Anti-Proliferative, Antioxidant And Anti-Inflammatory Potentials of *Cassia Nemophila* Flowers

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Research Article

Keywords: Cassia nemophila, anticancer, antioxidant, anti-inflammatory, in vitro assays

Posted Date: September 23rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-880001/v1

Abstract

Cancer is characterized by abnormal growth and uncontrolled growth of cells. Adverse effect on various body functions. It is a multifaceted disease with many causes. Medicinal plants have the potential to treat disorders. The study was designed to examine the properties of ethanolic and n-hexane flowering plants of Cassia and living flowers. C. Nophila was selected for this study because it has a long history of traditional and modern Cassia drug discovery. The contribution of oxidants was confirmed using the DPPH radical scavenging assay. Anti-inflammatory activities were tested by the HRBC membrane-strengthening membrane. Cytotoxic effects are being investigated with respect to the activity of the HCT115 cancer cell line. Efforts are being made to identify potential AMPK agonist operations. To adjust the crystal structure of AMPK and its ligand, AMBER18 software was used. ligands developed by the Molecular Operating Environment (MOE2018). Viewing and analysis of AMPK and its users using PyMOL. For authentication software packages, Chimera and AutoDock Vina were used. The results revealed the medicinal value of C flowers. Absolutely, flowers of C. and life have many active ingredients that can be used as effective anticancer drugs. More research needs to be done to explore the many properties of these plant species.

Introduction

The uncontrolled growth and differentiation of body cells is cancer. Any body cell with the capability of growth and division may lead to cancer. It is not an abrupt process, but it is a multistep process that may take many years (Ali et al. 2013). Cancer is slowly becoming the most common cause of death throughout the world. This is an alarming situation because of the living standards and lifestyles of modern society, such as a poorly balanced diet, stress, competitiveness, smoking, alcoholism, and adulterated food. Moreover, environmental hazards also play a vital role (Anand et al. 2008).

The main therapies used for cancer treatment are chemotherapy, radiotherapy, and surgeries with side effects. The use of traditional drugs to control and kill cancerous cells and treat the damage caused by them is gaining popularity due to the lack of side effects (Madhuri et al. 2009). Most cancers are one of the foremost concerns in ultramodern fitness gadgets. In 2018, 18 million new cancer instances were registered, and 9.5 million human beings died of cancer. Cancer is one of the main reasons for death worldwide, surpassing simplest in cardiovascular disorders. Exploring new drugs to fulfill the desires of drug-resistant sicknesses is still a warm topic in medical chemistry. Due to drug resistance, the cutting-edge scenario is alarming, and new tablets are urgently needed to triumph over cutting-edge issues together with facet consequences. At gift, clearly stimulated products are nonetheless a vital supply of new drug treatments. Cancer is a multifactorial and multidimensional group of diseases with multiple causes. It is a more diverse disease affecting a variety of body processes. Similarly, it is not enough to consider a single factor involved in its development. Oxidative damage and prolonged inflammatory disorders can contribute well to the promotion of various categories of cancers. Oxidative damage is caused by the overproduction of free radicals in the body. This causes an imbalance between the antioxidant defense mechanism of the body and free radical production. These free radicals may

destabilize the membranous system, DNA, RNA, and other macromolecules by snatching radicals from them, as they are highly reactive species. This brings irreversible damage to body tissues (Halliwell et al. 2015). The body needs a fair supply of antioxidants to keep check on excess free radical production, thus helping the body combat a number of disorders, such as atherosclerosis, neurodegeneration, cancer, aging, pulmonary disorders, cataracts, heart diseases, diabetes, etc. (Aruoma et al. 2010). Cancer is one of the deadliest illnesses worldwide, especially in Western countries. According to the International Cancer Observatory, an estimated 9.9 million people have died by 2020 from cancer. Cancer is a complex disease commonly defined as uncontrolled growth and growth of cells in the connective tissue and microenvelo (a tumor) that can grow into a whole organ or systematically in other tissues (metastasis). This abnormal cell behavior may be the result of a genetic mutation or epigenetic-driven mutations of key genes (oncogenes) associated with the cell cycle and cell death (apoptosis). Cancer cells also appear to be incompatible with planned apoptosis and malignant behavior of microtubules because they are involved in the mitotic process. The World Health Organization identifies them as the leading causes of random cancer mutations, ionizing radiation, active oxidative stress, and many chemical agents. (Garcia et al 2021)

Inflammation is a complex response of the body against any foreign invasion. It results in phagocyte activation and the production of a considerable number of free radicals and lysosomal enzymes to produce an inflammatory response. for fighting against foreign invasion. The inflammatory response can be kept under strict control; otherwise, it may lead to several disorders, including cancer. Recent studies have shown a close link between inflammation in living systems and oxidative stress, autoimmune diseases, brain dysfunction, etc. (Eming et al. 2007). Inflammation is the body's response to a pathogen attack mediated by the release of mediators, including cytokines, histamine, prostaglandin, and leukotrienes, as well as blood vessels, leading to leukocytes in inflammation, leading to the removal of bacteria. However, if inflammation is not properly controlled, it can cause a variety of diseases, including autoimmune diseases, neurological diseases, heart disease, cancer, diabetes, and disease (Pham et al 2021). In step with the world fitness agency (who), persistent inflammation is one of the main threats to different continual sicknesses. Irritation acts as an important part of the frame's response to harm and infection because the immune machine acquires and removes harmful materials even as wound recovery begins. Persistent infection, or continual irritation, is characterized through lengthy term staying power from months, years to many years. Similarly, persistent irritation is linked to more critical ailments, which include most cancers, heart disorders, diabetes, and Alzheimer's disease. (Le, K. M et al 2021)

Life exists due to the close relationship between animals and plants. It is a long journey from wild unwanted plants to valuable therapeutic agents used for curing diseases. They are considered more effective with fewer side effects and better adaptability in living organisms than synthetic drugs (Janmeda et al. 2011). Natural products derived from natural sources are more biologically friendly than synthetic products because of their isolation from biological systems, which is why they face minute rejection problems in organisms (Balunas et al. 2005)(Drahl et al. 2005).

It is considered that 64% of the total world population in developing countries utilizes plants as drugs (Pal et al. 2003). Asian lands are rich in natural herbs, and most of them are used in treating tumors, ulcers, and sores in traditional medicinal ways. There is great margin in scientific study of these herbs and exploration of their benefits at the industrial level. Natural remedies that were widely used in the past are now used in the treatment of many diseases, serve as a source of interest in the raw form and serve as a template for modified transformation. This shows its industrial importance in treating different diseases, including a variety of cancers. Many of these herbs are used traditionally, but the benefits derived from them are less effective, as inefficient techniques are used by naive and inexperienced people (Arshad et al. 2005).

Cassia is a broad genus from the family Leguminosae and is widely distributed in Asia, Mexico, West Indies, and Brazil. Their numbers of species are used in traditional medicinal systems for treating wounds, tumors, inflammation, and hepatic disorders. It has antimutagenic and antioxidant properties (Kaur et al. 2014).

Extracts that have antioxidant activity also possess anti-inflammatory activity in addition to anticancer and antiproliferative capabilities. The study is based on investigating the anti-inflammatory, antioxidant, and cytotoxic activities of ethanolic and n-hexane extracts of *C. nemophila* flowers using different assays.

Materials And Methods

Plant material and extraction

Cassia nemophila flowers was collected from different wild regions of District Peshawar and Nowshera, KPK, Pakistan. This study complies with relevant institutional, national, and international guidelines and legislation. We have taken permission for the collection of *Cassia nemophila flowers* from the Department of Zoology Abdul Wali Khan University Mardan and Forestry Department of concerned Districts.

The wild *Cassia nemophila* plant was identified and authenticated by Dr. Mohib Shah in the Department of Botany Faculty of Chemical and life Science, Abdul Wali Khan University Mardan Kp Pakistan. The *Cassia nemophila* specimen was deposited in the institutional herbarium with voucher number AWKUM. BOT.54.2.11.

The dried flowers were powdered and extracted with n-hexane and ethanolic solvents. The solvents were evaporated and dried to obtain greenish black gummy extract and were used later to evaluate its antioxidant, anti-inflammatory, and cytotoxic properties.

MTT- cell proliferation assay

The cytotoxicity of the crude extract of *C. nemophila* flowers was studied using the (MTT) {three- (four, five-dimethylthiazol-two-yl)-two, five-diphenyltetrazolium bromide} assay. This assay was totally based

on the inhibitory effects of the extract on the viability of HCT115 cells received from an American lifestyle series (manassas, va, usa). The basic unit cell viability was directly proportional to the reduction of yellow soluble tetrazolium MTT to purple insoluble formazan. Mitochondrial dehydrogenase enzymes present in viable cells do this converse, common after treatment with cytotoxic drugs. After culturing, 5×10^{3} HCT115 cells in 200 µl DMEM were seeded per well in 96-well plates along with fetal bovine serum and 1% antibiotics (streptomycin and penicillin). The plate was incubated overnight at 37°C in humidified 5% carbon dioxide in an incubator.

Then, the extract was added at different concentrations and incubated for 24 hours. MTT reagent was added and allowed to set for 2–4 hours in a carbon dioxide incubator. The absorbance was observed at 570 nm using plate reader formula.

The experimental protocol was approved by the Research grants and Experimentation Ethics Committee of the Department of Zoology Abdul Wali Khan University Mardan on the use of human tissue samples and blood. "Informed consent was obtained from all subjects for the use of blood and tissues" Informed consent has been taken from healthy blood and tissue doners that this blood and tissue will be used for experimental purpose and most of the doners were students of the university of aged above 18 years. It was carried out in strict compliance with the National Research council guidelines on the care and use of laboratory.

All procedures complied with the standards for the care and use of human tissue samples and blood subjects as stated in the guidelines laid by Institutional Ethical Committee Abdul Wali Khan University Mardan Kp Pakistan.

Percentage inhibition was calculated as follows:

$$\text{Percentage viability} = \frac{\text{OD of control cells- OD of sample cells}}{\text{OD of vehicle treated cells}} \times 100$$

DPPH- (2,2-diphenyl-2-picryl hydroxyl)-free radical scavenging assay

(Two, two-diphenyl-two-picryl hydroxyl) DPPH-free radical scavenging assays were used to evaluate the antioxidant capability of the *C. nemophila* extract in vitro. DPPH can release free radicals, and these free radicals were scavenged by *Cassia nemophila* flower extract, indicating its antioxidant capabilities.

The reaction mixture was prepared by taking 3 ml of *C. nemophila* extract of different concentration in methanol (10, 20, 40, 60, 80 and 100 µg/ml) and 1 ml of 1 mM DPPH and set for 30 minutes in dark, vortexed it few times and the absorbance become determined at 517 nm, using UV 5100B spectrophotometer.

The percent scavenging of free oxidants by antioxidants was determined by using the following formula.

$$\text{Scavenging DPPH (\%)} = \frac{\text{absorbace control} - \text{absorbance sample}}{\text{absorbance control}} \times 100$$

The IC50 represents 50% scavenging of radicals by the test sample and was determined using GraphPad Prism software.

HRBC- membrane stabilization assay

The anti-inflammatory activity of *C. nemophila* flowers was studied using an HRBC (human red blood cell) membrane stabilizing assay. This method is based on stabilization of red blood cells against lysis in a saline environment. The activity of the drug was studied by estimating the cell content released in suspension after repeated centrifugation. Blood from healthy volunteers was collected and centrifuged several times at 3000 rpm for 12 minutes. A 10% v/v suspension of packed cells was prepared in hyposaline solution. Then, a mixture of 1 ml phosphate-buffered saline, 2 ml of 0.25% saline solution, 0.5 ml of packed red blood cell suspension and 0.5 ml of plant extract was mixed, centrifugation occurred for 15 minutes at 3000 revolutions per minute, and the supernatant of spectrophotometry was collected at 560 nanometers.

