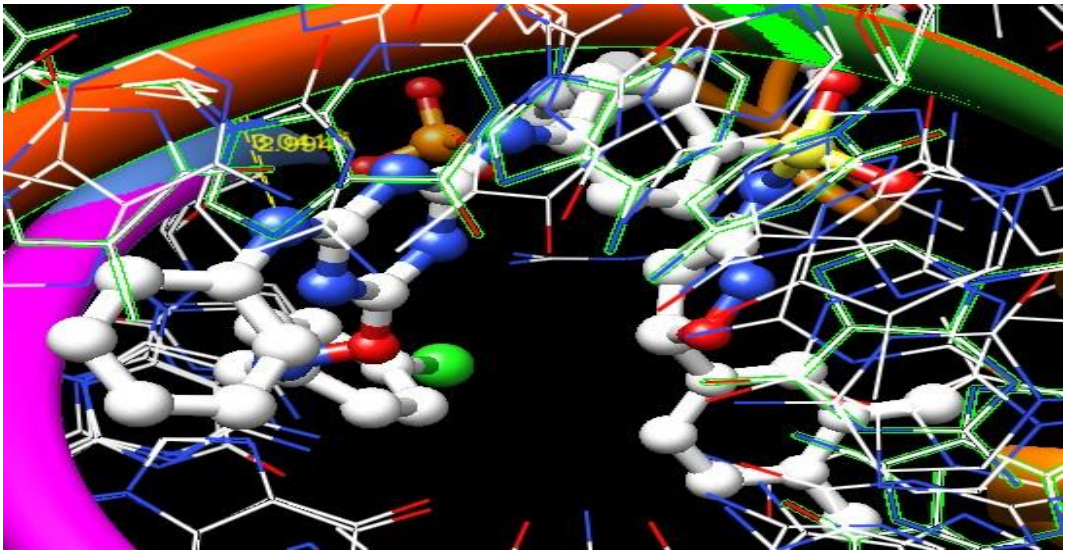


Figure 6: Docking pose of Standard (Etoposide) against Topoisomerase II as TargetEnzyme

DISCUSSION:

Quinones are ubiquitous in nature and are present in many drugs such as anthracyclines, daunorubicin, doxorubicin, mitomycin, mitoxantrones and saintopin, which are used clinically in the therapy of solid cancers. The quinone scaffold is presented in many currently used anticancer drugs. Particularly, substituted naphthoquinones are active quinone derivatives that are widely used as raw materials in pharmaceuticals and agrochemicals industries. A diverse array of bioactivities has been reported for substituted naphthoquinone derivatives to exert anticancer, antimicrobial, antifungal, antiviral, radical scavenging, antiplatelet and trypanocidal activities. Due to the prevalence, morbidity and mortality, cancer represent significant medical, social, financial burden on the society. Recently, great attention has been drawn on the research for new anticancer drugs in order to improve survival rates and wellbeing. The mechanism of action of naphthoquinones involves oxidants and electrophile properties influenced by their chemical structure. There are two important mechanisms of quinone cytotoxicity: stimulation of oxidative stress and alkylation of cellular nucleophiles, which encompass a large range of biomolecules. Reactive oxygen species (ROS) may react directly with DNA, lipids and proteins, leading to cell damage and shunting electrons toward oxygen, a futile pathway for reduction equivalents otherwise used for cytochrome P450 reductase-dependent reactions. Cellular damage can also occur through the alkylation of crucial proteins and nucleic acids. Besides the mechanism of ROS production, cytotoxic effect of clinically used quinone-based anticancer drugs are closely related to an inhibition of DNA topoisomerase II enzyme. The DNA topoisomerase II is an essential enzyme required for DNA replication, chromosome condensation and chromosome segregation. DNA unwinding process is a crucial step in which the coils of double stranded DNA need to be uncoiled thereby allowing further processes of DNA replication. In silico approaches play an important role in drug design and development. Quantitative structural activity relationship (QSAR) is a computational method for correlating chemical structures of compounds with their respective biological activities.Furthermore, QSAR studies could provide crucial pharmacokinetic information such as absorption, distribution, metabolism and excretion. which are important parameters in drug design and development. Here, a series of 2-amino-3-chloro substituted naphthoquinone derivatives (five) were synthesized using the nucleophilic displacement reaction of 2,3-dichloro-1,4- naphthoquinone by amines and subsequently investigated for their cytotoxic activity against for human breast cancer cell line MCF-7. The resulting experimental data were consequently used for QSAR analysis. In order to elucidate the effect of 2-substituted amino group on the 1,4-naphthoquinone scaffold, an additional set of 5 structurally modified compounds were virtually constructed. The structures of the synthesized compounds were studied using Autodock software against Topoisomerease II as target enzymes. The docking results showed that Compounds L3, L2, L4, and L1 were found to have significant binding score against target enzyme Topoisomerase compared to standard drug Etaposide.

CONCLUSION:

The present study deals with designing of some novel substituted 2 -amino 3- chloro naphthalene – 1,4 dione derivatives on the basis reaction between 2,3- dichloronaphthalene -1,4-dione and various secondary amine like 5-amino uracil (L1), 5-amino 1,3-dimethyl uracil (L2), 2-amino-chromen (L3), 2-amino- furan (L4) and isonicotinohydrazide (L5) in presence of ethanol. The synthesized compounds were characterized by IR, 1H NMR, 13C NMR, Mass spectrometry and elementary analysis. Preliminary QSAR study was carried out for the synthesized compounds. All the compounds obeyed the Lipnski rule of 5. Solubility characters of synthesized compounds were carried out by using various solvents. The tested compounds are freely soluble in DMSO, soluble in chloroform and methanol, slightly soluble in acetone, ethyl acetate and ethanol and insoluble in water. The structures of the synthesized compounds were studied using Autodock software against Topoisomerease II as a target enzymes. The docking results showed that Compounds L3, L2, L4, and L1 were found to have significant binding score against target enzyme Topoisomerase compared to standard drug Etaposide.

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