Molecular docking analysis of selected pyrimidine derivatives with human cyclin-dependent kinase 2

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Abstract: A series of pyrimidine were synthesized, characterized and evaluated for their antioxidant properties using the human cyclin-dependent kinase-2 protein model. Data shows that the pyrimidine derivatives (compound ID 4G) with para fluoro groups substitution at phenyl ring attached to the 4th position (IC50 98.5µg/ml), compound 4B bearing hydroxy group at para position of phenyl ring (IC50 117.8µg/ml) have significant antioxidant activity. Docking data infer that compounds 4c, 4a, 4h and 4b possess binding energy (-7.9, -7.7, -7.5 and -7.4 kcal.mol-1) with 1HCK (PDB ID) receptor.

Keywords: Chalcone, pyrimidine, spectral characterization, antioxidant activity, DPPH

Background: The development of degenerative diseases like atherosclerosis, ischemic heart disorders, ageing, diabetes mellitus, cancer, and many more is largely attributed to oxidative stress [1]. Oxidative processes lead to the development of free radicals such as superoxid (O2 -), hydroxyl-radical (OH -), and non-free radical (ROS) species such as H2O2 and singlet-oxygen [2]. The oxidation process leads to a reactive oxygen species formation. These reactive species of oxygen cause lipid peroxidation, protein peroxidation, and damage to DNA, and cell degeneration [3]. Throughout the pathology of several diseases, including brain disorders, aggregating platelets, inflammatory diseases, and a variety of other disorders, free radical reactions are therefore involved [4]. Antioxidants can curb free radical chain reactions [5]. In the field of drug design and discovery, therefore, sufficient attention is required when developing effective antioxidants. Phenolic compounds are widespread in plants, protect against the light of UV, insects, bacteria and viruses while preventing competitive plants from developing [6]. Their ability to eliminate hydroxyl radicals, and to help prevent diseases linked to oxidative stress in membranes, proteins and DNA, have traditionally been considered powerful natural antioxidants [7]. Chalcones abound from ferns to higher plants in nature [8]. Aromatic compounds with an unsaturated side chain are frequently in vitro cytotoxic [9]. It was also reported that Chalcones were anti-inflammatory, analgesic and anti pyretia3. Some Chalcones have bactericidal and antifungal activity and some of their derivatives have anti mutagenic activity [10]. Chalcones are 1,3- diphenol-2, propene-1-one, in which a three-carbon α,β- in saturated carbonyl system is connected to two aromatic rings [11]. These are abundant and considered the precursors of flavonoids and iso flavonoids in edible plants. Chalcones have been extensively studied and developed as one of the pharmaceutically significant molecules, both synthetic and natural. Chalcone derivatives have been screened for anti-inflammatory activity [12], chemo phytosis [13], cardiovascular disease [14], anti-cancer activity [15], and cytotoxic activity [16] and anti proliferative activity [17].
Heterocyclic compounds are a class of cyclic organic compounds that are heteroatomically compatible with the carbon framework, such as nitrogen, sulphur, oxygen, etc. Heterocyclic compounds are used in the treatment of a variety of diseases, and possess various pharmacological activities [18]. The heterocyclic ring as the principal structural component for most therapeutic agents used in the current therapy. The heterocyclic rings containing nitrogen are characterised by their easy synthesis, but also by their wide distribution and biological profiles [19]. A literature survey has shown a broad range of bioactivities such as antimicrobial, antioxidant, anticancer, antimalarial, antidepressant, antihistaminic, antimicrobial and anti-inflammatory, and nitrogen-based heterocycles, i.e. isoxazole and dihydropyrazole [20]. Pyrimidine is a six-member heterocyclic compound that contains two nitrogen atoms at positions 1 and 3. Pyrimidine derivatives have showed various biological activities such as antimicrobial, antitumor, antifungal, and antileishmanial activities and are also useful for the treatment of thyroid and leukemia [21]. Therefore, it is of interest to document the Molecular docking analysis data of selected pyrimidine derivatives with human cyclin-dependent kinase 2 (PDB ID: 1HCK) for further consideration in this context.