The percent stabilization of red blood cell membranes was determined using the following formula.

$$\text{Percent Protection (\%)} = \frac{\text{absorbance control} - \text{absorbance sample}}{\text{absorbance control}} \times 100$$

IC50 values represent the concentration at which the sample shows 50% membrane protection.

Identification of potential agonists, crystal structure, ligand for AMPK activation and doking of Chrysophanol:

AMBER18 software (case, 2018) was used to refine the AMPK crystal structure and its ligand. Adjustment of ligands was accomplished via molecular operating environment (MOE) 2018. Viewing and Evaluation of ampk and its users, using PyMOL (Delano, 2002). In authentication software programmes, (chimera) (pettersen et al., 2004) and (car dock vina) (trott et al., 2010) have been performed.

AMPK's crystallographic structure and its ligand with atomic quadrants of Homo sapiens ampkα1β1γ1 with PDB identification 6c9f were obtained from the RCSB PDB online website (https://www.rcsb.org) (Yan et al., 2019). Its structure was in the form of diffraction X-ray. The amplification of the AMPK crystal structure is 2.92 ångström with a value of 0.245 without R. Cleansing the missing loops and poor hydrogen connection of the crystal frame using structural adjustments. With the addition of lacking hydrogen atoms and the inclusion of tether restrictions on system atoms, the short prep protocols in moe2018 have been used to refine the 6c9f crystal shape. The Amber10 eth energy field became used in moe2018 for short preparation and construction preparation.

Results

Effect of Cassia nemophila flower extract on the viability of the HCT115 cell line

The anticancer activity of ethanolic and n-hexane *C. nemophila* flower extracts was evaluated by investigating their cytocidal ability using the MTT assay. The results obtained were in favor of the cytotoxic abilities of both extracts, as shown in Figs. 1 & 2.

The extract was used at increasing concentrations (50–200 µg/ml). At 50 µg/ml, the ethanolic extract of flowers showed 90.72% cell viability, which was reduced to 16.89% viable cells at 200 µg/ml. In the case of the n-hexane extract of *C. nemophila*, the cell viability was 51.93% at 50 µg/ml, which was reduced to 11.45% at 200 µg/ml. The results shown by the standard drug Dastanib were comparable with those of the test extracts, and the maximum cell viability was estimated to be 78.48% at 50 µg/ml, which was reduced to 52.9% at 200 µg/ml.

Effect of Cassia nemophila on free radical scavenging

The antioxidant Hobby of ethanolic and n-hexane extracts of *Cassia nemophila* flowers was calculated by determining the extent of free radicals scavenged by the test extracts. The results showed an increase in antioxidant activity with an increase in concentration (10–100 µg/ml). Second, the scavenging capabilities of the ethanolic extract were higher than those of the n-hexane extract of *C. nemophila* flowers, as shown in Figs. 3 & 4.

The highest percent DPPH activity in the ethanolic extract was 49.64% at 100 µg/ml, and the IC50 value was 43.28 µg/ml. The n-hexane extract showed a maximum activity of 33.61% at 100 µg/ml, and the IC50 value was 62.44 µg/ml. Ascorbic acid used as a standard drug showed a maximum activity of 54.63% at 100 µg/ml, and its IC50 value was 91.67 µg/ml.

Effect of Cassia nemophila flowers on membrane stabilization

The anti-inflammatory activity of n-hexane and the ethanolic extract of *C. nemophila* flowers was determined by membrane stabilization of red blood cells under saline conditions. The results suggested an increase in membrane stabilization with increasing concentrations (5-100 µg/ml). The red blood cell membrane stabilization activity was more prominent in the case of the ethanolic extract than in the n-hexane extract, as shown in Figs. 5 & 6.

The percent stabilization of ethanolic and n-hexane extracts at 100 µg/ml was 47.01% and 38.28%, respectively. The IC50 values were 74.24 µg/ml and 89.57 µg/ml for the ethanol and n-hexane fractions, respectively, which were comparable with that of the standard drug (indomethacin), showing a maximum stabilization of 54.72% at 100 µg/ml and an IC50 value of 99.89 µg/ml.

Crystal structure of AMPK and its agonist:

The protein structure produced by AMP-activated kinase AMPK (α1β1γ1) is made up of 1024 amino acids with pdb identity: 6c9f with a solution of 2.92 ångström. Its visual look ligand of the ampk and (r34, from merck 991) is proven in Fig. 8. The ligand interplay with the lively amp package is shown in Fig. 7, wherein hydrogen bonding and lively site residues are truly shown.

Table 1 indicates that the energetic asn50 for Ampk binds with ligand with a hydrogen bond with a low power of 0. Eight kcal/mol, phe29 combines a ligand with hydrogen bonding with − 0.8 kcal/mol electricity. Lys33 has a very high potency of -1.0 Kcal/Mol and forms a pi-Hydrogen bond.

Table 1
Ampk energetic website online interacting residues with kind, distance of bond and binding strength

Bond (type)	Residues	Bond (Distance)	Energy (E)(kcal/mol)
Hydrogen	ASN 50	3.87	-0.9
Hydrogen	PHE 29	3.19	-0.7
Hydrogen	GLY 30	2.68	0.4
Pi-Hydrogen	LYS 33	3.69	-1.01

Properties of AMPK protein:

By using MOE2018 protein properties, different properties of the AMPK crystal structure were calculated. The conditions of the property descriptor for AMPK are shown in Table 2. The pH of 7.4 and temperature 298k were kept constant. Properties based on protein sequence or solvent properties are shown in Table 3.

Table 2
Setup of AMPK protein for characteristic calculation.

Conditions	Value
Temperature (K)	298.00
Viscosity of Solvent (cP)	0.00089
Dielectric of Solvent	78
Ionic strength of Solvent (M)	0.001
pH of Solvent	7.40
Salt	NaCl
Concentration of Salt (M)	0.003

Table 3
Characteristics totally based on protein solvent or sequence properties:

Property	Value
Mass of Protein (kDa)	97.24
Coefficient of Extinction (280 nm)	104630
Screening length of Debye (A)	95.90
Henry's function f(ka)	1.03
Isoelectric point (pI)	7.81
Percent helicity	31.4

Its characteristics are calculated from atom charges, places which are shown in Table 4. Its pH was kept constants for all characteristics; the salt was NaCl, and the temperature was 298 K.

Table 4
Computed Characteristics from atom charges and places:

Properties	Values
Gyration Radius (A):	34.57
Radius of Hydrodynamic (A):	133.13
Eccentricity:	0.25
Surface area of VdW (A^2):	39146.6
Surface area of Hydrophobic (A^2):	19104.8
Surface area Hydrophilic (A^2):	17784.4
Volume of VdW (A^3):	90062.3
Constant of Sedimentation (s):	7.00E-14
Mobility (10^{-5}cm^2/V. s):	-30
Frictional coefficient (kg/s):	5.80E-12
Diffusion coefficient (cm^2/s):	7.10E-06
Isoelectric point (pI):	8.54
Average net charge (Z):	16.12
Apparent charge:	10.77
Dipole moment (D):	882.72
K-D Hydrophobicity moment:	4001.4
Zeta potential (mV):	57.19
Dipole moment of zeta:	0.33
Quadrupole moment of zeta:	31.57

Electrostatic map of AMPK with ligand:

Moe (2018) calculated the electrostatic map of the lively website online of ampk and its agonist. The hydrogen bond acceptor areas are proven in pink (above), and the atoms that deliver hydrogen bonds are shown in blue, as proven in Fig.09. The residues leu20, gly21, gly27, gly30, asp90 and asp108 are shown with the aid of hydrogen bond donors. At the same time, residues lys33, lys53, arg83 and val13 have been proven to be hydrogen bond recipients.

Docking result of Chrysophanol:

The compound firoin (pub-chem id 10208 and molecular system c15h10o4) has electricity of -five.7538538 kcal/mol (scoring feature). Table 5 shows the interactions of Chrysophanol with 6c9f energetic website residues. Figure 10 suggests 2d interactions of ligand with the active website residues of ampk, Fig. 11 indicates 3-d interactions of lignad with the active website residues of ampk and Fig. 12 shows the surface view of ligand attachment in ampk active website online

Table 5
Compound interaction with active residues of 6C9F.

Bond Type	Residues	Bond Distance (Å)	Energy E (kcal/mol)
H- donor	ASN 111	2.91	-1.0
Pi-H	LYS 33	3.84	-0.6
Pi-H	VAL113	3.75	-0.7
Pi-H	VAL113	4.49	-0.7

3.8. Docking result of physcion:

The compound firoin (pub-chem identity 10639 and molecular formulation c16h12o5) has a power of -6.14368916 kcal/mol (scoring feature). Table 6 shows the interactions of physcion with 6c9f energetic site residues. Figure 13 suggests 2d interactions of physcion with the energetic website online residues of ampk, Fig. 14 suggests 3-D interactions of ligand with the active web page residues of ampk, and Fig. 15 shows the surface view of ligand attachment in ampk lively website

Table 6. Interactions of compound Physcion with 6C9F active site residues.

Bond Type	Residues	Bond Distance (Å)	Energy E (kcal/mol)
H- donor	ASP 90	3.09	-0.9
Pi- H	LYS 33	3.88	-2.4

3.9. Docking result of Diethylhexylphthalate:

The compound Diethylhexylphthalate (pub-chem id 8343 and molecular formula C24H38O4) has an energy of -6.9882164 Kcal/mol (Scoring Function). FIGURE 16 shows 2D interactions of ligand with the active site residues of AMPK FIGURE 17 shows 3D interactions of ligand with the active site residues of AMPK and FIGURE 18 shows the surface view of ligand firoin attachment in AMPK active site.

Discussion

Close relationships exist between antioxidant, anti-inflammatory and cytocidal activities, shown by any agent and favored over time (Lucas et al. 2006). During our study, we conducted a vast literature survey in which a natural product source was evaluated for its anti-inflammatory, antioxidant, and cytotoxic

activities. This impelled us to think on the aspect that these processes are linked with one another and can share several factors and causes associated with them. Several disorders affecting body functions include cancer, aging, heart diseases and neurological disorders. The study showed the involvement of antioxidant and anti-inflammatory effects in one or other aspects.

Phytochemicals in extracts from plants can play vital roles in overcoming chronic diseases such as cardiovascular diseases and cancers. Increasing interest is observed throughout the world to investigate novel anticancer agents of natural origin, which are still in the hands of traditional medicinal systems (Parmar et al. 2013).

Pakistan's rich flora, specifically its northern areas where lush green mountains house wild plants yet to be identified with their innate potential of curing multiple diseases. Native people use them and the knowledge roams in generation. However, we are facing a major risk of losing them before they are carried out to the drug industry. Another main reason is considered the dominance of synthetic drugs in local and nonlocal markets (Arshad et al. 2005).

Currently, the trend is shifting, and people are becoming more aware of the use of herbal drugs in treating minor to major illnesses because of their easy, cheap access and minimal side effects compared to synthetic drugs. Herbal drugs not only act as a phytomedicine in crude form but also provide vast ground for modern drug discovery, as they are considered blueprints for many chemicals. Several medicines of plant origin are rich in phenolic compounds, acting as a barrier against carcinogenesis, especially phenols such as flavonoids, lignans, and stilbenes. Family Leguminosae is rich in such compounds (Khair 2009).

