Adıyaman University Journal of Science
https://dergipark.org.tr/en/pub/adyujsci
DergiPark AKADEMİK
ISSN 2147-1630
e-ISSN 2146-586X

A New Schiff Base Molecule Prepared from Pyrimidine-2-thione: Synthesis, Spectral Characterization, Cytotoxic Activity, DFT, and Molecular Docking Studies

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Received: 07.11.2021 **Accepted:** 01.04.2022 **Published:** 30.06.2022

Abstract

Schiff base derivatives are some of the most widely used organic compounds for industrial purposes and they exhibit a broad range of biological activities. In this paper, a new Schiff base derivative **(2)** synthesized from the condensation reaction of 1-amino-5-benzoyl)-4-phenylpyrimidine-2(1*H*)-thione **(1)** with 2-chlorobenzaldehyde. The new compound was characterized by ^{1}H, ^{13}C NMR, and FT-IR. The biological activity property of this compound was tested against two different cancer cell lines and a healthy human cell line. The results demonstrate that molecule **2** has antiproliferative activity. In molecular modeling studies, the interaction site was examined using the epidermal growth factor receptor (EGFR) tyrosine kinase domain. Alignment in molecular docking, surface mapping binding, amino acids, and binding energy calculated. Glide score energy was found to be -9.820 kcal/mol and it predicted that the bonding interaction is strong. Theoretical calculations were made to compare the experimental and theoretical data. These calculations were performed on the 6–31 G* basis set using the Density Functional Theory (DFT) method and Becke-3-Parameter-Lee-Yang-Parr (B3LYP).

* Corresponding Author DOI: 10.37094/adyujsci.1020171

Using this method, various parameters, such as frontier molecular orbitals, HOMO-LUMO energy levels, band gap, and chemical reactivity indices were found and interpreted.

Keywords: Cytotoxic activity; Pyrimidine-2-thione; 2-Chlorobenzaldehyde; Schiff base; Molecular docking.

Pirimidin-2-tiyondan Hazırlanan Yeni Bir Schiff Bazı Molekülü: Sentez, Spektral Karakterizasyon, Sitotoksik Aktivite, DFT ve Moleküler Doking Çalışmaları

Öz

Schiff bazı türevleri, endüstriyel amaçlar için en yaygın olarak kullanılan organik bileşiklerden bazılarıdır ve çok çeşitli biyolojik aktiviteler sergilerler. Bu makalede, 1-amino-5-benzoil)-4-fenilpirimidin-2(1*H*)-tion (**1**)'in 2-klorobenzaldehit ile kondenzasyon reaksiyonundan yeni bir Schiff bazı türevi (2) sentezlendi. Yeni bileşik, ^{1}H, ^{13}C NMR ve FT-IR ile karakterize edildi. Bu bileşiğin biyolojik aktivite özelliği, iki farklı kanser hücre hattına ve sağlıklı bir insan hücre hattına karşı test edildi. Sonuçlar, molekül **2**'nin antiproliferatif aktiviteye sahip olduğunu göstermektedir. Moleküler modelleme çalışmalarında etkileşim bölgesi, epidermal büyüme faktörü reseptörü (EGFR) tirozin kinaz alanı kullanılarak incelenmiştir. Moleküler dokingte hizalama, yüzey haritalama bağlanması, amino asitler ve bağlanma enerjisi hesaplandı. Glide skoru enerjisi -9.820 kcal/mol olarak bulunmuş ve bağlanma etkileşiminin kuvvetli olduğu tahmin edilmiştir. Deneysel ve teorik verilerin karşılaştırılması için teorik hesaplamalar yapılmıştır. Bu hesaplamalar, Yoğunluk Fonksiyonel Teorisi (DFT) yöntemi ve Becke-3-Parameter-Lee-Yang-Parr (B3LYP) kullanılarak 6–31 G* temel setinde yapıldı. Bu yöntem kullanılarak sınır moleküler orbitalleri, HOMO-LUMO enerji seviyeleri, bant aralığı ve kimyasal reaktivite indeksleri gibi çeşitli parametreler bulunmuş ve yorumlanmıştır.

Anahtar Kelimeler: Sitotoksik aktivite; Pirimidin-2-tiyon; 2-Klorobenzaldehit; Schiff baz; Moleküler doking.

