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Synthesis, Spectral Characterization and Molecular Docking Studies of Lawsone Derivatives as Protein Kinase Inhibitors

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Naphthaquinone moiety is present in various cancer drugs. We have synthesized two derivatives from lawsone using phenylenediamine and 4-amino phenol by ultra sound irradiation technique. The synthesized derivatives 10,12-dihydro-5-10-diazatetraphene-12-one and 2-(4-anilino)-1,4-naphthaquinone were characterized by elemental analysis and various spectral techniques like UV-visible, IR, NMR (^{1}H and ^{13}C) and gas chromatographic mass spectra. The study focus to predict the anticancer activity of the synthesized compounds by *in silico* molecular docking studies using Schrödinger software suit. The selected protein was protein kinase CK2 (PDB ID: 1M2R). Both the derivatives have better interaction with various amino acids present in active site of the protein than the parent compound lawsone. The new derivative 2-(4-anilino)-1,4-naphthaquinone exhibit lowest glide score of -2.8 kcal/mol. From the result, structural modification of the parent compound proved to be a lead compound for further drug design investigations.

Keywords: Lawsone, Ultra sound, Protein kinase.

INTRODUCTION

Quinone and naphthoquinone moieties are prevalent motifs in various natural products, which are associated with diverse biological activities. Natural and synthetic quinone derivatives have been widely investigated for cancer therapy. Many drugs containing a quinone moiety, such as mitomycin C and doxorubicin have received clinical approval for cancer treatment [1].

Quinones readily undergo addition or substitution reactions and are structurally transformed by interacting with lipids and enzymes [2,3]. The amino and substituted quinone derivatives have immense important in biology and pharmacology. Quinones are the building block for the synthesis of heterocyclic compounds. Heterocyclic ring systems have emerged as powerful scaffold for many biological evaluation and play an important role in design and discovery of new pharmacologically active molecule [4-7]. These compounds have importance in ATP site-directed kinase inhibitors [8].

The molecular docking has been employed to get information about interaction of compounds with protein. The selected target protein was protein kinase CK2. Protein kinase play key role in cell signaling, gene expression and metabolic regulation by the fact that making as high priority drug target, particularly in oncology [9]. In this present investigation structural modification of lawsone using two different amines by ultrasound irradiation. Ultrasound irradiation technique is good and efficient technique, which reduce the reaction time and solvent. New derivatives were characterized by various spectral data. *in silico* molecular docking studies were carried out with these compounds using Schrodinger software.

EXPERIMENTAL

2-Hydroxy-1,4-naphthaquinone (lawsone), *o*-phenylenediamine and 4-aminophenol were purchased from Sigma Aldrich. Silica gel for column chromatography (CC) (mesh 100-200) and silica gel G were purchased from Merck. Silica gel for thin layer chromatography was purchased. Methanol, ethanol and acetone which are commercially available, were purified by standard procedure [10]. Chloroform, benzene, ethyl acetate and methanol were used for column chromatography. Estimation of carbon, hydrogen and nitrogen in lawsone and its derivatives were carried out using an Elemental, various MICRO cube elemental analyzers. The electronic spectra of lawsone and its derivatives were recorded on Shimadzu UV-3600 UV-Vis-NIR spectrometer. The IR spectra of lawsone and its derivatives were recorded in KBr pellets in the range 4000-400 cm^{-1}. The spectra were recorded on Shimadzu IR Prestige-21 the ^{1}H and ^{13}C NMR spectra of lawsone and its derivatives were recorded using Bruker 500 MHz NMR

spectrometer using DMSO as solvents and TMS as the internal standard Gas Chromatographic Mass Spectra were recorded on GC–MS QP 2010 plus using electron impact method.

General procedure

Preparation of 10,12-dihydro-5,10-diazatetraphene-12-one (DHD): This compound was prepared by the addition of ethanolic solution of *o*-phenylenediamine (1.08 mmol) to an ethanolic solution of 2-hydroxy-1,4-naphthoquinone (1.74 mmol) followed by ultra sound irradiation for 0.5 h. The reaction mixture was kept overnight. The precipitate was subjected to column chromatography using the eluent benzene-ethanol (3:1 v/v) ratio and the purity was checked using TLC plates. Yellow coloured crystalline compound. m.p. 185 °C. The reaction is shown in **Scheme-I**. IR (KBr, ν_{max}, cm^{-1}): 3217, 3062, 1627, 1597, 763, ^1H NMR (DMSO, ppm) 3.5 (s, 1H) 6.1, (s, 1H), 7.2-8.2 (m), ^{13}C NMR (ppm) 198, 162, 120-140, 103.

