



In-silico Molecular Docking & ADME/Pharmacokinetic Prediction Studies of Some New Chalcone & Pyrazole Derivatives from N-acetyl-para-aminophenol: Promising as Anticancer Agents

Jessica Shlimoon Hanna^{1*}

Abstract

New N-acetyl-para-aminophenol (APAP) based analogs were designed, and primarily screened (*In silico*) for predicting their theoretical binding affinities with tyrosine kinase and poly(ADP-ribose) polymerase-1 (PARP-1) enzymes which mainly used as a targets for cancer treatment. The new analogs were designed by including pyrazole moiety into APAP to get the final compounds [p1-p7]. The intermediate chalcone derivative was compound [c]. The computational approaches comprising ADME evaluations were done by means of the SwissADME website to expect the pharmacokinetic properties of the new compounds. The outcomes presented that all compounds passively and extremely absorbed from the GIT. Moreover, all theoretically designed compounds fulfilled the Lipinski rule of five (RO5). GOLD suite software (v. 2021.3.0) were also used to test the compounds selectivity to EGFR and PARP1 proteins. The ligand's interaction with EGFR protein showed promising activity for compounds (p1, p6, p7) by binding with amino acid (AAs) in the active pocket with more PLP fitness values than the reference erlotinib. Meanwhile, for the PARP1 enzyme, the compounds (p1, p3, and p6) showed PLP fitness values more than the reference. Compounds (p1 & p6) give favorable results for both proteins [EGFR, and PARP1]. The current study's findings support the compounds' relevance as prospective candidates intended for the treatment of cancer, which may aid pharmaceutical professionals to design and synthesis of stronger compounds in the future. Furthermore, the study encouraged *in vivo* and *in vitro* assessment studies for the proposed developed compounds in order to validate the computational findings.

1104

Key Words: Molecular Modelling, Binding Affinity, Pharmacokinetics, Chalcone, Pyrazole.

DOI Number: 10.14704/nq.2022.20.8.NQ44121

NeuroQuantology 2022; 20(7):1104-1113

Introduction

Cancer is defined by abnormal cell cycle progression and fast proliferation of normal cells. Cancer has been designated as the world's second leading cause of death, behind only cardiovascular disease. (1,2). Cancers develop due to a combination of hereditary and environmental causes (3). In recent years, a new era of cancer treatment has emerged, focusing on the transition from cytotoxic to targeted molecular medicines(4). Members of the epidermal growth factor receptor

(EGFR) family have been identified as some of the most significant cancer molecular targets known to date (5,6). Overexpression of EGFR proteins has been observed in a variety of human malignancies, including those of the breast, ovary, and kidney but more frequently in lung cancers (7). The poly (ADP-ribose) polymerase-1 (PARP-1) enzyme is another protein that has been exploited as a target in cancer treatment. (8).

Corresponding author: Jessica Shlimoon Hanna

Address: ^{1*}Department of Pharmacy, AL-Rasheed University College, Baghdad, Iraq.

E-mail: ^{1*}Jessicahanna@alrasheedcol.edu.iq



Interestingly, overexpression of this protein has been seen in a diversity of cancer cells, including melanomas, and glioblastoma breast and ovarian cancer cells. However, PARP1 high levels were associated with triple-negative breast cancer (TNBC) (7,9).

Due to increasing the incidence of various types of cancer, studies on the anti-cancer properties of pyrazole and chalcone derivatives appear to be of particular interest (10).

Computational chemistry concepts for instance computer-aided drug design (CADD) may minimize the time required to find novel compounds while simultaneously lowering the cost of synthesis (11,12). Molecular docking is a CADD technique that expects a molecule's binding affinity in addition to the optimal binding pose with the active pocket of a target (receptor), which is critical for performing structure-based drug design (SBDD) (10,13).