1. Introduction

Schiff bases are long-known compounds that are easy to synthesize and purify. For this reason, it attracts the attention of many researchers. Therefore, many compounds have been synthesized and studied extensively. Let us examine the work done from past to present synthesis of such compounds started in 1910. Studies in these years focused on synthesis [1-6]. As the variety and number of synthesized bases increased, researchers began to study the different properties of these substances. In 1970, a series of Schiff bases was synthesized by a group of researchers. The activities of these compounds were screened against lymphoid leukemia in

mouse and Intramuscular Walker sarcoma in rats. None of the compounds showed activity against lymphoid leukemia in mouse. However, activity against Intramuscular Walker Sarcoma was detected in rats [7]. In another study, the researchers synthesized a series of Schiff bases with salicylaldehyde from various aniline derivatives under acetic acid catalysis. *In vitro* antimicrobial activities of these compounds included various Gram-positive (S. aureus, B. subtilis, B. cereus), Gram-negative (S. typhi, S. enterica, E. coli, P. aeruginosa) bacteria, and fungi (C. albicans, A. niger, and A. fumigatus). They used antibacterial cefadroxil and antifungal fluconazole as a standard drug. They also tested the cytotoxic activity of these compounds against the human colorectal carcinoma (HCT-116) cell line. They reported that the synthesized bases showed significant activity against Gram-positive, Gram-negative bacteria, and fungi. They reported that 4-((2-bromophenyl) diazenyl)-2-((4-nitrophenylimino) methyl) phenol (SBN-13) showed more cytotoxic activity against the cell line than the standard drug, 5-fluorouracil [8].

Pyrimidines and their derivatives as important fine chemicals have been frequently found in many natural products and drugs and have exhibited a wide range of biological activities, such as anticancer [9], antiviral [10], antimicrobial [11], anti-hepatitis [12] and anti-inflammatory properties [13]. Aminopyrimidine-2-one/thione derivatives appeared to be an important starting compound in synthetic organic chemistry. In recent years, the reactions of aminopyrimidine-2-one/thione derivatives with isothiocyanate [14, 15], 1,3-dicarbonyl compounds [16], aryl chlorides [17] and transition metal complexes [18, 19] have been reported. Nowadays, theoretical, and experimental comparison of N-aminopyrimidine-2-one derivatives have become popular [15, 20, 21]. Therefore, in the present study, the starting material **1** was obtained from the reaction of furan-2,3-dione and acetophenone thiosemicarbazone according to the procedure in the literature [17] (Scheme 1). In the second step, a new Schiff base **2** was synthesized and tested as *in vitro* towards human cell lines for learning it's anticancer activity (Scheme 2, Table 1).

It has been determined that the synthesis of new Schiff bases is of great importance for drug development studies [22, 23]. Recently, it has been added to theoretical (the parameters related to ground state calculations of HOMO and LUMO (electronic chemical potential and global hardness, global electrophilicity index and softness) studies in addition to experimental studies of new Schiff-based compounds [23, 24].

In cancer treatment using EGFR inhibitors, cancer cells destroyed, and the side effects of drugs reduced to a minimum. Therefore, they represent a valuable target for the design of an important class of potential anticancer agents.[25-27]. Numerous heterocyclic compounds have been reported as important tyrosine kinase (TK) inhibitors [28-31]. The cellular proliferation properties of EGFR make it heavily responsible in malignant tumors. Most of the abnormal forms

of EGFR in malignant tumors have been reported to occur in many types of cancer, such as lung cancer, colorectal cancer, breast cancer.[32-36].

Molecular docking of the synthesized compound was performed within the binding site of EGFR TK to gain insight into molecular interactions and possible modes of action. Molecular docking was performed using the EGFR protein (PDB ID: 6DUK) as a target [36]. Free energy, chemical hardness, chemical potential, dipole moment, softness, electronegativity, electrophilicity index, nucleophilicity, HOMO (highest occupied molecular orbital), LUMO (lowest unoccupied molecular orbital), and ΔE_{Gap} calculated to understand and interpret the electrostatic and chemical behavior of the synthesized compound.

2. Materials and Methods

2.1. Synthesis and characterization of a new molecule

The reagents and solvents purchased from different chemical companies and used without further purification. 1-amino-5-benzoyl-4-phenylpyrimidine-2(1*H*)-thione **(1)** and 2-chlorobenzaldehyde, used in the synthesis steps. The purities of the compounds routinely monitored using DC Alufolien Kieselgel 60 F254-Merck thin layer chromatography and Camag brand TLC lamp (254/366 nm). This study also used an electrothermal 9100 brand digital melting point device, Shimadzu Model 8400 FT-IR spectrophotometer, Bruker brand 400 MHz (for ^{1}H NMR) spectrophotometer, and 100 MHz (for ^{13}C NMR) spectrophotometer. Starting material **1** was prepared according to literature procedures [9]**.** The synthesis steps given in Scheme 1.

1-(2-Chlorobenzylideneamino)-5-benzoyl)-4-phenylpyrimidin-2(1*H*)-thione, (2)