Preparation of 2-(4-anilino)-1,4-naphthaquinone (ANQ): The derivative was prepared by same procedure using 4-aminophenol. Dark brown crystals were obtained with m.p. 220 °C. IR (KBr, ν_{max}, cm^{-1}) 3332, 3187, 1681, 1643, 1581 ^1H NMR (DMSO, ppm) 9.1 (s, 1H), 5.5 (s, 1H), 6.2 (s, 1H), 7.8-8.0 (m), ^{13}C NMR (ppm): ANQ, 182, 184, 160, 111, 125-135.

***in silico* molecular docking studies**

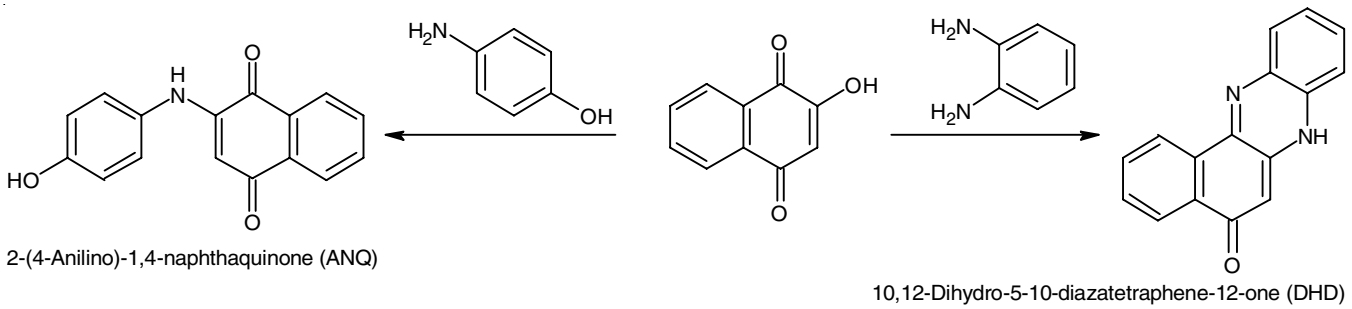
Preparation of protein and ligands: Atomic coordinates of protein kinase CK2 domains in complex with 5,8-diamino-1,4-dihydroxy-anthraquinone (PDB ID:1M2R), as obtained by X-ray crystallography with resolution 1.5 Å was downloaded from protein data bank. The structure of lawsone and its derivatives were imported into Maestro module available in the Schordinger pakage (10.2) and protein was optimized using the protein preparation wizard. The compounds were pre-processed by the LigPrep module of the Schordinger modelling software.

RESULTS AND DISCUSSION

Spectral characterization of derivatives: The derivatives 10,12-dihydro-5-10-diazatetraphene-12-one (DHD) and 2-(4-anilino)-1,4-naphthaquinone (ANQ) were synthesized in good yield by the reaction of lawsone with phenylenediamine and 4-amino phenol respectively under ultrasound irradiation. The structures of synthesized compounds were assigned based on their elemental analysis and spectral data. The elemental analysis data was in good agreement with the stoichiometry of derivative 10,12-dihydro-5-10-diazatetraphene-12-one experimental values were C (72.45), H (4.18) and N (5.28) and the theoretical values were C (72.25), H (4.22) and N (5.31) and another derivative 2-(4-anilino)-1,4-naphthaquinone have the experimental values were C (10.37) N (11.86), H (22.32) and the theoretical values were C (10.36), N (11.86), H (22.36). The electronic spectra of lawsone in methanol gave adsorption peaks observed at 248 and 275 nm corresponding to π-π* transition and another peak at 334 nm corresponding to n-π* transition. The UV visible spectra of the derivative DHD in methanol gave peak at 235 nm due to π-π*transition and n-π* transition observed at 285 nm [11,12]. In both derivatives peaks observed above 430 and 435 nm is due to extended conjugation, which indicates the formation of derivatives.