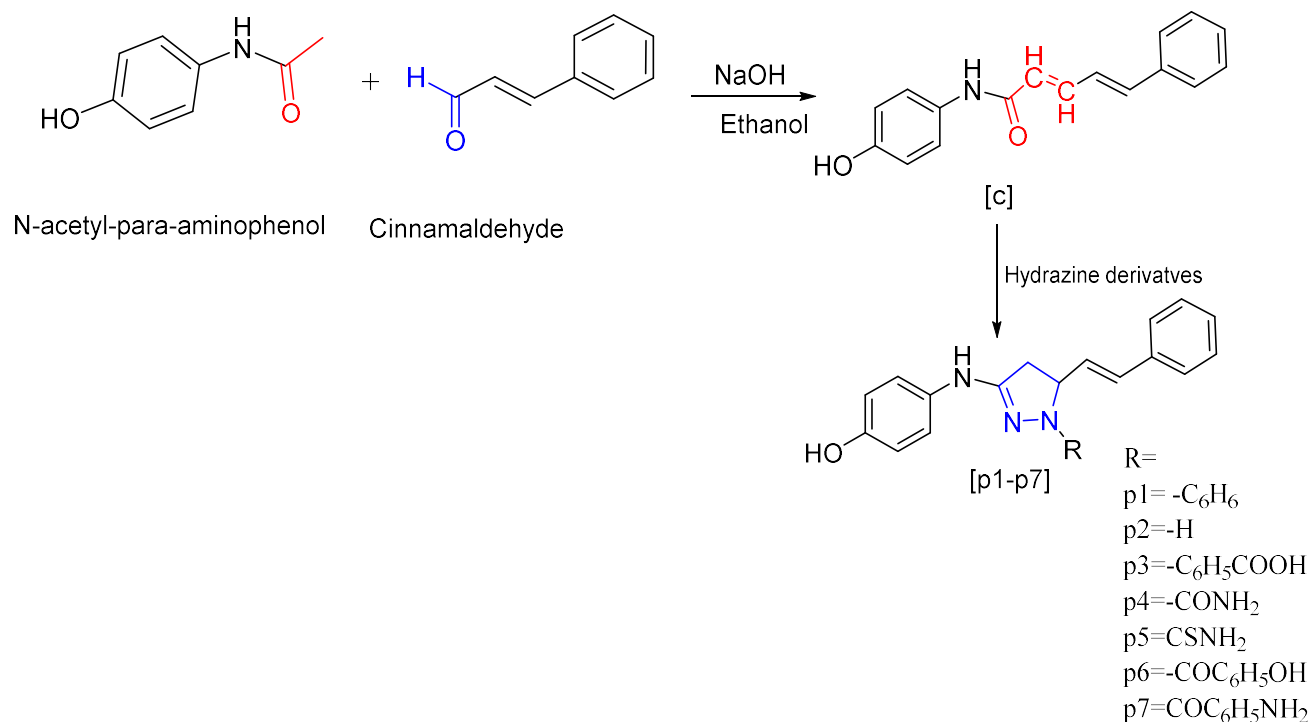
The goal of this study was to use a molecular docking strategy to virtual screen new pyrazole and chalcone derivatives with EGFR and poly (ADP-ribose) polymerase-1 proteins as the target

and to recognize a promising lead molecule as a template to create novel hypothetical compounds with higher binding affinities and superior molecular interactions with the target. Moreover, the compounds' drug-likeness properties & (ADME) absorption, distribution, metabolism, and excretion were assessed (*in-silico*). The other evaluations were physicochemical aspects of a molecule, for instance, saturation, solubility, lipophilicity, polarity, size, and flexibility, which provide essential evidence about if the molecule may be used as a drug at an initial stage of development.

Methodology

Chemical Synthesis

The assumed synthesis pathway for the intermediate chalcone derivative compound [c] and the final compounds [p1-p7], is derived from N-acetyl-para-aminophenol (APAP), as the following scheme:



Scheme 1. Hypothesized synthetic pathway of compounds [c] and [p1-p7]

Computational Methods

In-silico Pharmacokinetic Predictions

The SwissADME website was used to determine pharmacokinetic or ADME (Absorption,

Distribution, Metabolism, and Excretion) investigations along with other physicochemical aspects of our designed compounds.

The chemical structure of newly designed

compounds was drawn in chemAxon's Marvin JS and then transformed into the SMILE name.

Small molecules' lipophilicity and polarity are determined using BOILLED EGG (14,15).

Molecular Docking and Virtual Screening

Docking tests were carried out utilizing a full license of GOLD [Genetic Optimization for Ligand Docking] (v.2021.3.0), a Cambridge Crystallographic Data Center software (CCDC) (16). Molecular docking studies are an important technique for the discovery of novel drugs since they predict affinity, contact with receptors, and, most importantly, biological activity (17).

The CCDC GOLD Suite (v. 2021.3.0) includes Hermes visualizer software (v. 2021.3.0), which is used to help prepare input files for docking with GOLD. Additionally, visualize the receptors, ligands, interaction type (H-bond, hydrophobic...etc.), active site, bond length determination, position prediction, and obtain photos.

Ligand and Receptor Preparation

The molecular structure of our ligands was sketched using ChemDraw professional (v.16.0) software. The energy of our molecules was then minimized using Chem3D (v.16.0) software via the MM2 force field.

The new compounds were docked utilizing the (3D) three-dimensional structures of two active targets: the crystal configuration of EGFR receptor (PDB code: 1M17) complexed with erlotinib and the crystal configuration of PARP1 protein (PDB code: 5HA9) complexed with TP0 reference ligand. The receptors are supplied from the protein data bank (PDB) website into GOLD's Hermes. To validate the docking technique, the co-crystallized ligands were re-docked.

Polar Hydrogen-atoms were also introduced for precise tautomeric states and ionization of AAs residues. The EGFR kinase receptor and PARP1 proteins' structures are then constructed by eliminating the crystallographic water molecules that aren't engaged in the active site. In addition, the original ligands were extracted from the receptor's active site.

Molecular Docking Protocol

The receptors are docked using the Hermes visualizer tool from the CCDC GOLD package. The binding site is determined by the initial ligand

interaction location. For the docking method, the protein-binding site with all of the protein residues discovered inside the (10Å^o) of the reference ligand was used.

All parameters utilized throughout the docking method were set to default. The number of generated postures was set to ten, and the top-ranked solution was retained as the default; also, the early cessation option was inactivated. As a configuration template, Chemscore kinase was employed. As a scoring function, the piecewise linear potential (ChemPLP) is used.