Mixtures of compound **1** (1 mmol) 0.429 g and 2-chlorobenzaldehyde, (1 mmol) 0.141 g in 30 mL of ethanol refluxed in the presence of a catalytic amount of *p*-toluene sulfonic acid as catalyst for 6 h. The solvent evaporated after this reaction time. Then, the residue treated with dry diethyl ether and filtered. This Schiff base **2** was purified with crystallization in ethyl alcohol. Yield: 72%; m.p.: 240-242 °C; color: yellow. FT-IR: 3041 (aromatic C-H), 1677 (C=O), 1619.6-1601.9 (C=N and C=C), 1266 (C=S), 765-729 cm^{-1} (pyrim. ring). ^{1}H NMR (400 MHz, DMSO):-δ (ppm) = 9.28 (s, 1H, N=CH), 9.01 (s, 1H, pyrim. -CH) and 8.21-7.35 (m, 14H, Ar-H). ^{13}C NMR (100 MHz, DMSO): δ (ppm) = 192.02 (Ph-C=O), 190.51, 176.03, 167.00, 164.61, 146.78, 145.06, 136.62, 136.45, 136.29, 135.88, 135.58, 135.19, 135.06, 134.33, 131.45, 131.19, 131.10, 130.78, 130.49, 130.29, 130.14, 130.01, 129.70, 129.40, 129.33, 129.19, 128.89, 128.80, 128.59, 128.36, 120.53, 116.50, 112.33 (Ar-C). Elemental analysis for $C_{24}H_{16}ClN_3OS$ (429.92 g/mol) %: Found C: 66.95, H: 3.95, N: 9.60, S: 7.30. Anal. Calc. C: 67.05, H: 3.75, N: 9.77, S: 7.46.

2.2. *In vitro* cytotoxic activity studies

Human colon cancer cell line (DLD-1) (ATCC® CCL-221™), human liver hepatocellular carcinoma cell line (HepG2) (ATCC® HB-8065™), and human normal lung cell line (Wl-38) (ATCC® CCL-75™) were purchased from American Type Culture Collection (ATCC, USA).

The cytotoxic activity studies performed completely according to the literature [37, 38]. The DLD-1, HepG2, and Wl-38 cells were seeded into sterile 96-well plates at a density of 5 x 10^3 cells/well. After 24 h, cells were exposed to the Schiff base compound for 48 h. After this period was completed, 5 mg/mL of MTT stock solution was added to each well and the plates were incubated for 3 h.

2.3. Computational details

2.3.1. DFT calculations

Theoretical calculations for the **molecule 2** were calculated using the B3LYP/6 -31 G* basis cluster method [39] in the Spartan'08 package program [40]. Minimized structural parameters, HOMO, LUMO, ΔE_{gap}, chemical hardness (η) [41], chemical potential (μ) [42-44], dipole (debye) , softness (σ), electronegativity (χ) [41], electrophilicity index (ω), nucleophilicity (ε), energy values were determined in the vacuum [45, 46].

2.3.2. Molecular docking study

The Maestro software in the Schrödinger 2021-2 Glide program [47] was used to perform all molecular docking studies [48]. Before the molecular docking study, the crystal structure of the enzyme and the preparation [49] of the ligand are included. The crystal structure (PDB ID: 6DUK) to use as the EGFR obtained from the RCSB Protein Data Bank (https://www.rcsb.org/structure/6DUK). Water molecules optimized by removing heteroatoms and co-factors. **Molecule 2**, used as the ligand was prepared and optimized using the LigPrep wizard [50] of the Schrödinger Software Suite and minimized using the OPLS2005 force field [48]. Grid box size was set to 20 Å Radius using the Grid Generation receptor implemented in Glide [48]. Molecular docking calculations performed using the Standard Precision (SP) mode.

2.3.3. In silico ADME prediction

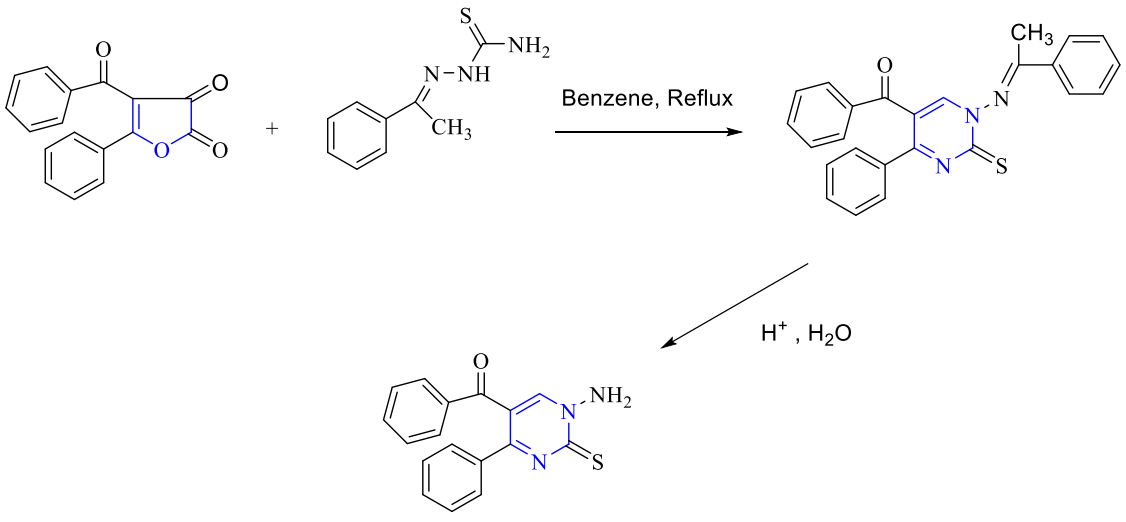
The synthesized compound Schrödinger Maestro 2021-2 QikProp [51] was used to calculate the synthesized compound's Absorption, Distribution, Metabolism, and Excretion (ADME) properties, which were selected together with the standard drug Lapatinib, for its pharmacological properties, such as drug similarity. Various predicted pharmacological parameters calculated.