Various members of the genus Cassia show the presence of anthrones, flavonoids, xanthones, sesquiterpenes, anthraquinones, glycosides, lactones, etc. The presence of such compounds made them effective antioxidant and anti-inflammatory drugs and impart diverse biological and pharmacological properties to them (Poole et al.2001). These properties can favor *cassia nemophila* to be selected for the presence of anti-inflammatory, antioxidant and cytocidal properties. This in turn supports the therapeutic properties of selected drugs in treating cancers. Oxidative damage is considered the primary cause of cancer, but it is a complicated problem associated with several factors. Inflammation is also considered one of its causes. It can provoke and attract many free radicals and immune components to the site, thus provoking an inflammatory response (Eming et al. 2007). *C. nemophila* reduces radicals to hydrazine when reacting with hydrogen donors according to the antioxidant principle. The results showed that the test compounds scavenged stable free radicals released by DPPH in a concentration-dependent manner in both solvent extracts. The activity of each extract can support *C. nemophila* as an active antioxidant agent. The antioxidant activity is due to the number of flavonoids, phenols, anthraquinones and lignins (Sarker et al. 2005).

The key role of chronic inflammation and tumor formation was highlighted by a vast range of evidence from experimental work. Clinical studies also support it. The process inside living systems is much more complex and intricate but is regulated well by genetic makeup. Any process or tissue of our body under

prolonged disturbance has more chances to undergo permanent damage. Chronic inflammation results in horrible diseases such as cancer (Gebhardt et al. 2008). The HRBC assay was selected because inflammation can destabilize the red blood cell membrane, promoting the release of several inflammatory cytokines and aiding in the dissemination of cancer.

The results are comparable with those of standard drugs, supporting their membrane stabilization capability and anti-inflammatory activity. This process is analogous to lysosomal membranes, which are stabilized to inhibit the release of hydrolytic and proteolytic enzymes, limiting the inflammatory response. The exact mechanism of membrane stabilization is not known and is only observed by inhibition of osmotic loss of intracellular electrolytes and fluid components (Menon et al. 2011).

Uncontrolled growth of cells is considered one of the most distinguishing features of cancer. The use of cytotoxic drugs in cancer treatment is an important aspect of chemotherapy, which induces apoptosis in cancerous cells. Cancerous cells are exceptional cells that easily escape the cytolytic pathway, grow, and metastasize, reducing the quality of life of humans. Cell viability was assessed using the MTT assay in the HCT115 cell line, which is a preliminary assay to estimate the cytotoxicity of *C. nemophila* flower extract and is usually correlated with its anticancer activity. Elevated concentrations of free radicals during inflammatory responses tend to be cytocidal and are detected by mitochondrial dehydrogenase in reducing MTT to blue formazan. Its formation is directly proportional to the number of viable cells because blue formazan interferes with mitochondria and cell viability

The results clearly showed the significance of the *C. nemophila* flower extract against cell viability in a dose-dependent manner, as the viability of cancerous cells decreased when the extract was used in a more concentrated form, in the case of both extracts. However, it was obvious that the n-hexane extract was more cytotoxic than the ethanolic extract of the test sample. The variable effect of the test extract against the HCT115 cell line can open the way to be explored against more cell lines. The overall discussion favors the extract as a source of choice and opens ways to explore it more.

Conclusion

The current study evaluated the antioxidant, anti-inflammatory, and cytotoxic activities of ethanolic and n-hexane extracts of *Cassia nemophila* flowers induced by chemical mediators and validated the potential and significance of folk medicine in primary health care. It also suggests the presence of potential compounds in our targeted plant species, which still needs to be explored and can be used as an effective therapeutic agent against different types of cancers imposing negligible side effects on living bodies. The potential activities of the extract were correlated with its flavonoid and phenolic contents. It also proved itself to be a good lead to carry out extensive *in vivo* studies on its anticancer properties.

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Figures

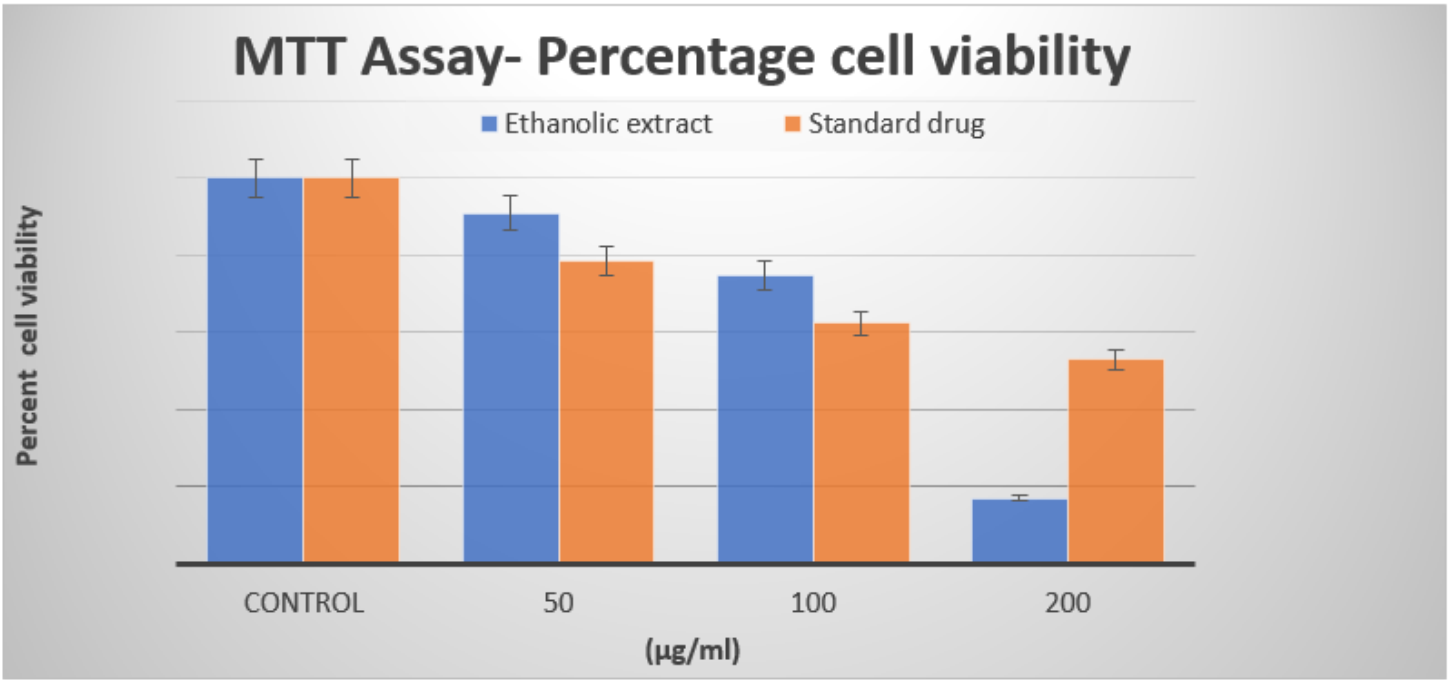

Figure 1

Effect of ethanolic extract of Cassia nemophila flowers on viability of HCT115 cell line

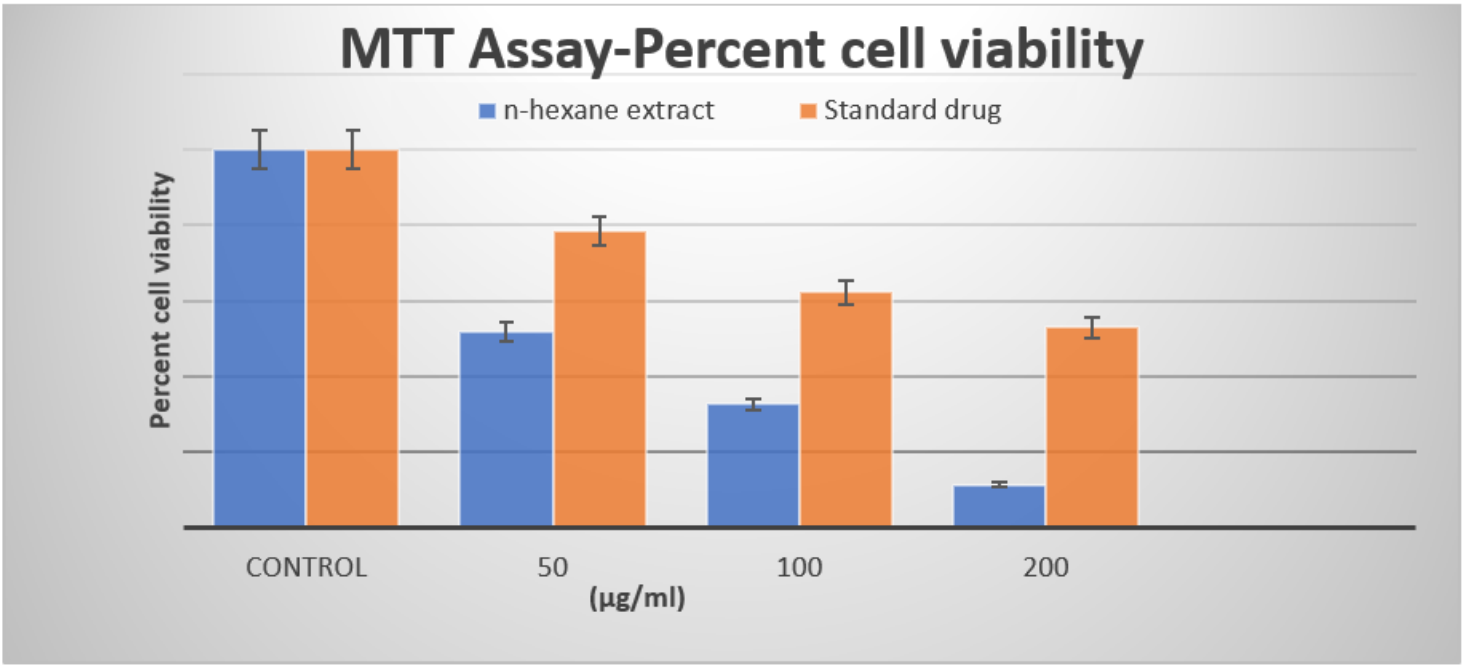

Figure 2

Effect of the n-hexane extract of Cassia nemophila flowers on the viability of the HCT115 cell line