The results were then saved as mol.2 files. This information includes the optimum binding method, binding free energy, and docked postures. These outcomes were carefully examined to determine the optimal binding and interaction of our proposed ligand with receptor amino acid residues (EGFR and PARP1).

Results and Discussion

Chemical Synthesis

The new analogs were designed by including pyrazole moiety into N-acetyl-para-aminophenol (APAP) to get the final compounds [p1-p7]. The intermediate chalcone derivative was compound [c]. The anticancer activity of chalcone and pyrazole moieties was proved in many research(18,19). The hypothesized synthetic pathway that might be used in the future is shown in scheme (1). The targeted Compounds were designed based on a research article (20,21). 1106

ADME Results Interpretation

SwissADME server (14) was used for in silico prediction of the physicochemical and ADME properties of designed compounds. It is a supportive and inexpensive method to detect the ADME properties before synthesis and biological testing and to exclude ligands inadequate with an acceptable pharmacokinetic profile (15).

These parameters include the topological polar surface area (TPSA), used to assess a drug's capacity to permeate cells, compounds with TPSA<140Å² that indicate high permeability and bioavailability (22). Outcomes revealed that all compounds have TPSA <140Å² ranging from (47.86-105.97) Å². However, all the compounds fulfilled the classical descriptor for lipophilicity (log Po/w). Meanwhile, molar solubility (log S) in water ranges from moderate soluble to poor



solubility. All the ligands are expected to passively and highly absorb from The GIT.

Another useful molecular descriptor is Lipinski's "rule of five" (RO5) briefly states that compounds should have a molecular mass of ≤ 500 Daltons, ≤ 5 H-bond donors, ≤ 10 H-bond acceptors, and $\log p \leq 5$ (octanol-water partition coefficient) to be absorbed orally otherwise will have poor bioavailability and permeability(23). Our results showed that all designed compounds satisfy the RO5 as shown in table (1). Additionally, the

bioavailability score of all ligands was 0.55.

The BOILED-EGG for designed compounds is represented in figure (1). Illustrate that the compounds do not permeate BBB which is Compounds [p3, p4, p5, p6, and p7], on other hand passively and highly absorbed from GIT. Compounds [c, p1, p2, p3, and p6] in red dots are not to be transferred out of the cells of the CNS by P-glycoprotein, meanwhile, compounds [p4, p5, p7] in blue dots are to be transferred out from CNS by P-glycoprotein.

Table 1. ADME results of the designed compounds

Comp.	H-donor	H-acceptor	MR	TPSA	GI Abs.	BBB permeability	Bio-availability	Lipinski violation
c	2	2	81.53	49.33	High	yes	0.55	0
p1	2	2	118.56	47.86	High	yes	0.55	1[MLOGP>4.15]
p2	3	2	93.42	56.65	High	yes	0.55	0
p3	3	3	101.62	90.95	High	No	0.55	0
p4	3	3	101.62	90.95	High	No	0.55	0
p5	3	2	108.82	105.97	High	No	0.55	0
P6	3	4	125.25	85.16	High	No	0.55	0
P7	3	3	127.63	90.95	High	No	0.55	0