3. Results and Discussion

3.1. Experimental

In this study, N-aminopyrimidine-2-thione derivative 1 was used as a key starting material. Compound **1** was prepared according to the literature method by a two-step process (**Scheme 1**) [9]. Reaction of **1** with 2-chlorobenzaldehyde afforded the Schiff base derivative **2** in satisfactory yields (76%) (see experimental section) (**Scheme 2**). The moderate yield of the reaction can be explained by the chemical behavior of aminopyrimidine-2-thione **1** towards the aromatic aldehyde. The carbonyl group represents the electrophilic site in the molecule of the aromatic aldehyde, which can interact with a nucleophile [13-16] (**Scheme 2**).

Molecule **2** was obtained from the reaction of compound **1** and 2-chlorobenzaldehyde in 76% yield. In the FT IR spectrum of molecule **2**, the C=O and C=S absorption bands observed at 1677 and 1266 cm^{-1}, respectively. The signals of N=CH and pyrim. -CH protons were observed at 9.28, 9.01 ppm as singlets in the ^{1}H NMR spectrum of molecule **2**. The aromatic protons of **2** were observed in the 8.21-7.35 ppm region. A resonated signal recorded by ^{13}C NMR spectrum at 192.02 ppm due to the presence of the (ph-C=O) group. Aromatic carbons of **2** were determined in the 190.51-112.33 ppm region. The results of measurements of **2** are given in the experimental section. The general outline of the reaction studied shown in **Scheme 1**.



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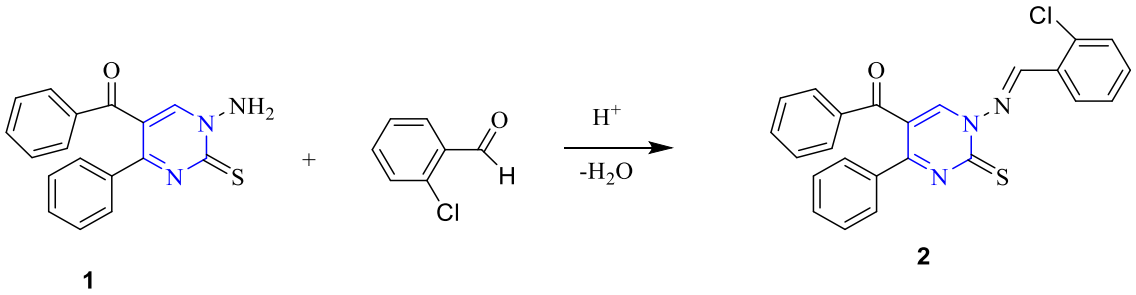
1-Amino-5-benzoyl-4-phenylpyrimidine-2(1*H*)-thione

Scheme 1: Synthesis of the compound **(1)**



Scheme 2: Synthesis of the compound **(2)**

3.2. *In vitro* cytotoxic activity studies

The cytotoxic activity of the Schiff base was tested at 5, 10, 20, 50, 100, and 200 µM concentrations against DLD-1, HepG2, and Wl-38. The results are given in Table 1.

Table 1: IC_{50} results of molecule in cell lines

Compounds	IC_{50} (µM)		
	HepG2	DLD-1	Wl-38
2	91.90	169.90	40.90
Cisplatin	65.23	77.04	28.71

As seen in the results in Table 1, molecule **2** had cytotoxic activity on the HepG2 and DLD-1 cancer cell lines with IC_{50} values of 91.90 and 169.90 µM, respectively. This molecule was found to have a more toxic effect on liver cells than on colon cells. Developed molecule **2** was found to have less activity against both cell lines than cisplatin, which was used as a positive control. Furthermore, molecule **2** did not show selectivity when tested on healthy lung cells, Wl-38. It inhibited the proliferation of the Wl-38 cells. The dose-dependent effect of molecule **2** on cell viability is given in Fig. 1.

In our recently published work, similar eight compounds were synthesized and tested against colon (DLD-1) and breast (MDA-MB-231) cancer cell lines for 48 h [52]. Except for one, the others showed similar results to the study here. The compound, namely 1-(2,4-dichlorobenzylideneamino)-5-benzoyl-4-phenylpyrimidin-2(1*H*)-one, demonstrated higher toxic effect on colon cancer cells in comparison to cisplatin, with an IC_{50} value of 34.41 µM. In a conducted study by Devim and coworkers, similar pyrimidine-based compounds were developed and screened against breast and colon cancer cell lines for 48 h [53]. They found that the IC_{50} values of the molecules ranged from 118.90 µM to >200 µM in the DLD-1 cell line. In this study, we found that the efficacy of the molecule **2** was in this range against colon cancer cells with IC_{50} value of 169.90 µM.