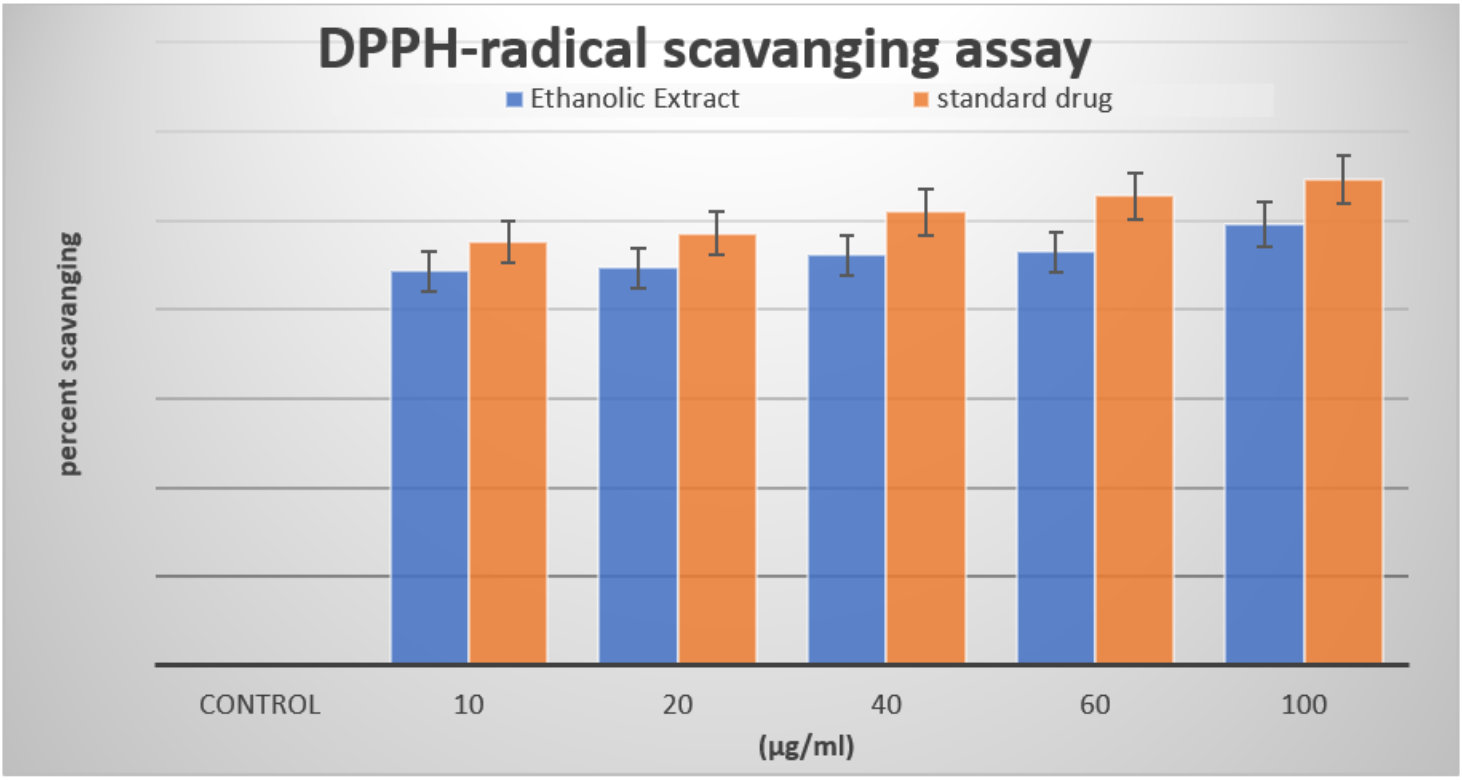

Figure 3

Antioxidant activity of ethanolic extract of cassia nemophila flowers

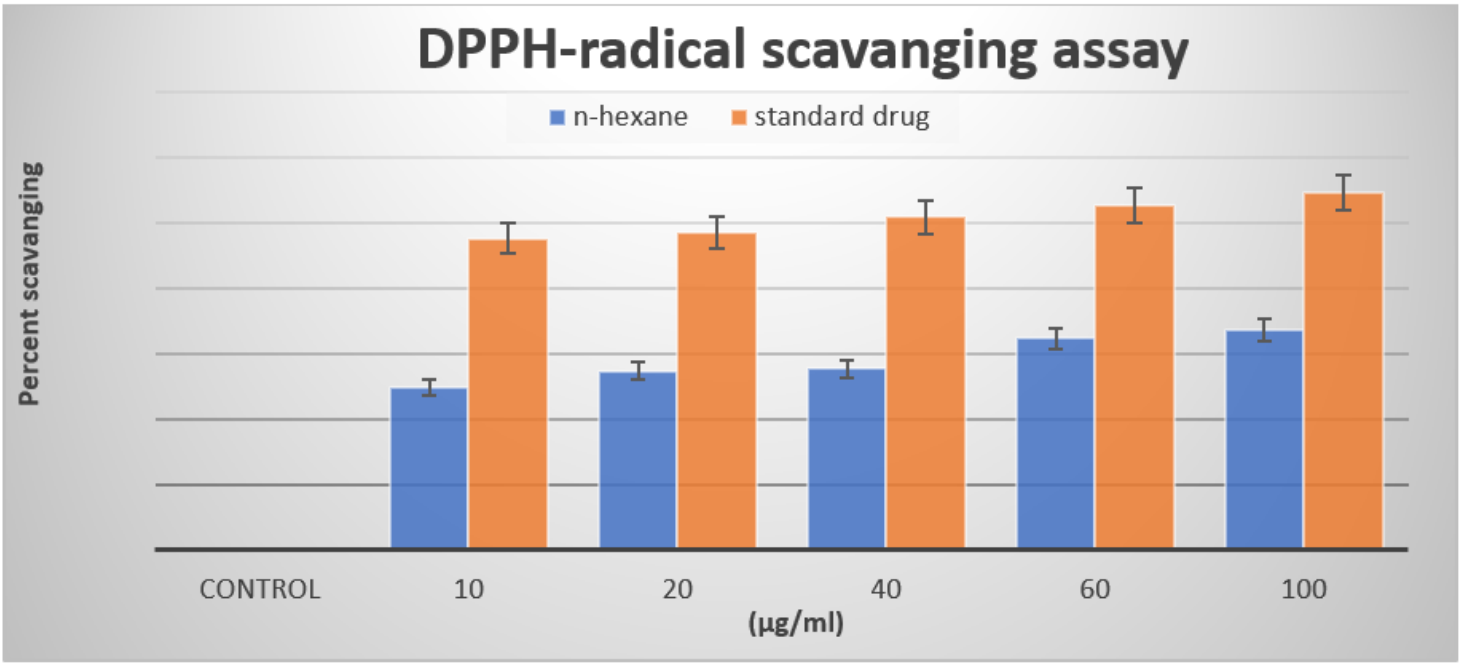

Figure 4

Antioxidant activity of the n-hexane extract of Cassia nemophila flowers

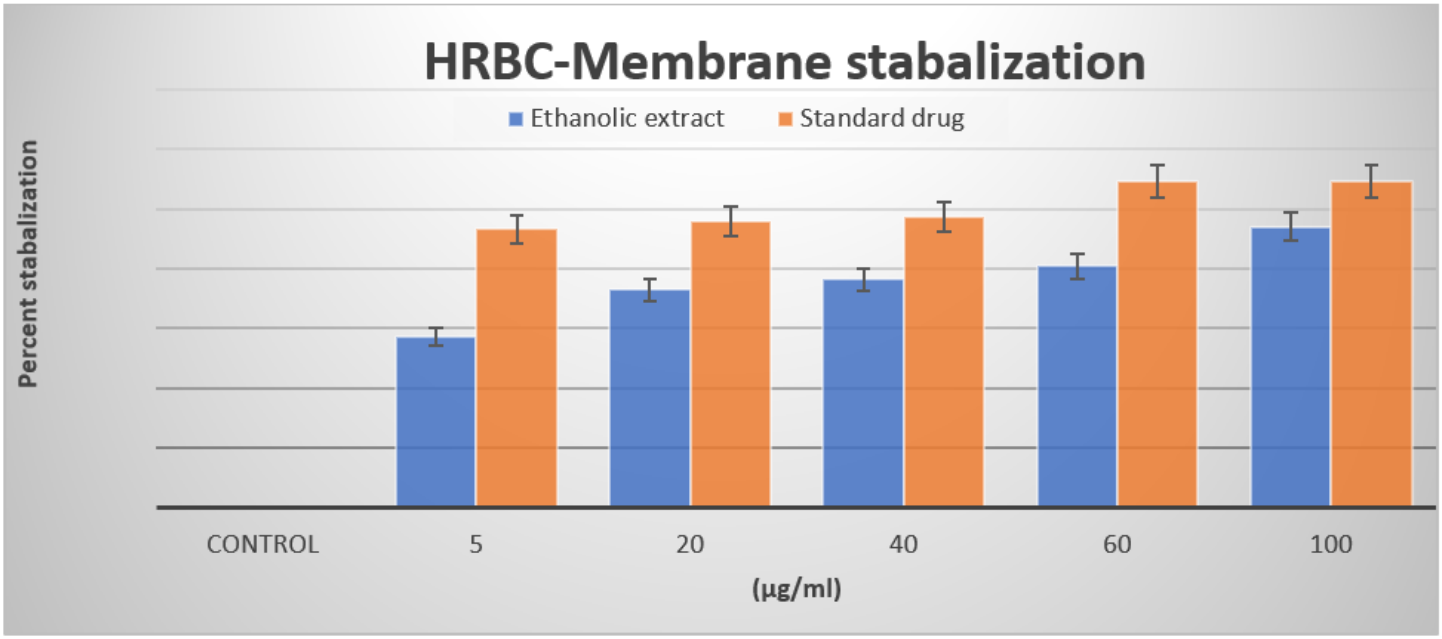

Figure 5

In vitro anti-inflammatory activity of the ethanolic extract of Cassia nemophila flowers

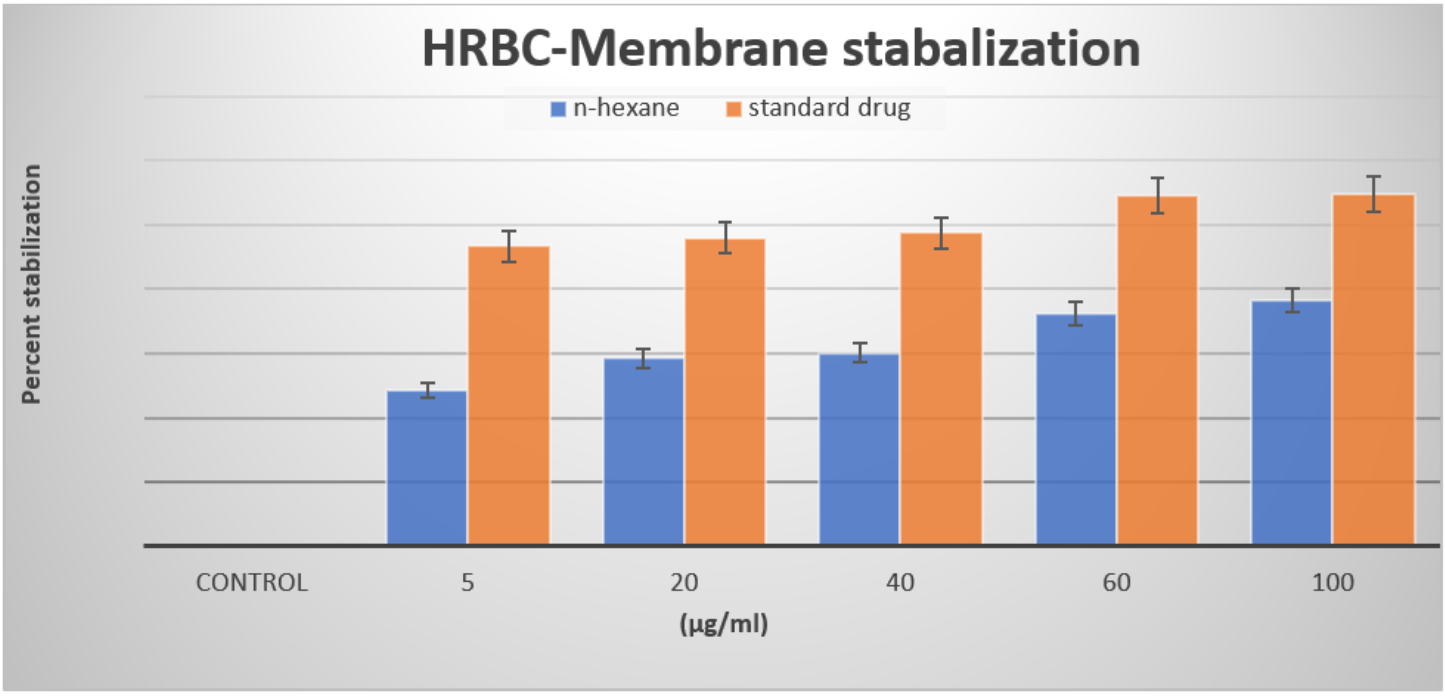

Figure 6

In vitro anti-inflammatory activity of the n-hexane extract of Cassia nemophila flowers