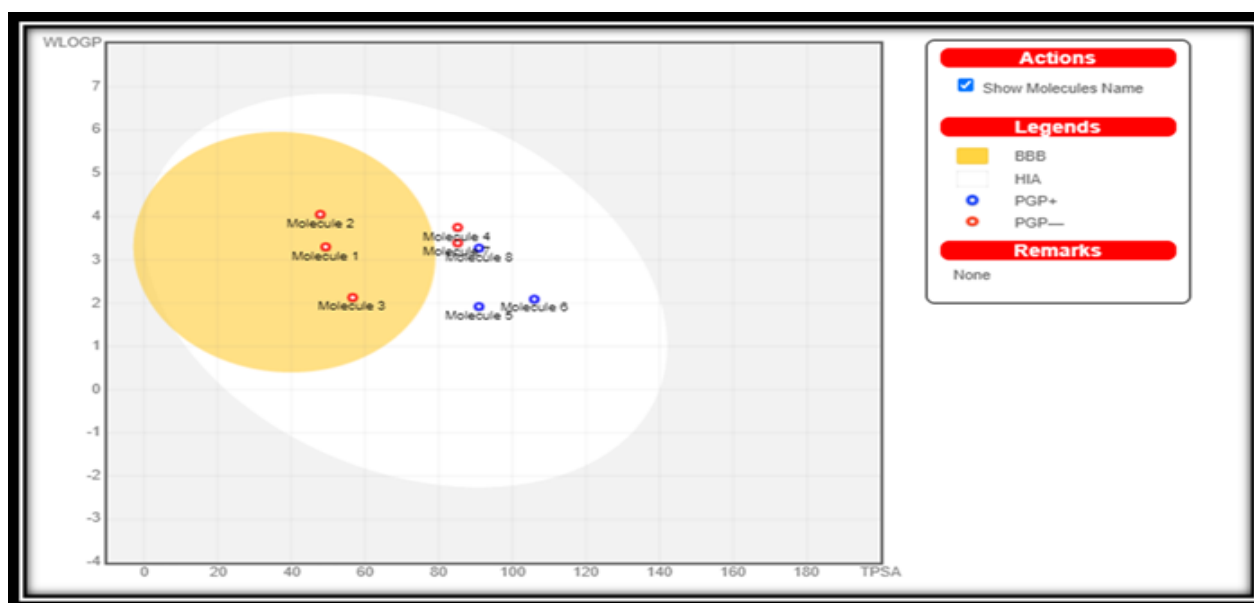


Figure 1. BOILED-EGG for designed compounds. **Yellow ovule (yolk):** Are molecules expected **passively cross** through blood brain barriers. **White ovule (white):** Are molecules expected **passively absorb** by the GIT. **PGP+:** **Blue dots** are for molecules expected to be **effluated** by the P-glycoprotein (P-gp) from the CNS. **PGP-:** **Red dots** are for molecules expected **not to be effluated** by the P-glycoprotein (P-gp) from the CNS

Interpretation of Docking Results

GOLD define as "Genetic algorithm for docking flexible ligands into protein binding sites"(24). Generally, GOLD predict the pose and gives exceptional outcomes for virtual screening(16). GOLD is part of the GOLD suite, that comprises further software such as Hermes, CSD python,

mercury, ConQuest.. etc.

Energy optimization methods were used to locate stable and minimum energy conformation by changing the geometry of the structure.

The docking studies result in the prediction of binding energies and selectivity of the designed compounds to proteins e.g. (EGFR, PARP1) through



studying the molecular interaction between the active binding sites of the proteins, and designed compounds.

The EGFR and PARP1 inhibitory activities of intended compounds, erlotinib, and TP0, were ranked based on their PLP fitness included in the complex formation at the active sites. Tables (2) and (3) show The PLP fitness of the docked compounds on EGFR, and PARP1 proteins, respectively.

GOLD software also gives the distance of hydrogen bonding between our designed ligands and a specific protein as well as all bonds length was $\leq 3\text{\AA}$ (17).

Docking solutions reveal that the intended compounds have appropriate binding energies with the receptor active site and likely promising action with EGFR and PARP1 proteins because it binds to the amino acids (AAs) residues in the active site via H-bonds and other short contacts. However,

compounds [p1, p6, and p7] with EGFR protein illustrate the highest PLP fitness value (73.13, 77.53, 73.48) respectively, and H-bonding with AAs as demonstrated in Table (2).

All other ligands reveal less binding energies than the reference Erlotinib give (71.5) PLP fitness value and form H-bonding with AAs (Met769, Leu764).

Docking results for PARP1 protein show the most important interaction inside the active site was for compound p3 with (94.14) PLP fitness value which forms H-bond with amino acids (ASP109, HIS201, TYR28, ARG204, GLU102). Compounds (p1 & p6) also give high PLP fitness values (92.24, 91.98) respectively than the reference ligand (TP0). There were no H-bond contacts for the reference ligand (TP0), only short contacts (3 contacts) with AAs [TYR228, SER203, TYR246 (5)]. The best scoring of the designed derivatives and reference control were represented in Tables (2 & 3) & figures (2 to 9) for EGFR & PARP1 proteins respectively.

Table 2. The PLP fitness for APAP analogs & the reference drug docked with EGFR

Compound	EGFR binding energy (PLP fitness)	Amino acid included in H-bonding	Amino acid included in a short contacts
C	58.14	Pro770	Leu764, Pro770
P1	73.13	Met769	Leu768(3)*, Met769
P2	58.78	Met769	Met769, Val702, Leu764
P3	71.33	Met769	Met769, Ala719, Leu694, Leu768(3)*, Phe699
P4	66.39	Met769, Lys721, Asp831	Leu768(5)*, Lys721, Asp831, Met769
P5	63.21	Met769	Lys721, Leu768(3)*, Met769
P6	77.53	Met769 (2)*, Leu764, Ala719, Thr766	Val702, GLY695, Lys721, Ala719, Thr766, Leu764, GLY772, Met769 (2)
P7	73.48	Met769	Leu964(2)*, Leu768(2)*, Ala719, Leu820, Met769
Erlotinib	71.5	Met769, Leu764	Met769, Leu764(2)

*Number in brackets denote to the number of bonds.

Table 3. The Binding Energies of APAP analogs docked with PARP1 receptor

Compound	PARP1 binding energy (PLP fitness)	Amino acid included in H-bonding	Amino acid included in a short contacts
C	69.15	Ala219, ASP105, ASP109	ASP109, TYR228(5)
P1	92.24	GLY233(2), ALA219	ASP105, GLY233(4), HIS201(2), SER243(2), TYR235, ALA219
P2	65.98	TYR28, ASN106	ASN106, ARG204, SER203(2), TYR264, TYR235(4)
P3	94.14	ASP109, HIS201, TYR28, ARG204, GLU102	ASP109, HIS201, TYR28, ARG204, ILE211
P4	79.98	TYR28, ASN106, SER243, ASN206	MET299(2), SER243, TYR264, ASN106, SER203, GLY202
P5	79.48	TYR28, SER243	TYR28, SER243, MET229(2) ARG204, SER203
P6	91.98	SER243, TRP200	GLU202, HIS201, SER243
P7	90.33	SER243, GLN98	GLN98
TP0	70.18	-	TYR228, SER203, TYR246(5)

*Number in brackets denote to the number of bonds.