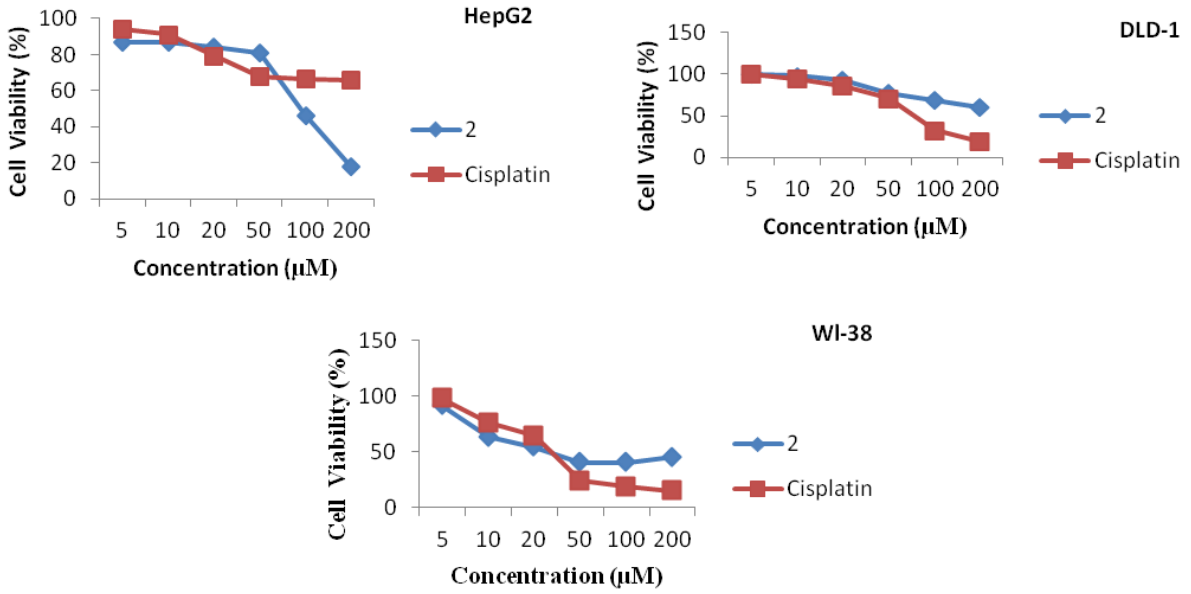


Figure 1: Dose-dependent antiproliferative effect of molecule **2** and cisplatin on HepG2, DLD-1, Wl-38 cells for 48 h

As seen in Fig. 1, the viability ratio of cells changed depending on the concentrations tested of molecule **2** and cisplatin for the three cell lines. The highest cell viabilities were seen in 5, 10, and 20 µM concentrations. Viability rates decreased further in concentrations after 20 µM. At 100 µM concentration of molecule **2**, cell viability ratios were calculated as 46.48%, 68.56%, and 40.49% for the HepG2, DLD-1, and Wl-38 cells, respectively. At 200 µM concentration of molecule **2**, the viability rates decreased further and were calculated as 17.86%, 60.13%, and 45.10% for the HepG2, DLD-1, and Wl-38 cells, respectively. Some pictures taken for the HepG2 cells are given in Fig. 2.