Lawsone possessing three electrophilic centers has proven to be an important building block for the construction of various heterocyclic frameworks; lawsone is converted into the corresponding benzo[a]phenaxazone [13]. In the IR spectrum, the parent compound lawsone contributed a broad spectrum at 3526 cm^{-1} shows the presence of hydrogen bonded hydroxyl group. This band was absent in the spectrum of derivative DHD, indicating that substitution has taken place at the hydroxyl group of lawsone. In lawsone, the 1,4-naphthoquinone carbonyl absorption bands observed at 1676 cm^{-1} (C=O hydrogen bonded) and at 1642 cm^{-1} (C=O free). Where as in derivative DHD one carbonyl peak was present at 1627 cm^{-1} is due to the hydrogen bonded carbonyl frequency. This gives evidence that one of the carbonyl group and OH group participated as reactive sites in derivative formation [13]. The NH asymmetry stretching frequency observed at 3217 cm^{-1}. A new band observed at 1597 cm^{-1} is due to C-N bond formation. In the derivative ANQ, 1,4-naphthaquinone carbonyl absorption bands remained as lawsone and bands observed at 1643 cm^{-1} (C=O hydrogen bonded) and at 1681 cm^{-1} (C=O free) as well as the hydroxyl group of parent compound was absent in derivative. These data gave the clear evidence that substitution has taken part in hydroxyl group of lawsone. Another peak observed at 3178 cm^{-1} due to the NH stretching frequency.

In ^1H NMR spectrum of derivative DHD, the singlet peak observed at 3.2 ppm (1H) is due to NH proton, another singlet observed at 7.3 ppm indicates the C-3 proton. A broad singlet observed at 7.2 ppm may be indicating the keto-enol tautomerism of the compound [14]. Aromatic protons observed at 7.8 to 8.2 is due to benzinoid and naphthoquinone protons respectively. In derivative, ANQ the singlet peak observed at 5.5 ppm (1H) is due to NH proton, another singlet observed at 7.3 ppm indicates the C-3 proton. A broad singlet peak observed at 9.1 ppm is due to the phenolic proton.



2-(4-Anilino)-1,4-naphthaquinone (ANQ)

10,12-Dihydro-5-10-diazatetraphene-12-one (DHD)

Scheme-I

^{13}C NMR spectrum of DHD gives presence of carbon atoms in the compounds. Aromatic carbons are observed at 120-140 ppm in the spectrum. In lawsone two carbonyl peaks are observed at 184 and 180 ppm. In the derivative DHD only one carbonyl peak observed at 198 ppm gives an evidence of one of the carbonyl group take part in substitution reaction. The peak at 103 ppm is due to carbon atom is in the β-position of electronegative nitrogen atom. ^{13}C NMR spectra of ANQ give aromatic carbons observed at 110-185 ppm. In the derivative, ANQ gives presence of two carbonyl peaks at 184 ppm corresponding to free carbonyl peak and 181 ppm is due to the hydrogen bonded carbonyl group. The peak at 111 ppm is due to carbon atom is in the β position of electronegative nitrogen atom

GC-MS spectrum of DHD gives only one peak is indicating presence one compound. Molecular peak may be unstable it can confirm from conformation stability calculations. The compound gives molecular ion peak at 246 *m/z*. The fragmentation as follows from the expected molecular ion elimination of carbonyl group gives peak at *m/z* at 219 corresponding to the molecular mass $C_{15}H_{13}N_2$ and gives a peak at 141 *m/z* corresponding to molecular mass C_9H_5O. The peak at 108 *m/z* indicates the presence of phenylenediamine group. GC-MS spectrum of ANQ the molecular ion peak was obtained at 264 *m/z* and the fragmentation as follows from the expected molecular ion elimination of carbonyl group gives peak at 239 *m/z* at corresponding to the molecular mass $C_{15}H_{13}NO_2$ another fragmentation peak at 141 *m/z* indicates elimination of carbonyl group and it corresponding to molecular mass C_9H_5O. The structure of the compounds were assigned as Figs. 1 and 2.