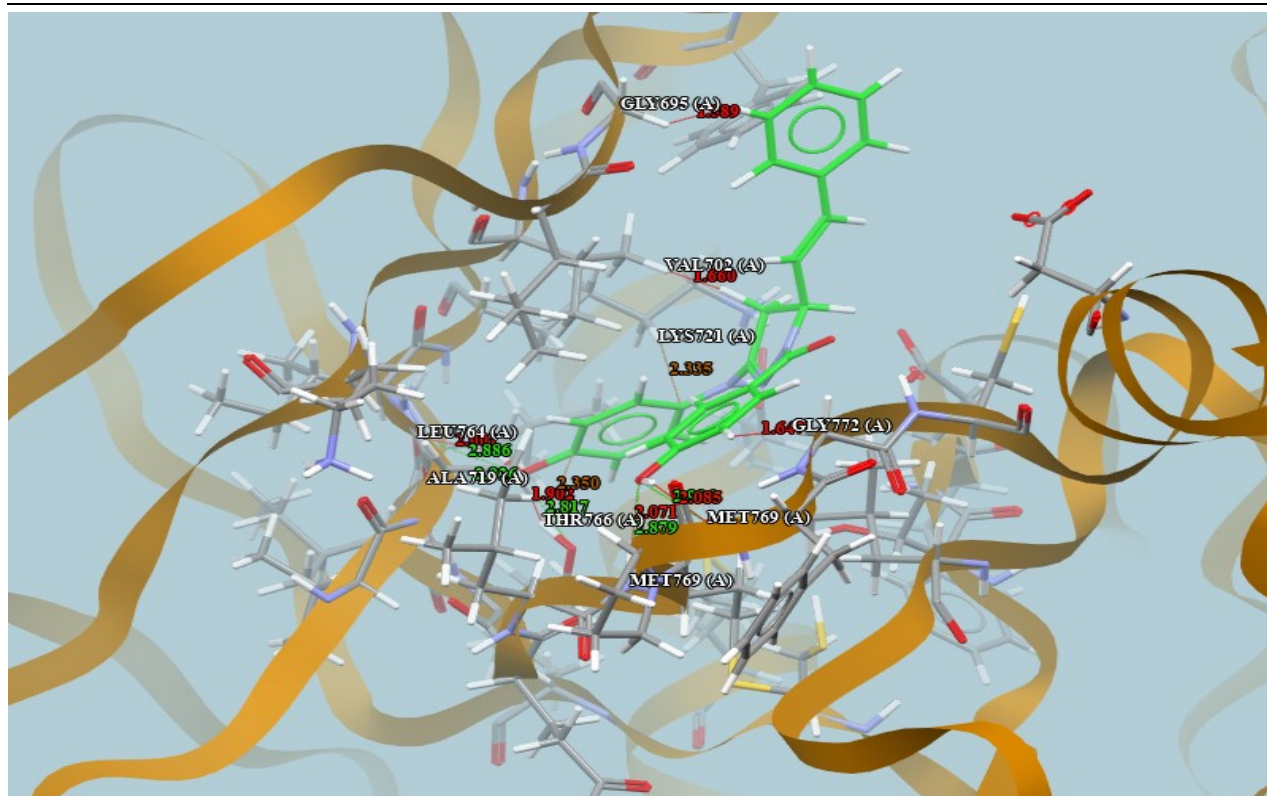


Figure 4. Show compound [P6] Hydrogen bond and hydrophobic interaction with EGFR protein (PDB code: 1M17). The contact between them by Hydrogen bond [Met769 (2)*, Leu764, Ala719, Thr766] indicates in a green whereas for hydrophobic in red. [Compound [P6]: ball and stick style, whereas amino acids as capped sticks]