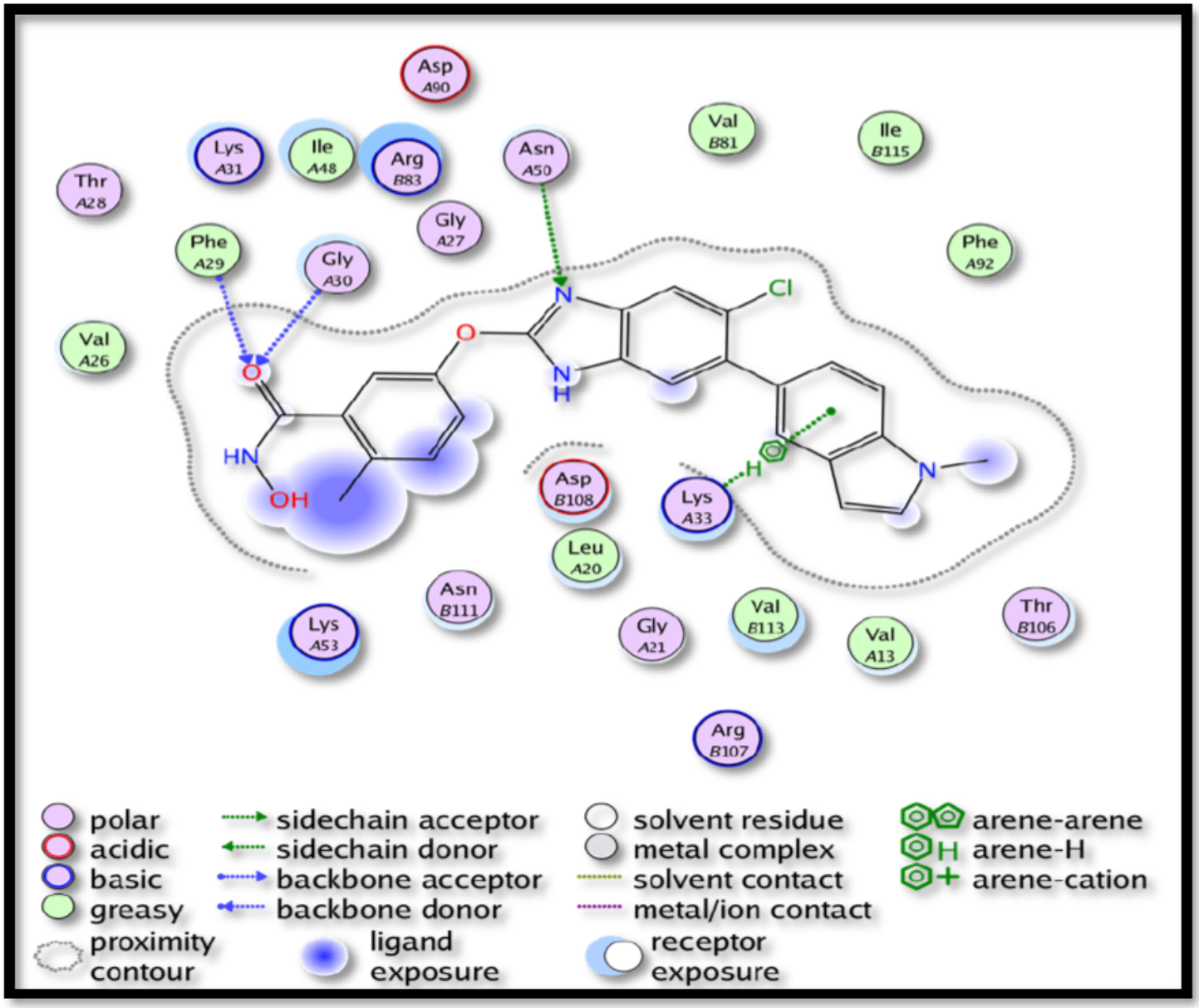

Figure 7

2D interaction of active AMPK ligand with residues.

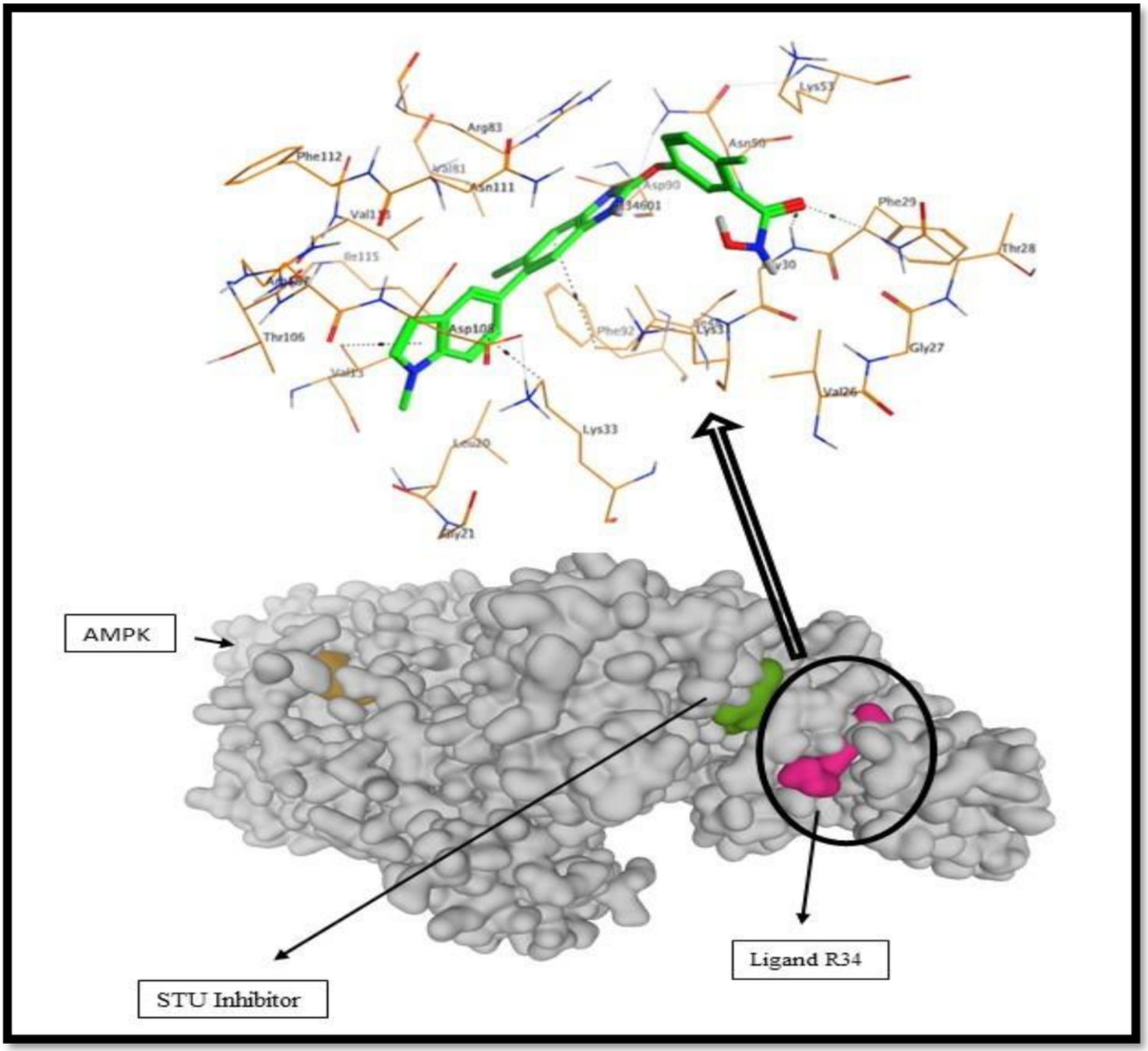

Figure 8

Surface view of Ampk and agonist meeting its active site.

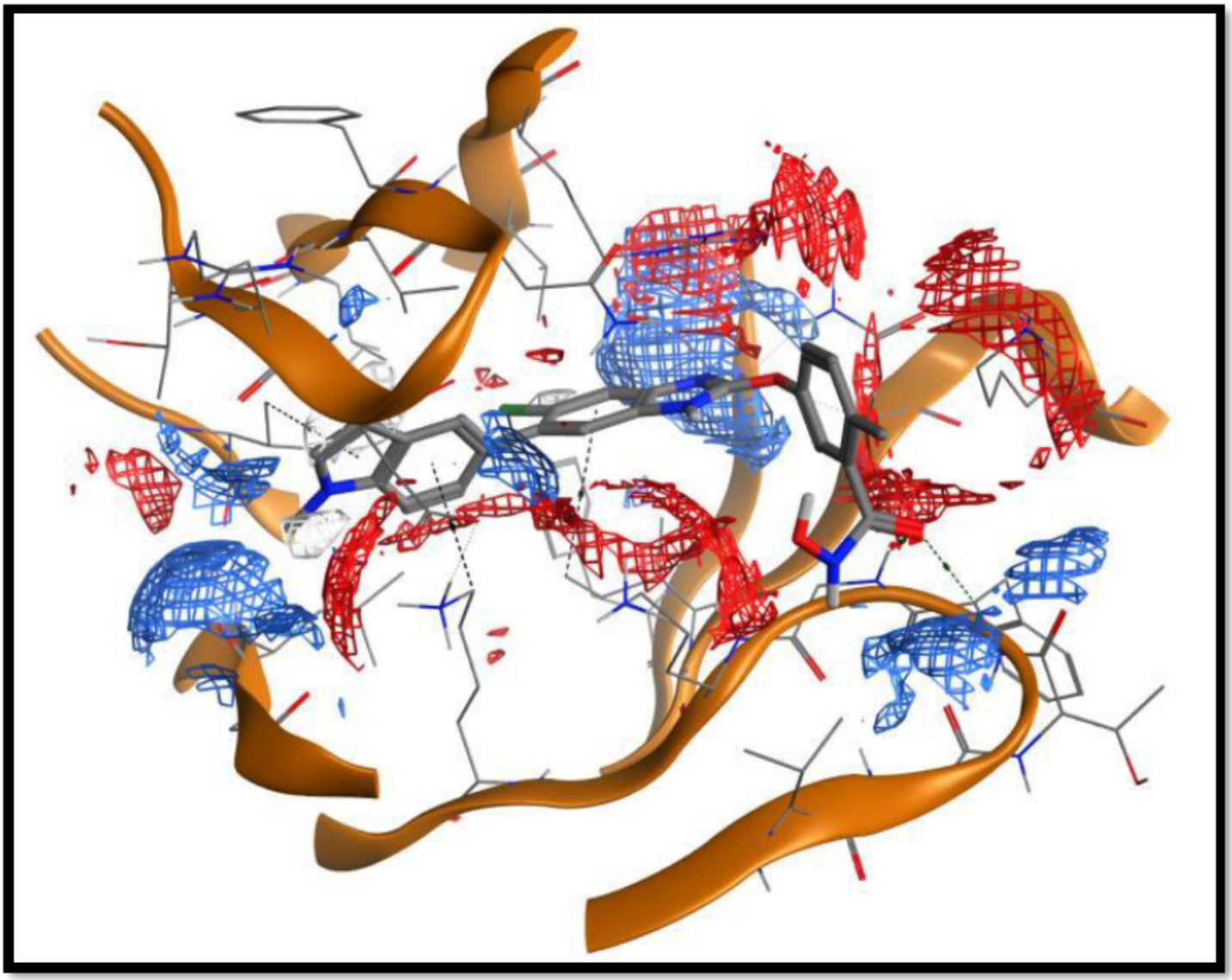

Figure 9

Electrostatic maps of the supplier of hydrogen bonds and acceptance atom active sites of AMPK.