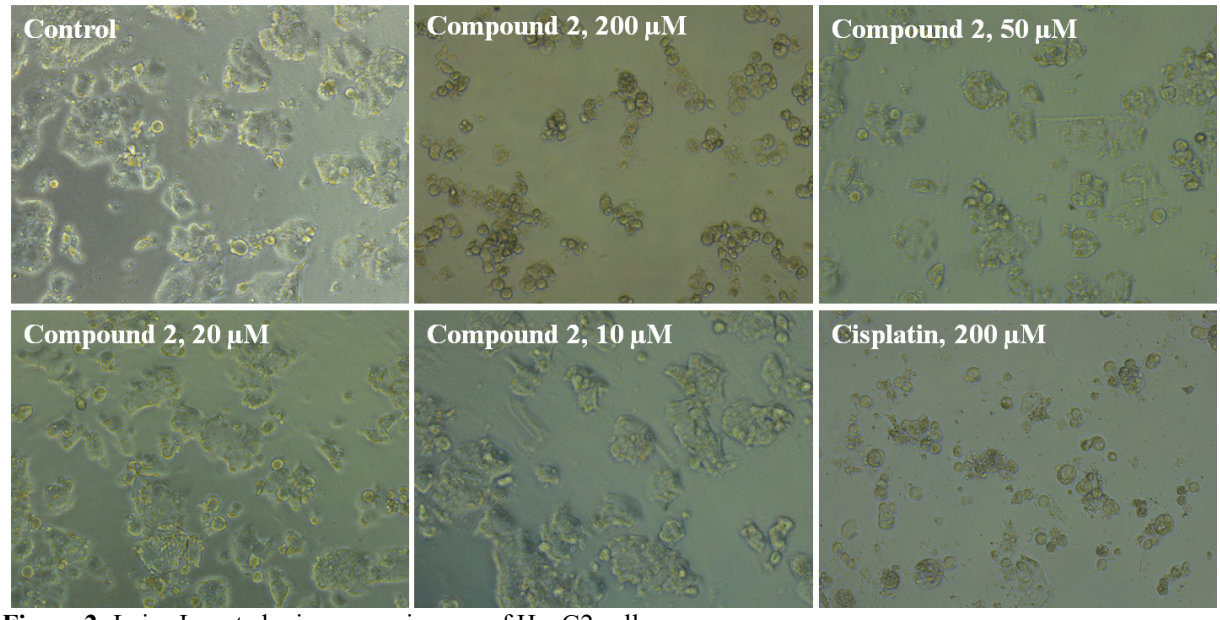


Figure 2: Leica Inverted microscopy images of HepG2 cells

It is seen that the cells in the control grow normally (Fig. 2). However, the cells affected by molecule **2** and cisplatin varied depending on the concentration. It is seen that cell viability rates are high at low concentrations, while viability rates are very low at high concentrations.

3.3. DFT calculations

Quantum chemical calculations used to explain the electronic and chemical properties of compounds. Molecule **2** B3LYP/6 -31 G* optimized in the gas phase using the DFT method. The purpose of calculating the frontier molecular orbital energies leads to the identification of the chemically active sites of the molecule. The value of the energy gap plays an important role in the case of organic molecules as it relates to the specific movement of electrons from one energy state to another [54]. In Fig. 3, the energy gap value of molecule **2** is calculated as 3.1896 eV. The energy gap reveals the stability important for the structure [55] and reflects the chemical activity of the molecule [56].

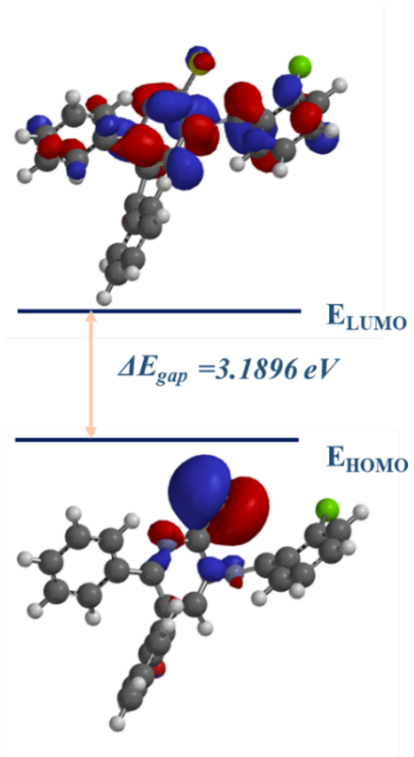


Figure 3: Frontier molecular orbitals of (HOMO-LUMO) with the energy gap of the molecule **2** in vacuum

It is responsible for the kinetic energy and chemical reactivity of the compound, where the energy gap between the HOMO-LUMO orbitals defines the chemical stability and reactivity of the molecule [57]. The energy difference between the investigated compound's HOMO-LUMO is 3.1896 eV. This value indicates that the compound is soft, unstable, and reactive. In Fig. 3, the energy difference between HOMO and LUMO is the data used to interpret the

properties of molecule **2,** such as activity and reactivity. Initially, the structure and optimal energy of studied molecule **2** were calculated using the DFT theory**.** Table 2 shows the calculated energies of the different frontier molecular orbitals and the molecular reactivity descriptors for ΔE_{Gap} and molecule **2**.

The electrophilicity index, ω value of the molecule, is a value of its electron ability take. A high ω value indicates good electrophilicity, while a low ω value indicates a poor electrophile [58].The ω value found is 2.7528eV (strong electrophiles with ω> 1.5 eV [59]), confirming that it is a strong electrophile. The chemical potential, μ gives information about the reaction mechanism. Through the parameters specified in Table 2, the reaction mechanism of molecule **2** can be predicted by defining the interactions and bonds.

Table 2: The parameters of studied molecule **2**

Parameters	**Vacuum**
Energy	-5308751.29 kcal/mol
E_{HOMO}	-5.4781 eV
E_{LUMO}	-2.2885 eV
ΔE_{Gap}	3.1896 eV
I	5.4781 eV
A	2.2885 eV
Chemical Hardness (η)	2.7391
Dipole (debye)	6.8673
Chemical Potential (μ)	-3.8833
Softness (σ)	0.3651
Electrophilicity index (ω)	2.7528
Electronegativity (χ)	3.8833
***Nucleophilicity* (ε)**	0.3633

3.4. Molecular docking study

The purpose of molecular docking analysis was to define parameters such as the interaction region, binding energy, Glide gscore, docking score, and glide emodel in the binding region of molecule 2, which is the ligand in our study with the suitable protein. Figure 4 shows the binding site of the ligand candidate, which can be an EGFR TK inhibitor, after interacting with 6DUK in silico. Accordingly, amino acid residues in the binding site were determined. In Fig. 5-a, it interacts with amino acids such as Ala743, Ile744, Leu747, Glu749, Thr751, Ile853, Thr854, Asp 855, Gly857, Leu858, and Leu861 in the active binding site. In

addition, there is an H bond interaction with Lys745 amino acid and H_2O and a π-π interaction with Phe856 amino acid.