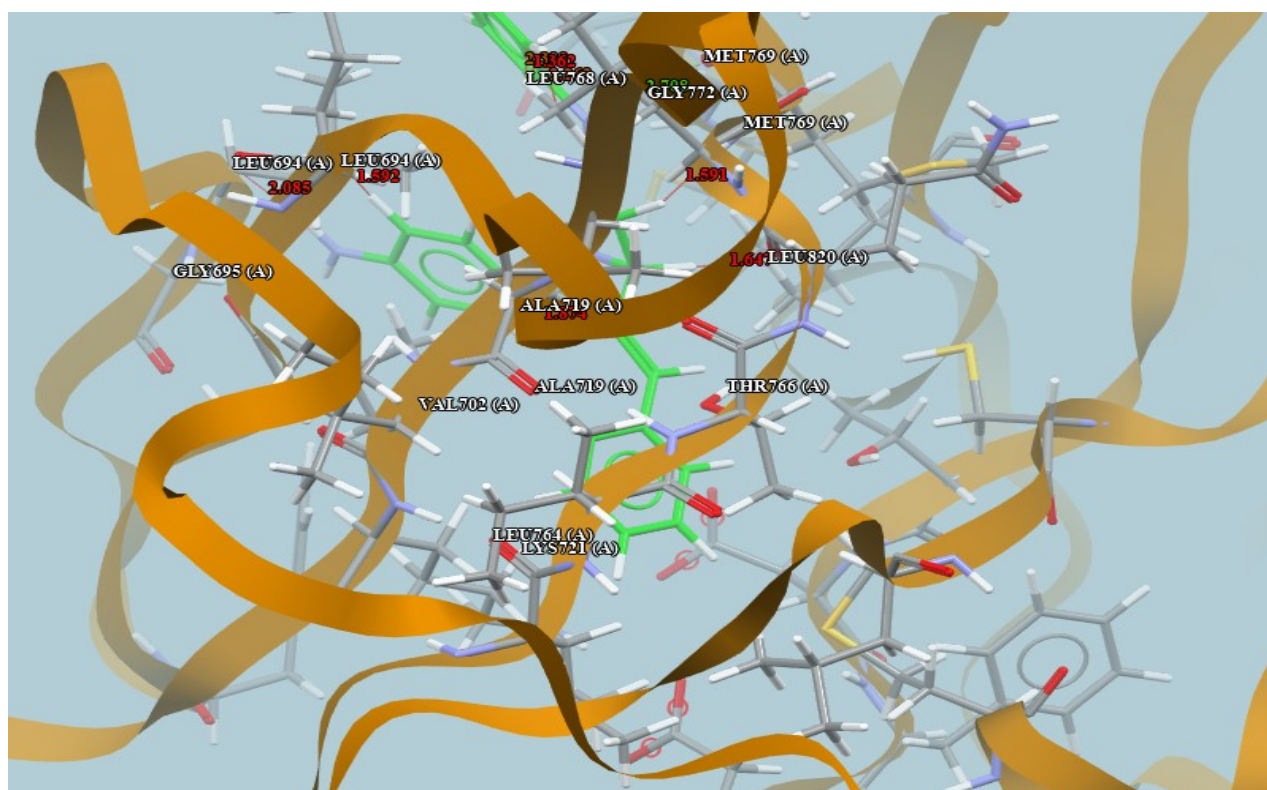


Figure 5. Show compound [P7] hydrogen bond and hydrophobic interaction with EGFR protein (PDB code: 1M17). The interaction between them by Hydrogen bond [Met769] indicates in a green whereas for hydrophobic in red. [Compound [P7] is represented in ball and stick style, whereas amino acids as capped sticks]



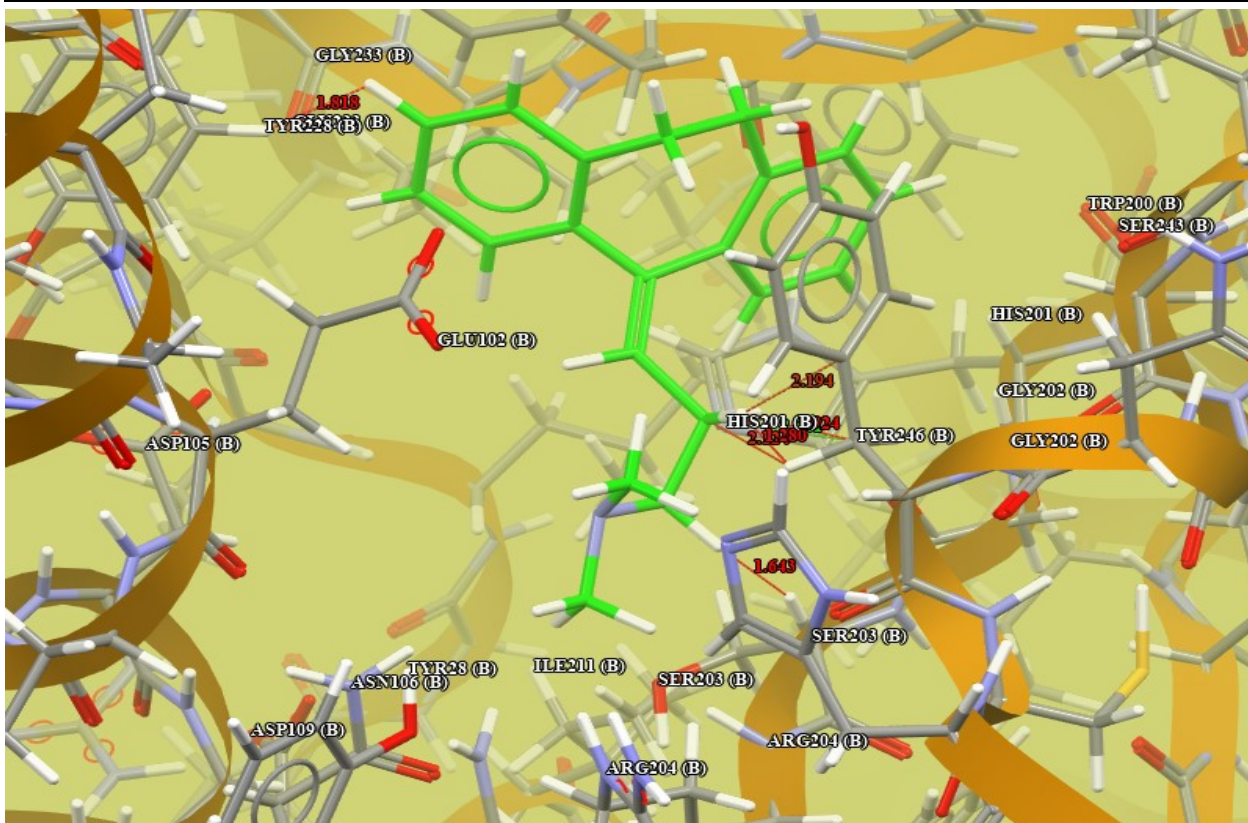


Figure 6. Depicts the reference TP0's hydrogen bond and hydrophobic interaction with the PARP1 protein (PDB code: 5HA9). The interaction between the reference and amino acid residues is indicated in red for hydrophobic. [TP0: ball and stick, whereas amino acids are in capped sticks] 1111