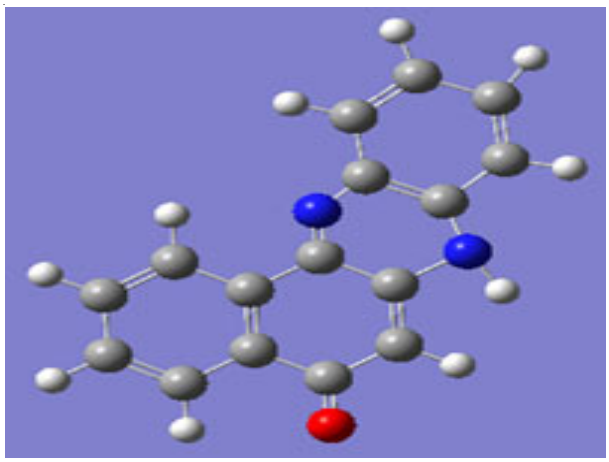


Fig. 1. 10,12-Dihydro-5-10-diazatetraphene-12-one (DHD)

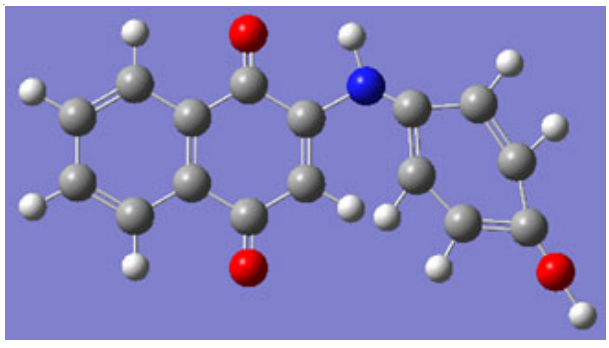


Fig. 2. 2-(4-Anilino)-1,4-naphthaquinone (ANQ)

Molecular docking studies: Rational drug design helps to speed up the drug designing process, which involves a wide variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target). The selected protein kinase (PDB ID: 1M2R) three dimensional structure was retrieved from protein data bank. Protein kinase plays a central role in controlling nearly all cellular functions, with special reference to signal transduction, gene expression and DNA synthesis and repair. They make up one of the largest families of enzymes, with more than 500 members encoded by the human genome and often unscheduled and/or constitutive activation of individual protein kinase underlies pathologies [15]. Numerous reports describing the discovery of new pharmaceutical substances targeting protein kinase have been publishing during last few years. Consequently, the development of efficient and selective inhibitors of protein kinase represents a powerful tool for unravelling the functional implication of individual kinase [16,17]. The active site residues are present in Protein Kinase was THR320, PRO322, HIE321, LYS303, ARG306, GLU311, GLU317, LEU313, ARG312, ARG10 and ASP299.

The protein was pre-processed before docking and a grid was generated for the active sites of protein to which ligand are to be bound. Docking was performed in extra precision and binding of ligand to the protein were listed out in the XP visualize. The interaction of ligand with protein was shown in various interactions like van der Waals interaction, hydrogen bonding and electrostatic interaction. The result was tabulated in Table-1. The compound ANQ has the lowest docked binding energy of -2.8 kcal/mol and forms various interaction with active site residue THR320, PRO322, HIE321, LYS303, ARG306, GLU311, GLU317, LEU313, ARG312 and ARG10, shown in Fig. 3. 10,12-Dihydro-5-10-diazatetraphene-12-one has the lowest docked binding energy -1.93 kcal/mol and interacted with active site THR320, GLU317, HIE321, LEU313 and LYS303 shown in Fig. 4. The compound DHD also exhibit