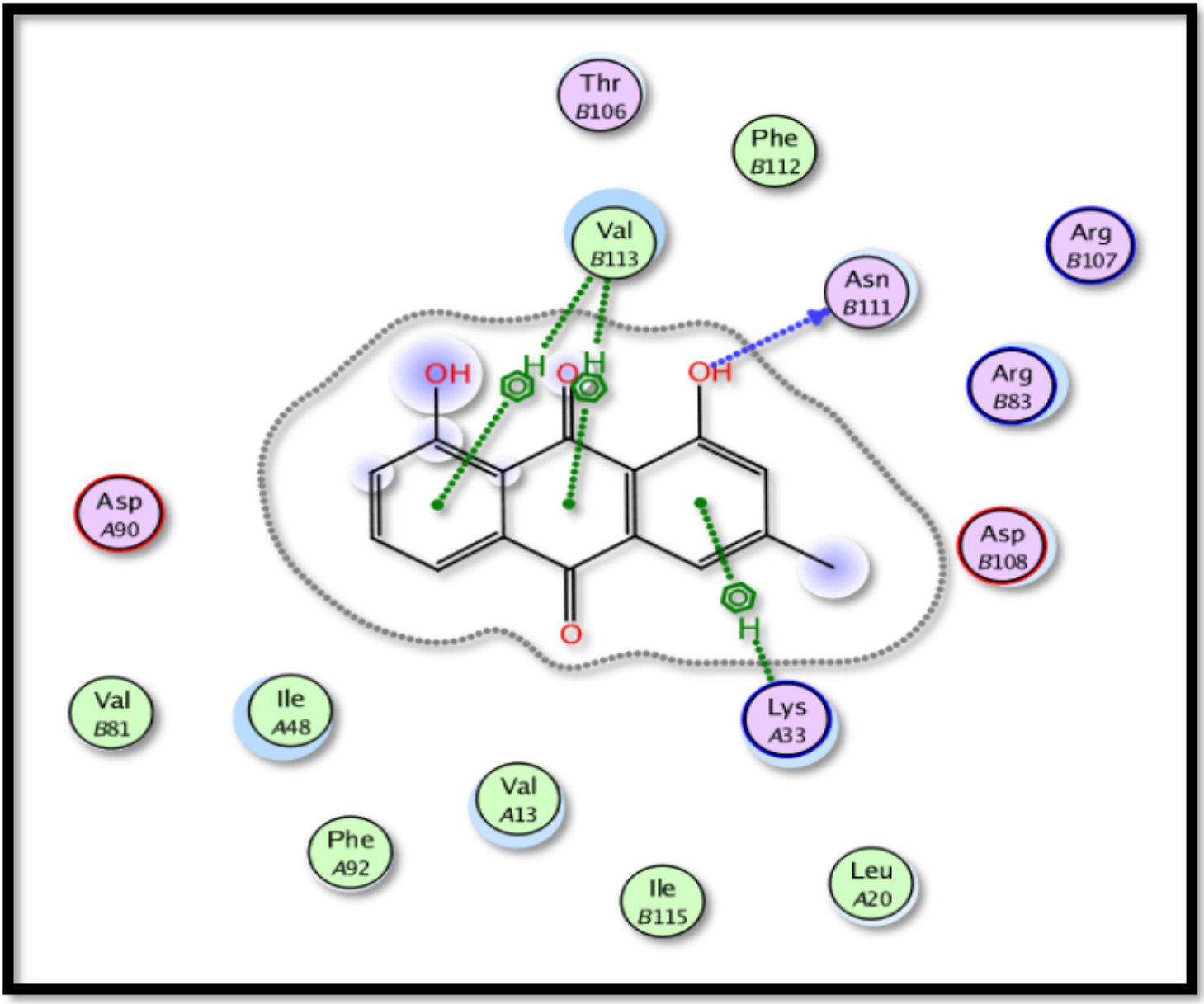

Figure 10

2D ligand interaction with functional residues of the AMPK site.

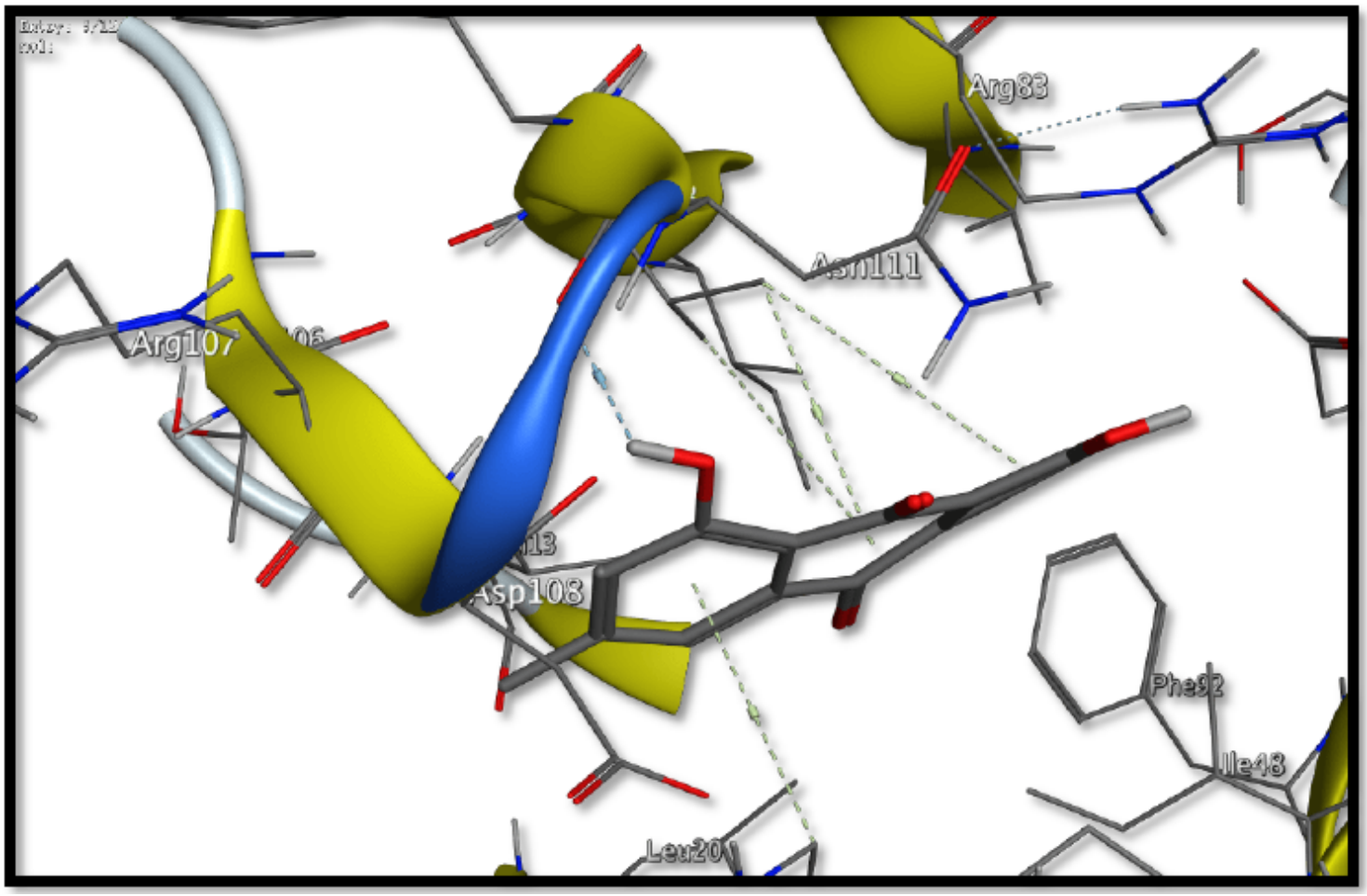

Figure 11

3D interaction with active AMPK residues.

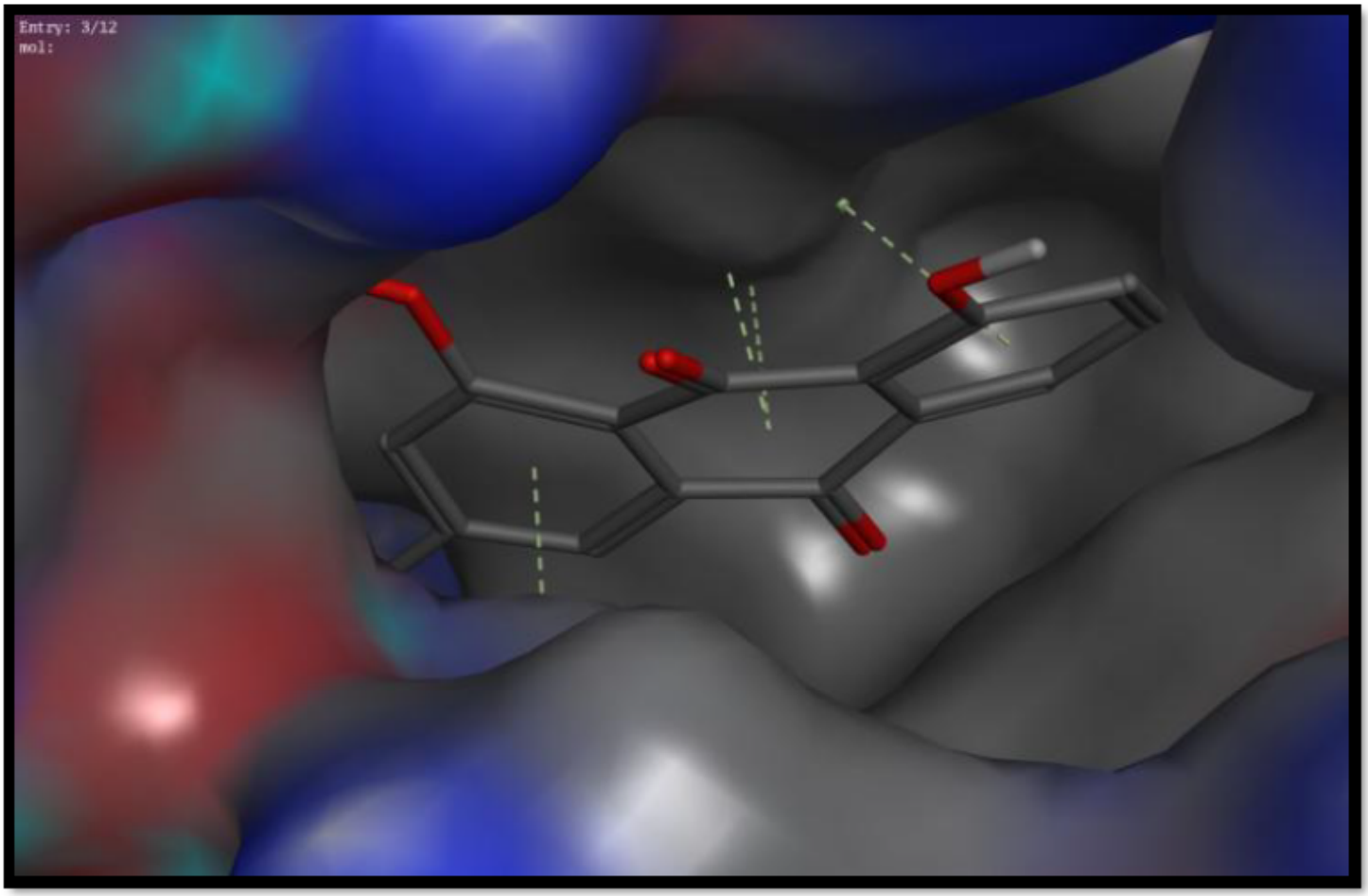

Figure 12

Surface view of ligand attachments on the active AMPK site.