Figure 7. Show compound [P1] Hydrogen bond and hydrophobic interaction with PARP1 protein (PDB code: 5HA9). The contact between them by Hydrogen bond [GLY233 (2), ALA219] denotes in a green whereas for hydrophobic in red. [Compound [P1] is represented in ball and stick style, whereas amino acids as capped sticks]



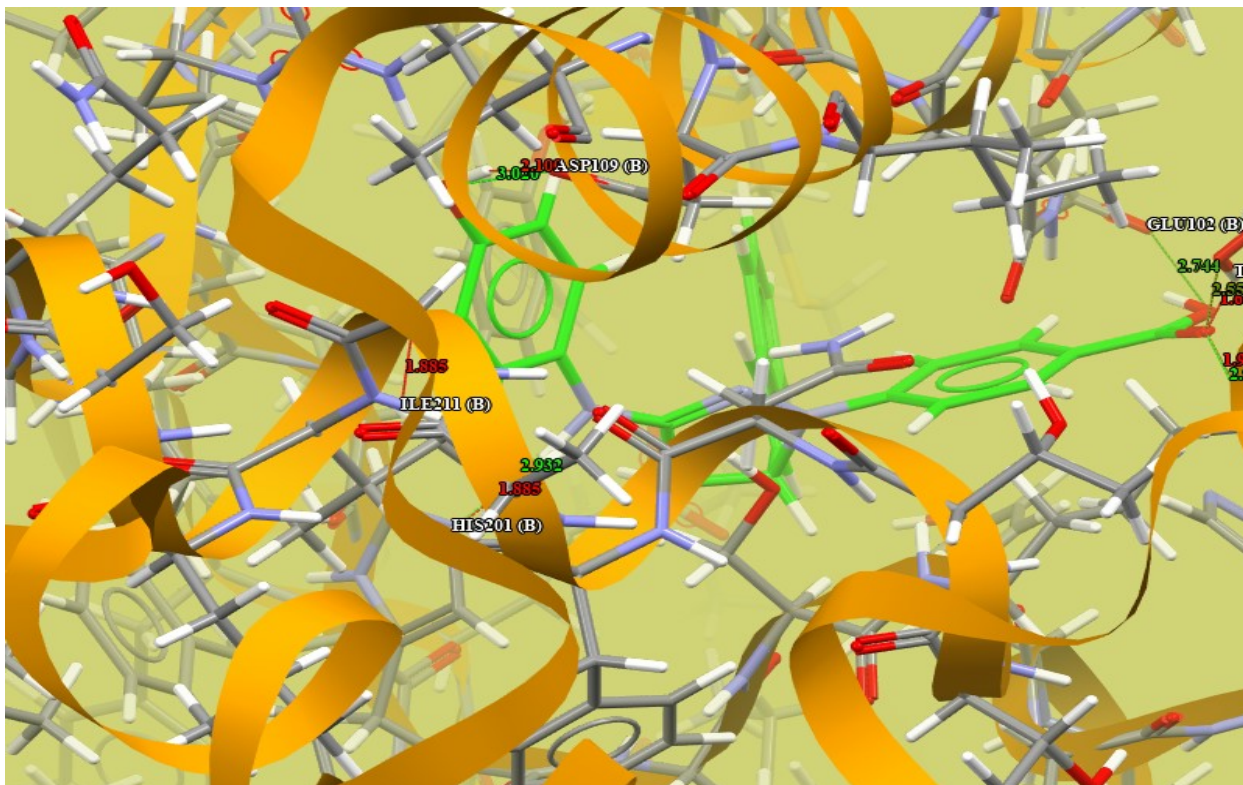


Figure 8. Show compound [P3] Hydrogen bond and hydrophobic interaction with PARP1 protein (PDB code 5HA9). The contact between them by Hydrogen bond [ASP109, HIS201, TYR28, ARG204, GLU102] denotes in a green whereas for hydrophobic in red. [Compound [P3] is represented in ball and stick style, whereas amino acids as capped sticks]



Figure 9. Show compound [P6] Hydrogen bond and hydrophobic interaction with PARP1 protein (PDB code: 5HA9). The contact between them by Hydrogen bond [SER243, TRP200] denotes in a green whereas for hydrophobic in red. [Compound [P6] is represented in ball and stick style, whereas amino acids as capped sticks]



Conclusion

In conclusion, a new series of chalcone and pyrazole derivatives [c, p1-p7] were designed from N-acetyl-para-aminophenol. *In Silico* experiments including ADME studies predicted that all designed compounds passively and highly absorbed from the GIT. Also, all the compounds fulfilled the RO5. The docking studies for ligands interaction with EGFR protein showed promising activity for compounds (p1, p6, p7) by binding with AAs in the active pocket with more PLP fitness values than the reference erlotinib. Meanwhile, for PARP1 protein the designed compounds (p1, p63, and p6) show PLP fitness values more than the reference ligand. Compounds (p1 & p6) give favorable results for both proteins [EGFR, and PARP1].