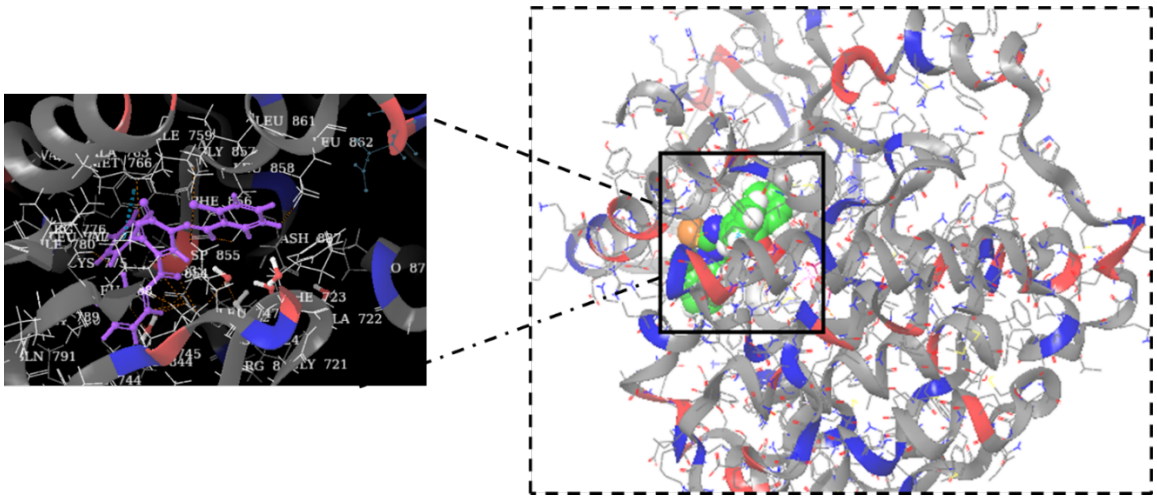


Figure 4: The binding mode of the compound used as molecule **2** with EGFR TK and the binding pattern in its active site

Descriptor parameter values in the binding region such as Glide energy, energy, Glide gscore, docking score, Glide emodel, Glide Lipo, Glide evdw, and Glide erotb given in Table 3. Here, among the conformers of molecule **2**, the conformer with the highest Glide gscore and the best active binding site was selected. With these values, the inhibitory property of ligand molecule **2** on EGFR TK can be interpreted.

Table 3: Parameter values found because of molecular docking of the molecule **2**

Parameters	**Values**
Glide Energy	-44.683 kcal/mol
Energy	45.229 kcal/mol
Glide Gscore	-9.820 kcal/mol
Docking Score	-9.820 kcal/mol
Glide Emodel	-44.854
Glide Lipo	-5.091
Glide evdw	-42.427
Glide Erotb	0.538

The interaction of the 6DUK protein downloaded from the protein data bank (https://www.rcsb.org/) with the **JBJ 1103** ligand is shown in Fig. 5-b. Figure 5-b, it was clearly observed that the ligand **JBJ 1103** entered the same pocket region as molecule **2**.