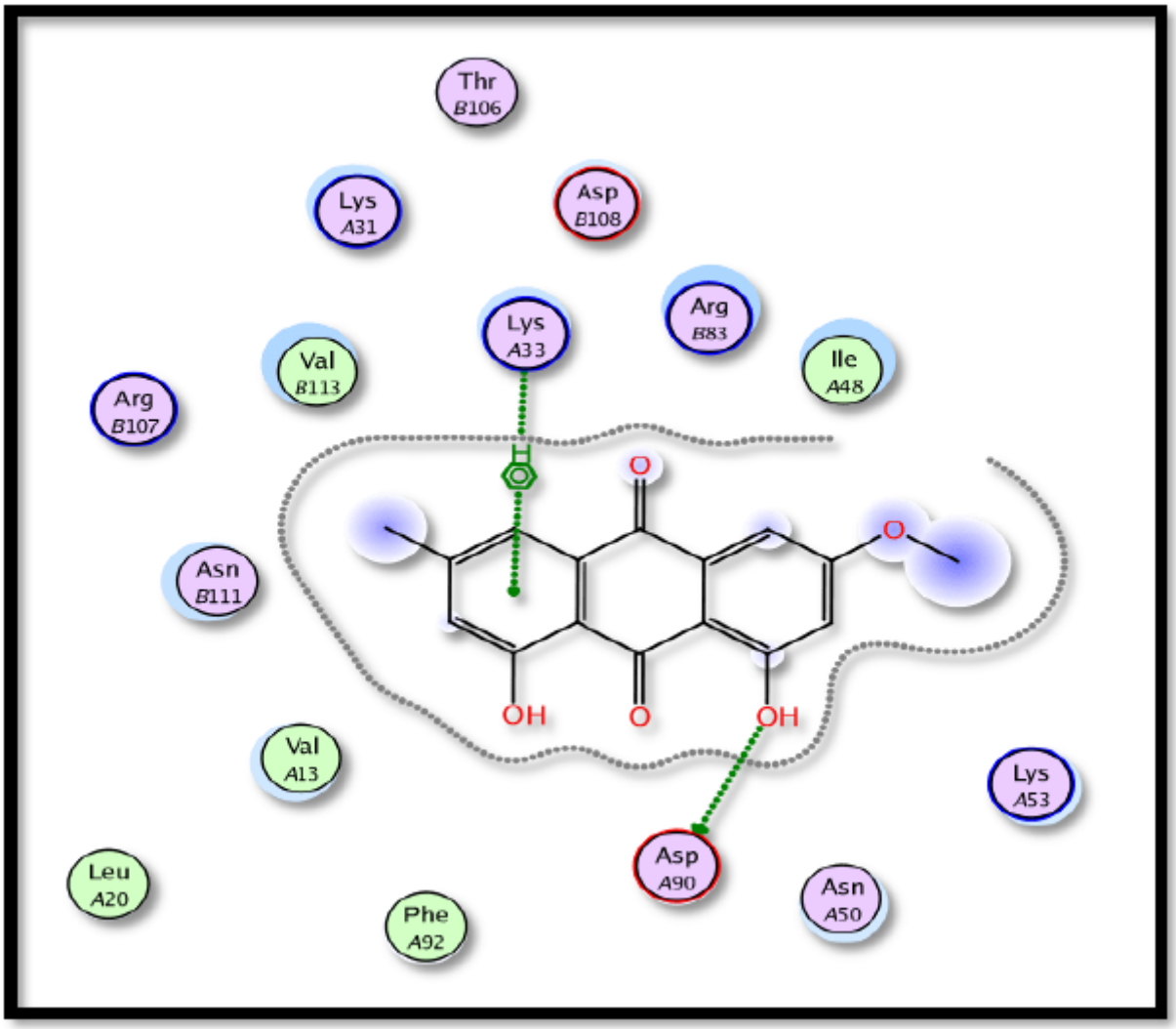

Figure 13

2D physcion interaction with functional remnants of the AMPK site.

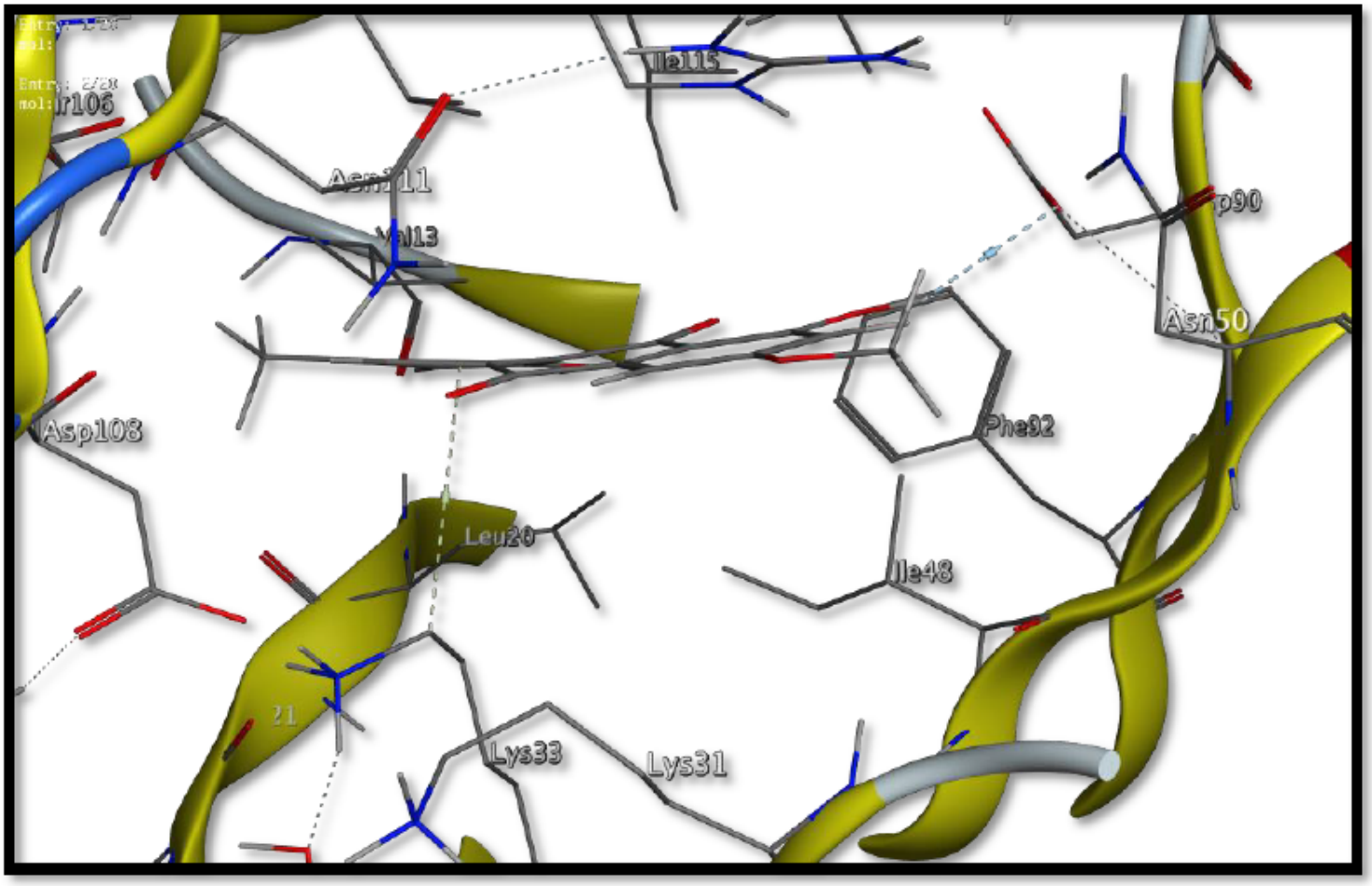

Figure 14

3D ligand interaction with functional remnants of the AMPK site.

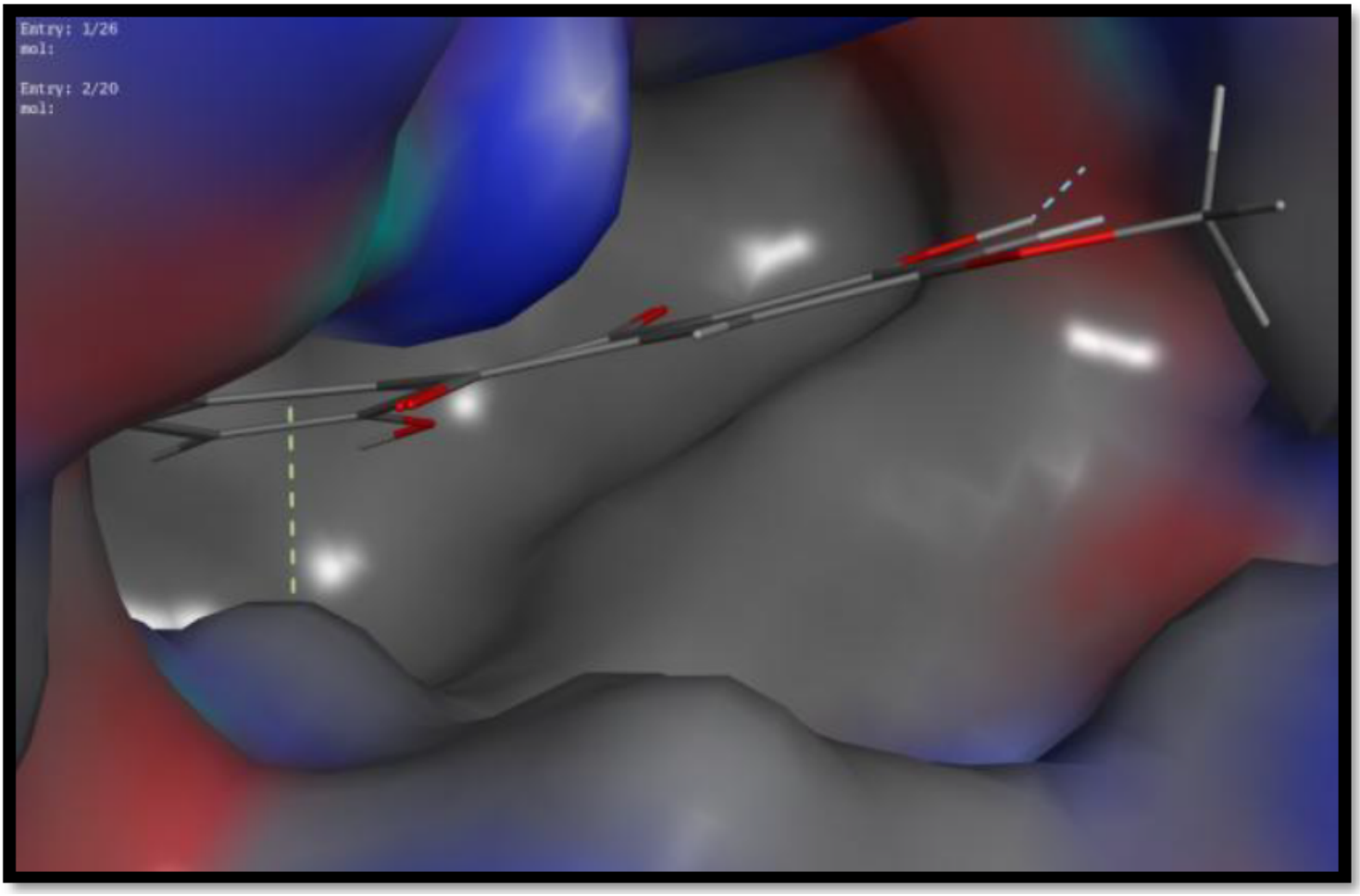

Figure 15

Surface view of ligand attachments on the active AMPK site.