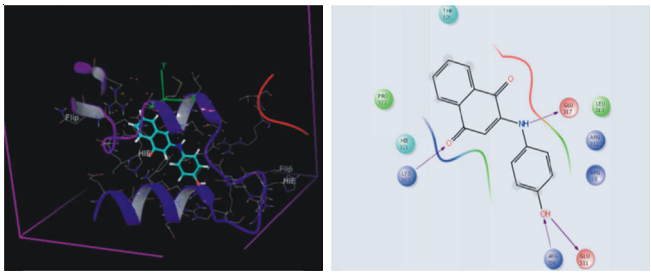


Fig. 3. Interaction between ANQ and amino acids

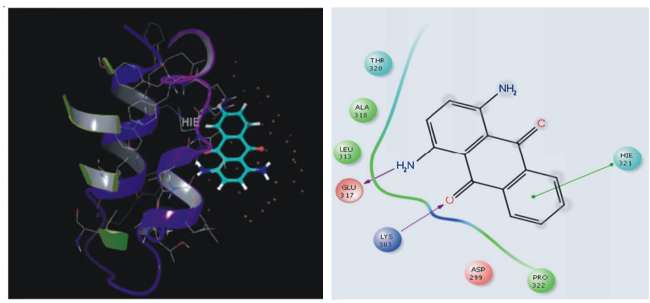


Fig. 4. Interaction between DHD and amino acids

TABLE-1
INTERACTION OF LIGANDS AND AMINO ACIDS

Ligand	Glide score	Dock score	van der Waals interaction	Hydrogen bond	Electrostatic interaction	Amino acid interacting to ligand
Lawsone	-2.07	-2.07	-1.02	-0.35	-0.35	THR320, PRO322, HIE321, LYS303, LEU313, GLU317
DHD	-1.93	-1.93	-0.99	-0.7	-0.7	THR320, GLU317, HIE321, LEU313LYS303
ANQ	-2.8	-2.8	-0.86	-1.33	-1.33	THR320, PRO322, HIE321, LYS303, ARG306, GLU311, GLU317, LEU313, ARG312, ARG10,
STD	-1.6	-1.6	-0.77	-0.95	-0.22	THR320, ALA318, LEU313, GLU317, LYS303, ASP299, PRO322, HIE321

compare score as the parent compound lawsone as shown in Fig. 5. Lawsone and its derivatives have better score than standard compound shown in Fig. 6. Interactions of ligand and amino acid in the active site of CK2 was visualizes by XP visualizer.

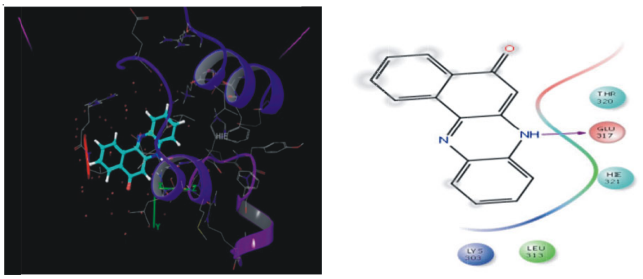


Fig. 5. Interaction between DHD and amino acids

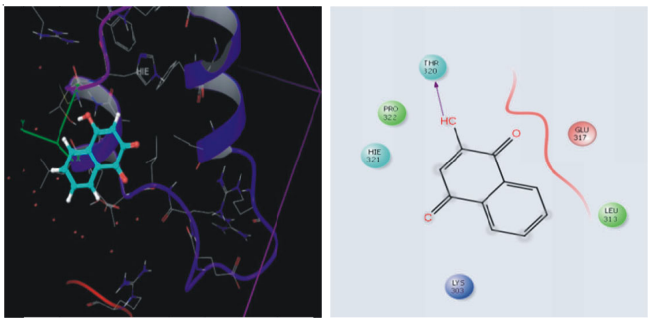


Fig. 6. Interaction between lawsone and amino acids

Conclusion

In the present work, we have synthesized two new derivatives of lawsone using ultrasound technique. The new compounds were characterized by UV-visible, IR, NMR (^{13}C, ^{1}H) and gas chromatography-mass spectrum spectral techniques. From these spectral data structure of the compounds were assigned. One of the new derivatives belongs to phenaxozone family; it has many pharmacological activities, especially, high anticancer activity. For predicting anticancer activity, we adopted molecular docking using Maestro Schrodinger Suite (10.2). For that selected protein was protein kinase (CK_2), the inhibition of protein kinase was important in drug designing. The protein structure was retrieved from Protein Data Bank (PDB ID: $1M_2R$). From the result compound ANQ exhibit more activity than lawsone and derivative DHD. Also have activity comparable to parent compound lawsone. So the structure modification is good tool to develop new drugs. The new derivatives were lead compound for new drug design.

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