Materials and Methods:
Elemental analysis was carried out using PERKIN ELMER 240 CHN analyzer. All the series of the synthesized product group were characterized by infrared spectroscopy, which are recorded on SHIMADZU IR spectrometers. The test sample mixed with KBr pellet technique was adopted to record the spectra. 1H and 13C NMR spectrum were recorded on BRUKER AVANCE III AMX-400 spectrometer for the synthesized compound. The frequency of 400 MHz for the proton NMR spectrum and 100 MHz frequencies for 13C NMR spectrum, CDCl3 and DMSO-D6 used as a solvent.

Antioxidant activity of synthesized derivatives using DPPH assay:
Hydrogen donating or free radical scavenging capacity of the prepared compounds characterized the in vitro antioxidant potential by 1,1-biphenyl-2-picrylhydrazyl radical (DPPH) method [22]. The antioxidant potential of test sample was measured by estimating the reduction in the absorbance of methanolic solution of DPPH (53 mg in 1L) was prepared using methanol and 5ml of this stock solution was added to 1 ml of test at various concentrations (500, 250, 125, 62.5, 31.5µg/ml). After 60 min, absorbance was measured at 517nm at different conc. (500, 250, 125, 62.5, 31.5µg/ml) in 62.5, 31.5µg/ml). After 60 min, absorbance was measured at 517nm at different conc. (500, 250, 125, 62.5, 31.5µg/ml).

Results and Discussion:
Spectral analysis of compound 3a-h:
The strong absorption stretching frequency band appeared at 1629 - 1699 cm\(^{-1}\) is due to C=O of chalcone moiety and a stretching frequency band strongly appeared at 1551 - 1600 cm\(^{-1}\) is due to CH=CH of the chalcone. The stretching frequency band present in the region of 3408 - 3479 cm\(^{-1}\) is due to N-H bond. Stretching frequency range at 3066 cm\(^{-1}\) is reveals that the presence of aromatic C-H group. The strong singlet appeared at 4.51 – 4.89 ppm is due to ring NH\(_2\) proton. The H-\(\alpha\) and H-\(\beta\) protons of chalcones occur as two doublets in the ranges 6.96 - 7.41 ppm (H-\(\alpha\)) and 7.61 - 7.91 ppm (H-\(\beta\)) in the \(^1\)H NMR spectra. The other aromatic protons usually appear in between \(\delta\) 6.97 - 9.09, depending on the type of aromatic/ heteroaromatic ring and also based on the electronic effects of the substituents present on these rings. The carbonyl carbon of the chalcones usually appears \(\delta\) 181.9 - 195.0 in its \(^13\)C NMR spectrum. The \(\alpha\)- and \(\beta\)- carbon atoms with respect to the carbonyl group give rise to characteristic signals in between \(\delta\) 121.6 - 126.4 and \(\delta\) 135.1 - 148.0 respectively. Phenyl ring aromatic carbon signals are appeared from 108 to 146.9 ppm.

**Spectral studies of compound 4a-h:**

A strong stretching absorption band appeared at 1620 cm\(^{-1}\) is due to C=N of pyrimidine ring. Stretching frequency band present in the region of 3062 cm\(^{-1}\) is due to aromatic C-H protons. A strong stretching absorption band appeared at 3441 cm\(^{-1}\) is due to NH2 of pyrimidine ring. The pyrimidine ring NH2 proton singlet peak appeared at 6.26 ppm. The aromatic protons appeared from 6.37 - 7.93 ppm. The NH2 protons of the phenyl ring appeared 5.32 ppm as a singlet. The pyrimidine ring C=N (C (3)), carbon is appeared at 158.9 ppm. Aromatic phenyl ring carbons chemical shift value from 105.1 - 144.3 ppm. The pyrimidine ring C-NH2 (C (1)), carbon is appeared at 162.4 ppm.

**Figure 2:** 2D & 3D representation of interactions between protein receptor and synthesized derivatives (4a-c)

**Figure 3:** 2D & 3D representation of interactions between protein receptor and synthesized derivatives (4d-f)

**Figure 4:** 2D & 3D representation of interactions between protein receptor and synthesized derivatives (4g-h & ascorbic acid)
Antioxidant activity of chalcone derivatives (4a-h):
Antioxidants are the compounds that protect the cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite [25]. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson’s and Alzheimer’s) [26]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scaveng free radicals such as peroxide, hydroperoxide or lipid peroxidation and thus inhibit the oxidative mechanisms that lead to degenerative diseases [27]. ROS (reactive oxygen species) is capable of generating free radicals. Mostly it includes hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻) and hydroxyl radical (OH). Chalcone groups play a vital role in trapping the free radicals [28]. Table 3 shows the percentage of DPPH radical scavenging activity of pyrimidine derivatives 4a-h.