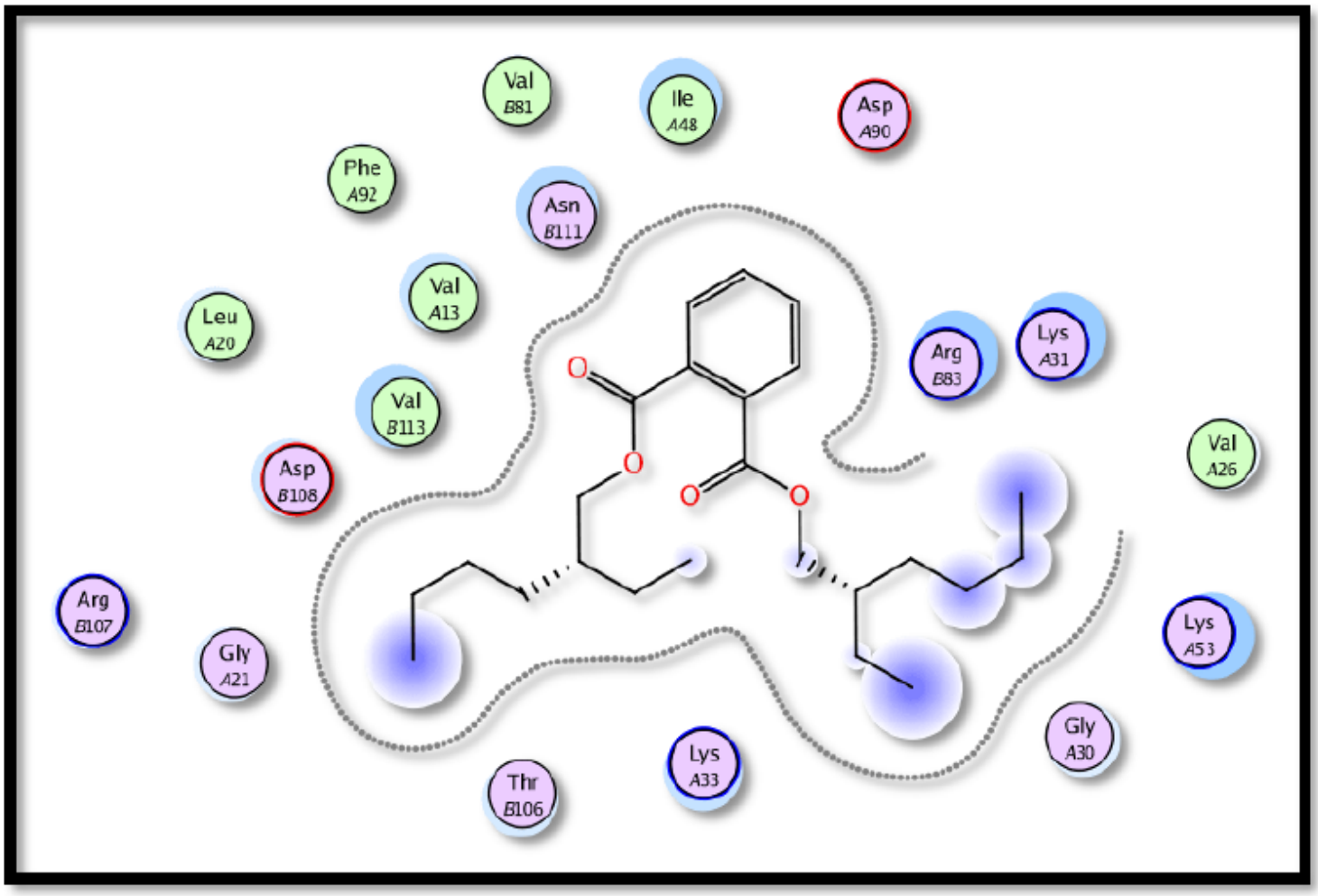

Figure 16

2D interaction of Diethylhexylphthalate with active residues of the AMPK site.

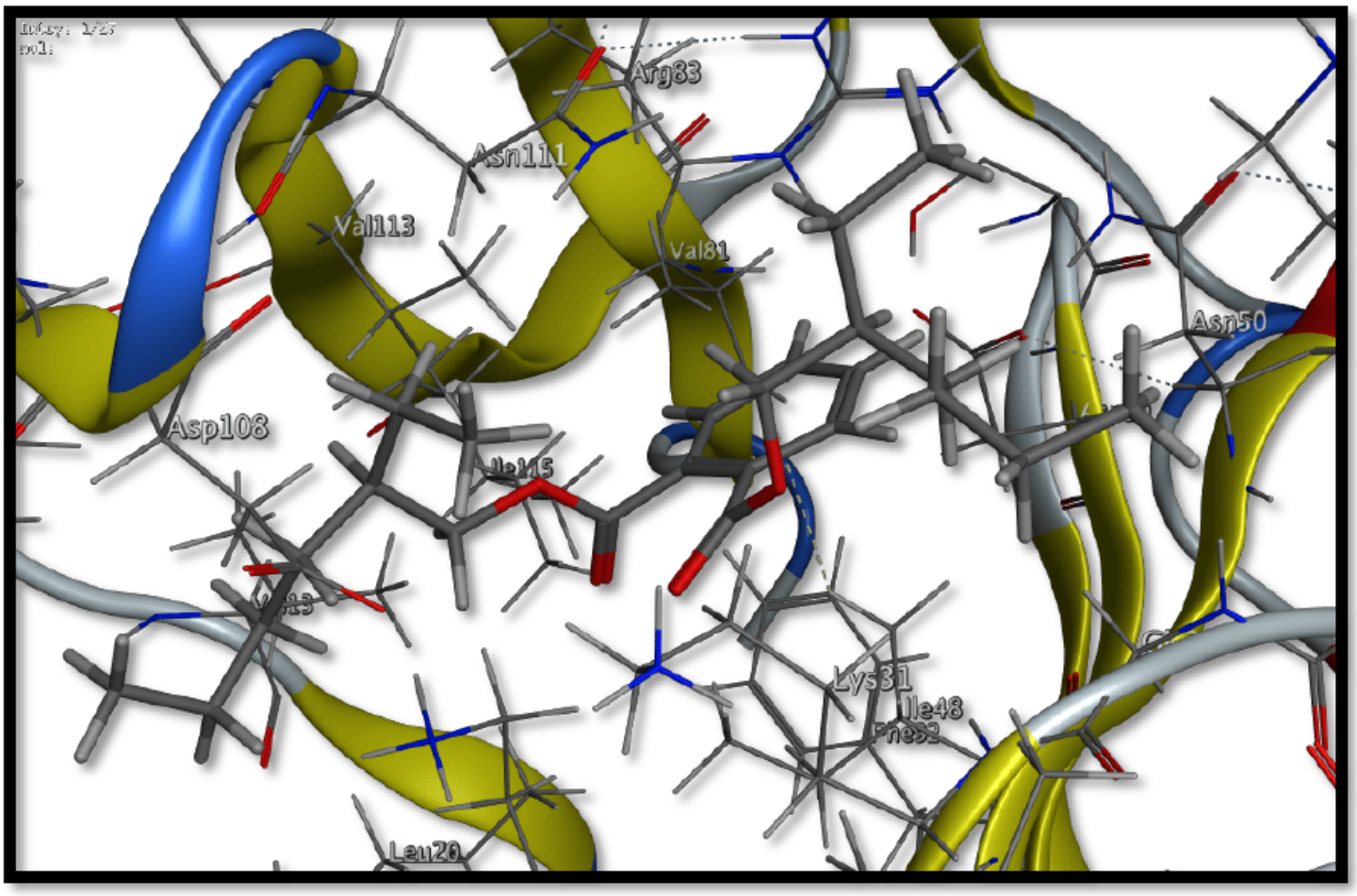

Figure 17

3D ligand interaction with functional remnants of the AMPK site.

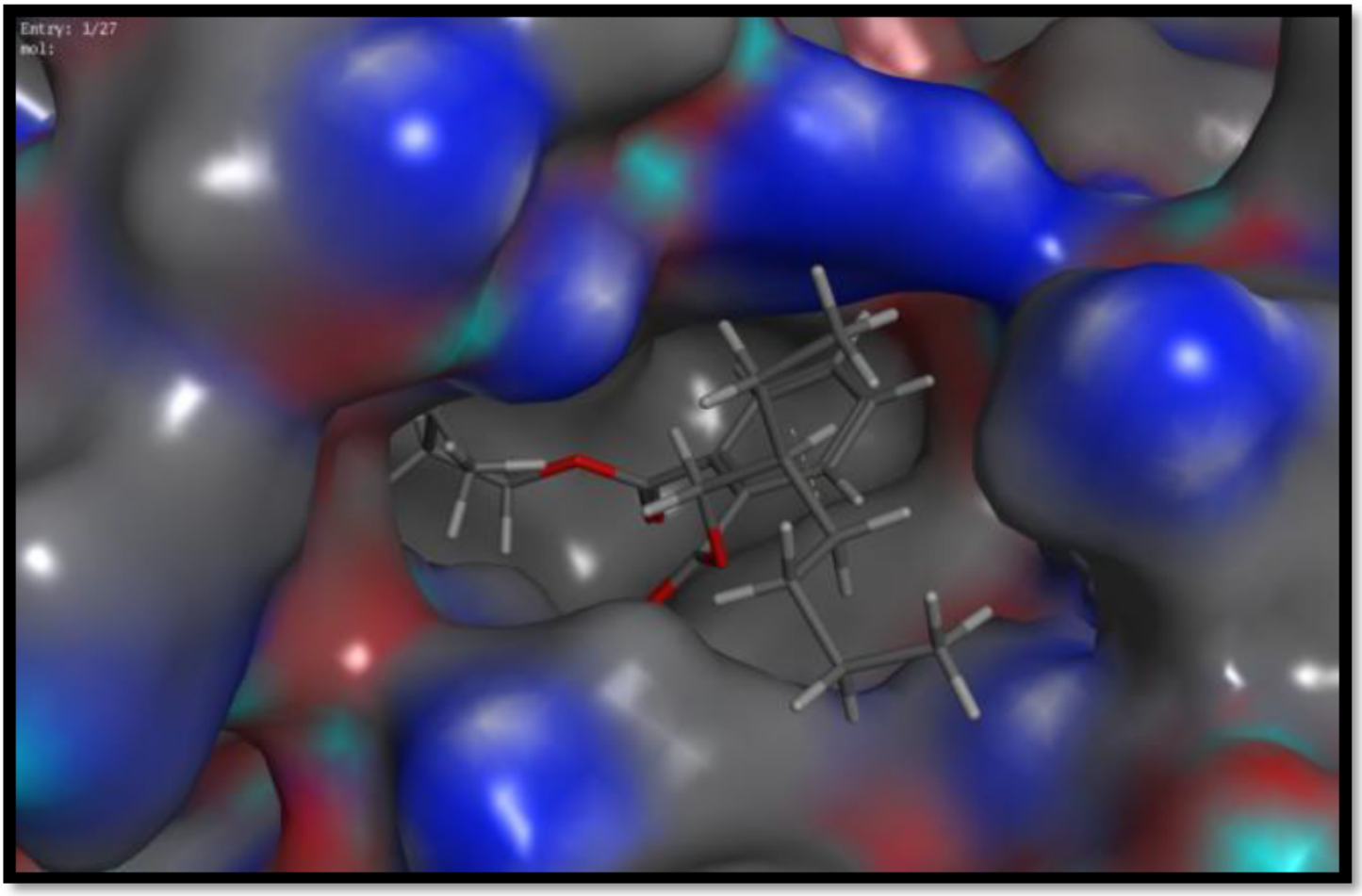

Figure 18

Surface view of ligand attachments on the active AMPK site.