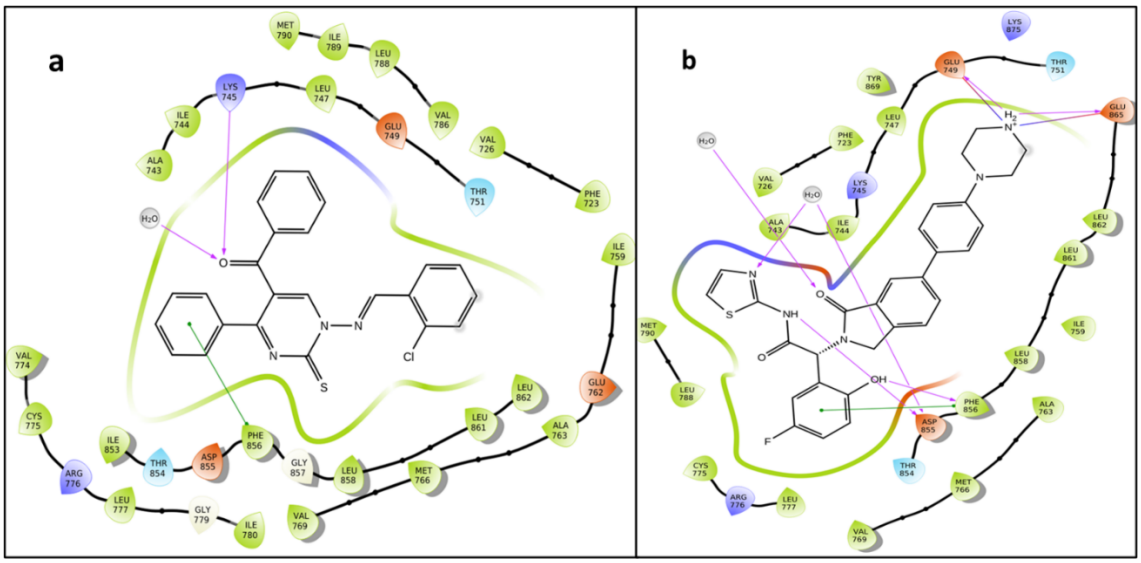


Figure 5: 2D structures of ligands docked into the PDB ID: 6DUK receptor, respectively: a) Molecule **2**, b) Interaction of JBJ 1103 ligand

In Fig. 6, the binding interaction of the ligand in the binding site shown with the binding surface and the electron charge distribution there is determined. It understood from which region the ligand enters the active site of the 6DUK protein and what type of electron density it has.

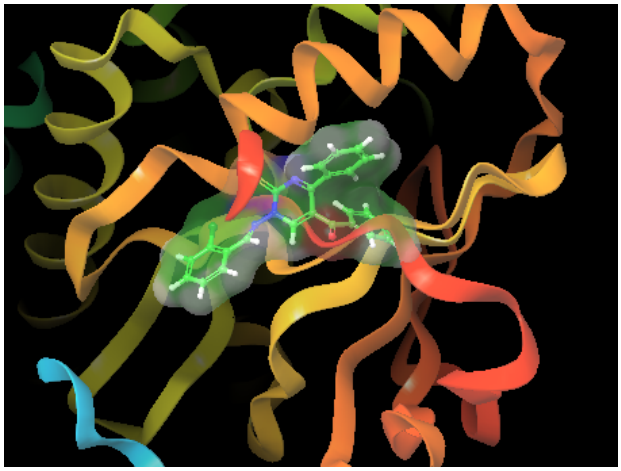


Figure 6: Docked pose of molecule **2** with EGFR (PDB ID: 6DUK)

3.5. In silico ADME prediction

To better understand the general properties of the synthesized compound, lipophilicity (octanol/water distribution coefficient, log P) calculated using the Schrödinger Maestro 2021-2 program. Theoretical estimation of the ADME properties of the compound (molecular weight, log P, number of hydrogen donors and acceptors, etc.) was performed and is shown in Table 4.

Table 4: Physicochemical properties of the synthesized compound for "drug similarity" for the standard drug lapatinib predicted by QikProp

Physicochemical Properties	Compound	Lapatinib
MW	429.923	581.060
SASA	714.633	859.153
HBD	0.000	1.000
HBA	8.250	6.500
QPloGPoct	20.069	27.196
QPlogPw	10.489	12.945
QPlogPo/w	5.249	5.842
QPlogS	-5.951	6.358
#rotor	10	6
QPlogBB	-0.088	-0.069
QPPMDCK	7666.211	3887.880
QPlogKhsa	0.367	0.189
PSA	55.651	42.261
QPlogKp	-0.210	-2.640
Rule of 5	1	2
Rule of 3	1	1

4. Conclusion

In this study, new benzylideneamino-pyrimidin-2(1H)-thione derivative, including pyrimidine core, was synthesized from the condensation reaction of aminopyrimidin-2(1H)-thione derivative 1 with 2-chlorobenzaldehyde (Scheme 2). The structure of this molecule **2** was determined from the FT-IR, 1H, 13C-NMR spectroscopic data, and elemental analysis. Furthermore, the newly synthesized compound was tested against three different cell lines, and it was found to have a toxic effect on cancer cells. In addition to the synthesis and cytotoxicity studies of molecule **2**, theoretical calculations, such as DFT, molecular docking, and ADME were made. Atomic charges of molecule **2** studied by the DFT method and values, such as HOMO-LUMO energies, ionization potential, electron affinity, electronegativity, electrophilicity index, hardness, and chemical potential calculated.

The molecular docking studies revealed that molecule **2** interacts strongly with the EGFR TK protein, with a Glide gscore and docking score of -9.820 kcal/mol. The docking score of -9.820 kcal/mol shows that it interacts well with Lys745 and H_2O through hydrogen bonding and that the π-π interaction with Phe856 is good. In addition to the molecular docking studies, ADME studies were compared with the data obtained by comparing ligand molecule **2** with the commercially available Lapatinib drug.

Acknowledgements

Burçin Türkmenoğlu would like to thank Erzincan Binali Yıldırım University, Basic Sciences Application and Research Center (EBYU-EUTAM) for the Schrödinger Maestro 2021-2 program. Senem Akkoç would like to thank Suleyman Demirel University Research Fund (TSG-2021-8458) for financial support.

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