Conflict of Interests

The authors declared no conflict of interest.

References

- Mohamed MFA, Abuo-Rahma GEDA. Molecular targets and anticancer activity of quinoline-chalcone hybrids: Literature review. *RSC Adv.*, 2020; 10(52): 31139–55.
- Hanahan D, Weinberg RA. The hallmarks of cancer. Vol. 100, *Cell.*, 2000, p. 57–70.
- Das M, Manna K. Chalcone Scaffold in Anticancer Armamentarium: A Molecular Insight. *J Toxicol.*, 2016; 14.
- Xu J, Mao W. Overview of Research and Development for Anticancer Drugs. *J Cancer Ther.*, 2016, Sep 22; 07(10): 762–72.
- Masuda H, Zhang D, Bartholomeusz C, Doihara H, Hortobagyi GN, Ueno NT. Role of epidermal growth factor receptor in breast cancer. *Breast Cancer Res Treat.*, 2012; 136(2): 331–45.
- Bethune G, Bethune D, Ridgway N, Xu Z. Epidermal growth factor receptor (EGFR) in lung cancer: An overview and update. *J Thorac Dis.*, 2010; 2(1): 48–51.
- Arora A, Eric M. scolar. Role of tyrosine kinase inhibitors in cancer therapy. *J Pharmacol Exp Ther.*, 2005; 315(3): 971–9.
- Xin M, Sun J, Huang W, Tang F, Liu Z, Jin Q, et al. Design and synthesis of novel phthalazinone derivatives as potent poly (ADP-ribose) polymerase 1 inhibitors. *Future Med Chem.*, 2020; 12(19): 1691–707.
- Tian Y, Xie Z, Liao C. Design, synthesis and anticancer activities of novel dual poly (ADP-ribose) polymerase-1/histone deacetylase-1 inhibitors. *Bioorganic Med Chem Lett.*, 2020; 30(8): 127036.
- Chouiter MI, Boulebd H, Pereira DM, Valentão P, Andrade PB, Belfaitah A, et al. New chalcone-type compounds and 2-pyrazoline derivatives: Synthesis and caspase-dependent anticancer activity. *Future Med Chem.*, 2020; 12(6): 493–509.
- Abdullahi M, Adeniji SE. In-silico Molecular Docking and ADME/Pharmacokinetic Prediction Studies of Some Novel Carboxamide Derivatives as Anti-tubercular Agents. *Chem Africa.*, 2020; 3(4): 989–1000.
- Ekinci D. *Medicinal Chemistry and Drug Design.* Intech., 2012.
- Rungta D. A Review of Computational Tools for Designing Drugs Used by General Practitioners. *J Gen Pract.*, 2014; 02(06): 4–6.
- Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.*, 2017; 7: 42717.
- Hou T, Wang J, Zhang W, Xu X. ADME evaluation in drug discovery. *J Chem Inf Model.*, 2007; 47(1): 208–18.
- Verdonk ML, Cole JC, Hartshorn MJ, Murray CW, Taylor RD. Improved protein–ligand docking using GOLD. *Proteins.*, 2003; 52: 609–23.
- Alvarez J, Shoichet B. Virtual screening in drug discovery. *Virtual Screening in Drug Discovery*, 2005, 1–470.
- Abdelgawad N, Ismail MF, Hekal MH, Marzouk MI. Design, Synthesis, and Evaluation of Some Novel Heterocycles Bearing Pyrazole Moiety as Potential Anticancer Agents. *J Heterocycl Chem.*, 2019; 56(6): 1771–9.
- Sankappa Rai U, Isloor AM, Shetty P, Pai KSR, Fun HK. Synthesis and in vitro biological evaluation of new pyrazole chalcones and heterocyclic diamides as potential anticancer agents. *Arab J Chem.*, 2015; 8(3): 317–21.
- Hanna JS, Khan AK, Essa HJ. Synthesis, Molecular Docking, and Cytotoxic Evaluation of Some Novel 1H-Pyrazole Derivatives from Pentoxifylline. *Int J Pharm Res.*, 2020; 12(02): 3158–68.
- Alawad KM, Mahdi MF, Raauf AMR. Molecular Docking, Synthesis, Characterization and Adme Studies of Some New Five-Member Ring Heterocyclic Compounds With in Vitro Antiproliferative Evaluation. *J Hunan Univ Sci.*, 2021; 48(9): 367–82.
- Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. *J Am Soc Exp Neurother.*, 2005; 2(4): 541–53.
- John M. Beale J john HB. Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry. 12th ed, 2004, 1–1022.
- Gareth Jones1, Peter Willett, Robert C. Glen ARL and RT. Development and Validation of a Genetic Algorithm for Flexible Docking Gareth. *J Mol Biol.*, 1997; 267: 727–48.