The synthetic pyrimidine derivatives 4a-h was tested for their free radical scavenging potential. All compounds showed various degrees of radical scavenging activity in DPPH radical scavenging assay, and their IC50 values ranged between 117.8 to 259.2µg/ml. Compound 4g shows better IC50 value (98.5µg/ml) compared with reference drug ascorbic acid. Derivatives 4b and 4c with IC50 values of 117.8 and 132.4 µg/ml, respectively, showed free radical inhibitory activity that is many folds better than the standard ascorbic acid with IC50 value of 67.5µg/ml, as depicted in Figures 1 and Table 1.

Compounds 4b and 4c showed good to moderate activities (Figures 1 & Table 1). The remaining derivatives, including 4a, 4e and 4h showed weak inhibitory activities (Figures 1 & Table 1). A structure-activity relationship established for all compounds that confirmed substitution of various functionalities at the aromatic ring confers free radical scavenging activity to each particular pyrimidine analogue. Analogue 4g, a 4-flurophenyl analogue was found to be the most active pyrimidine among the series, which with an IC50 value of 98.5µg/ml, corresponding to 72.18% radical scavenging activity of the standard drug (Table 1). The high activity shown by analogue 4g is due to the position of fluoro groups present an aromatic moiety. Thus, the antioxidant activities could be caused by hydrogen atom transfer and/or electron transfer followed by a proton transfer mechanism in a substituent dependent manner.

Docking study of chalcone derivatives with 1HCK (PDB ID):
The results of docking studies infer that compounds 4c, 4a, 4b and 4b possess binding energy (-7.9, -7.7, -7.5 and -7.4 kcal.mol⁻¹) with 1HCK receptor. Compound 4c form a hydrogen bond with THR 165, GLU 12, LYS 33 and THR 14 of 3LN1 through F ---- H (THR 165), NH2 ---- O (GLU 12), Pyrimidine N ---- H(LYS 33),Pyrimidine N ---- H(THR 14),Pyrimidine NH2----O(THR 14).

The protein residues of VAL 63, LYS 129, VAL 18 and ILE 10 form alkyl - pi interactions and several VdW and polar / electrostatic interactions. The compound 4a formed a hydrogen bond with LYS 33, THR 14, TRH 165 and GLU 12 through Pyrimidine N ---- H (LYS 33),NH2 ---- O (THR 14), Pyrimidine N ---- O(THR 14), CN ---- H (THR 165),NH2 ---- O (GLU 12).

The protein residues of ILE 10, VAL 18, GLA 131 and LYS 129 form alkyl - pi interactions and several VdW and polar / electrostatic interactions were also observed with compound 4a. In addition, compound 4h have a binding energy -7.5 kcal.mol⁻¹. The compound 4h formed a hydrogen bond with THR 14 and ILE 10 through Pyrimidine NH2 ---- O (ILE 10), Br ---- H(THR 14).

The residues PHE 80, ASP 145, ALA 144, ALA 31, LEU 134 and VAL 18 have common VdW and polar / electrostatic interactions in compound 4h. The compound 4b formed a hydrogen bond with GLU 12 and THR 14 through NH2 ---- O (GLU 12), pyrimidine NH2 ---- O(THR 14). The protein residues of LYS 129, GLY 11, ILE 10 and VAL 18 form alkyl - pi interactions and several VdW and polar / electrostatic interactions were also observed with compound 4b.

Docking confirmation of other compounds like 4b, 4d, 4e and 4f is shown in Figures 2, 3 & 4. We can understand H-bonding donor and acceptor capacity from Table 2.A seen from the results, we concluded that our designed compounds 4c, 4a, 4b and 4b are more active than reference ligand ascorbic acid (-7.9 kcal.mol⁻1).

Table 1: Free radical scavenging assay in percentage for the pyrimidine derivatives 4a-h by DPPH radical scavenging methods

| Concentration | 4a | 4b | 4c | 4d | 4e | 4f | 4g | 4h | Ascorbic acid |
|---------------|----|----|----|----|----|----|----|----|---------------|
| 500           | 68.49 | 79.6 | 84.11 | 70.34 | 66.85 | 75.12 | 72.18 | 69.56 | 89.36 |
| 250           | 52.51 | 61.5 | 65.06 | 58.22 | 54.21 | 63.26 | 62.2 | 49.2 | 74.52 |
| 125           | 40.87 | 50.2 | 48.36 | 46.69 | 40.78 | 52.84 | 53.37 | 52.43 | 57.14 |
| 62.5          | 29.44 | 34.7 | 31.59 | 32.24 | 31.47 | 34.25 | 42.54 | 22.5 | 55.41 |
| 3.15          | 13.62 | 21.9 | 14.96 | 17.39 | 16.12 | 28.32 | 28.14 | 14.25 | 29.57 |
| IC50 Value    | 217.4 | 117.8 | 132.4 | 167.9 | 197.7 | 114.8 | 98.5 | 259.2 | 67.5 |

Table 2: Amino acid residue interaction for compounds 4a-h with 1HCK

| T. No Compounds | Binding score (kcal/mole) | Hydrogen bond Interactions | Interacting atoms | Other Interactions | No of Hydrogen bond Interactions |
|-----------------|---------------------------|----------------------------|-------------------|-------------------|---------------------------------|
| 1 4a            | -7.7                      | LYS 33, THR 14, THR 165, GLU 12 | Pyrimidine N ---- H (LYS 33),NH2 ---- O (THR 14), Pyrimidine N ---- O(THR 14), CN ---- H (THR 165),NH2 ---- O (GLU 12) | ILE 10, VAL 18, GLA 131, LYS 129 | 4 |
| 2 4b            | -7.4                      | GLU 12, THR 14, THR 165, GLU 12 | NH2 ---- O (GLU 12), pyrimidine NH2----O(THR 14) | VAL 63, LYS 129, VAL 18, ILE 10 | 4 |
| 3 4c            | -7.9                      | GLU 12, THR 14, THR 165, GLU 12 | NH2 ---- O (GLU 12), pyrimidine NH2----O(THR 14) | GLN 131, VAL 18, ALA 144, LEU 134, ALA 31 | 2 |
| 4 4d            | -8.4                      | THR 14, ILE 10 | Br ---- H(THR 14), Pyrimidine NH2----O(ILE 10) | GLU 81, ALA 31, VAL 64, LEU 134, VAL 18, ILE 10 | 2 |
| 5 4e            | -6.5                      | LEU 83, ASP 86 | NH2 ---- O (ASP 86), Pyrimidine NH2----O(LEU 83), | GLN 131, ALA 144, ILE 10, LEU 134, LYS 89 | 4 |
| 6 4f            | -6.1                      | ASP 86 | NH2 ---- O (ASP 86) | LYS 89, HIS 84, LEU 134, ILE 10, LYS 33, VAL 18, GLN 131 | 2 |
| 7 4g            | -6.9                      | ASP 86, LEU 83 | NH2 ---- O (ASP 86), Br ---- H(LEU 83), | PHE 80, ASP 145, ALA 144, ALA 31, LEU 134, VAL 18 | 2 |
| 8 4h            | -7.5                      | THR 14, ILE 10 | Pyrimidine NH2----O (ILE 10), Br ---- H(THR 14), | LYS 89, HIS 84, LEU 134, ILE 10, LYS 33, VAL 18, GLN 131 | 2 |
Conclusion: 4-(2-amino-3, 5- dibromophenyl)-6-(4-substitutedphenyl) pyrimdin-2-amine (4a-h) have been synthesized by treating guanidine hydrochloride with various substituted chalcones (E)-3-(2-amino-3,5-dibromophenyl)-1-(4-substitutedphenyl) prop-2-en-1-one (3a-h) in ethanol solvent. Biological activities of the synthesized compounds were evaluated by antioxidant activity. The methyl and fluoro substituted compounds 4f and 4g show better activity in antioxidant activity. All the ligand inhibition activity was measured by molecular docking studies. On the basis of molecular docking studies compounds 4c (chloro) and 4a (cyano) have maximum binding score in the case of 1HCK receptor. Finally, Compounds having electron withdrawing (cyano) have maximum binding score in the case of 1HCK receptor. 

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