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Medicinal Chemistry & Drug Discovery

Synthesis, In Vitro Cytotoxicity, and DFT Studies of Novel 2-Amino Substituted Benzonaphthyridines as PDK1 Inhibitors

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The present work emphasizes the utility of 2,4-dichloro-5-methylbenzo[*h*][1,6]naphthyridine as starting precursors. The reaction of 2,4-dichloro-5-methylbenzo[*h*][1,6]naphthyridine with a variety of aliphatic and aromatic amines yielded 2-amino substituted 2,4-dichlorobenzo[*h*]naphthyridines. All the compounds were examined for their *in vitro* anticancer activity against six human cancer lines and docked with PDK1 inhibitors. The structure-activity relationship studies are revealed that the compounds holding aminocarbazole moiety and triazole amine moiety improve the activity profile. All the structures of synthesized compounds were optimized at B3LYP-D3/6-31G(d) level in the water. Furthermore, the electronic properties and biological reactivity of the synthesized compounds are explored using computational techniques.

Introduction

Nitrogen containing heterocycles provide with their significant importance in the drug design and drug discovery approved by the FDA database.[1,2] These nitrogen containing heterocycles holding amine scaffolds revealed a significant role in the drug discovery, which showed potential anticancer activity against different human cancer cell lines.[3–5] The 3-phosphoinositide-dependent protein kinase 1 (PDK1) inhibitors play a significant role in cancer cell growth, survival, and tumor angiogenesis for drug discovery.[6–10] PDK1 inhibitors represent a promising target for anticancer drugs in small molecules.[9–12] Among all nitrogen heterocycles, 1,6-naphthyridine analogues[13,14] are important class of compounds possessing diverse types of biological properties.[15,16] Recently, 1,4-dihydro-4-oxo-1,6-naphthyridine analogues have been described as antibacterial agents.[16–20] 5-Substituted-8-hydroxy-1,6-naphthyridine-7-carboxamides are useful as HIV integrase inhibitors for the treatment of HIV infection (AIDS).[21,22] A series of novel 1,6-naphthyridine derivatives have been prepared as potential inhibitors of human topoisomerase I,[23] antiproliferative, antitumor,[24,25] anticonvulsive agents,[26] p38 mitogen-activated protein kinase inhibitors,[27] JAK2 inhibitors,[28,29] and spleen tyrosine kinase inhibitors.[30] Recently significant attention focused on procedures for the modification of 1,6-naphthyridine derivatives with the aim of looking for new biological active compounds.[31,32] It is a small wonder therefore that significant efforts were made to discover and optimize new reactions that facilitate the construction of [1,6]naphthyridine derivatives. The significant role of PDK-1 in naphthyridine analogues plays a key signaling pathways involved in the evolution of cancer prompted us to investigate the use of PDK-1 inhibitors as anticancer agents.[12,33]

Recently[34] various metal catalyzed reactions of aryl halide with amine have been developed.[35–43] A comprehensive review of the literature points out that the reaction of 2,4-dichlorobenzonaphthyridines were aimed to get substituted naphthyridines having biological activity one among them is the amination reaction involving aliphatic and aromatic amines thereby deriving the respective aliphatic and aromatic aminonaphthyridines. With the importance of these compounds in mind, we targeted the synthesis of a system that bio labile 1,6-naphthyridines with the expected significant biological activity. All the compounds were subjected to cytotoxic evaluation against six human cancer cell lines (A549, HCT-15, T47D, C6, Hep-G2, and Hep-2) compared with cisplatin as a reference drug. Further, the literature reports on the representation of PDK1 in lung and breast cancer cells instinctively prepared to focus on the naphthyridines for its inhibition.[12] Also, the synthesized compounds have been docked with PDK 1

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inhibitors. Additionally, computational analyses[44–47] are done at B3LYP-D3/6-31G(d) level in the water. All the synthesized compounds are optimized, and the ground state of these compounds is obtained. Furthermore, the electronic properties of these compounds are investigated using a contour plot of frontier molecular orbital and molecular electrostatic potential (MEP) maps.

Results and discussion

Chemistry

Synthesis of 2,4-dichlorobenzo[h]naphthyridine from 4-aminoquinoline

With the intention to prepare various 2-substituted benzo[*h*]naphthyridines, 2,4-dichloro-5-methylbenzo[*h*][1,6]naphthyridine (**3**) was taken as a precursor. The starting precursor was derived from 4-aminoquinoline (**1**) and malonic acid (**2**) reflux in $POCl_3$ for 8 h as depicted in Scheme 1. We got a single spot-on TLC. Analyzing the product through various spectral and analytical techniques, the FT-IR spectrum showed stretching frequencies at 1644 cm^{-1} and 1606 cm^{-1}, were due to the presence of two C=N functional groups. Its ^{1}H NMR spectrum showed two singlets at δ 3.31 and δ 7.60, which due to C_5-methyl and C_3–H of the naphthyridine moiety respectively. All other aromatic protons appeared in the region between δ 7.65 and δ 8.94. Its ^{13}C NMR spectrum showed the presence of 13 carbons. From its elemental analysis the molecular formula of the compound was found to be $C_{13}H_8Cl_2N_2$. All the spectral and analytical results revealed the product as 2,4-dichloro-5-methylbenzo[*h*][1,6]naphthyridine (**3**) (Scheme 1).

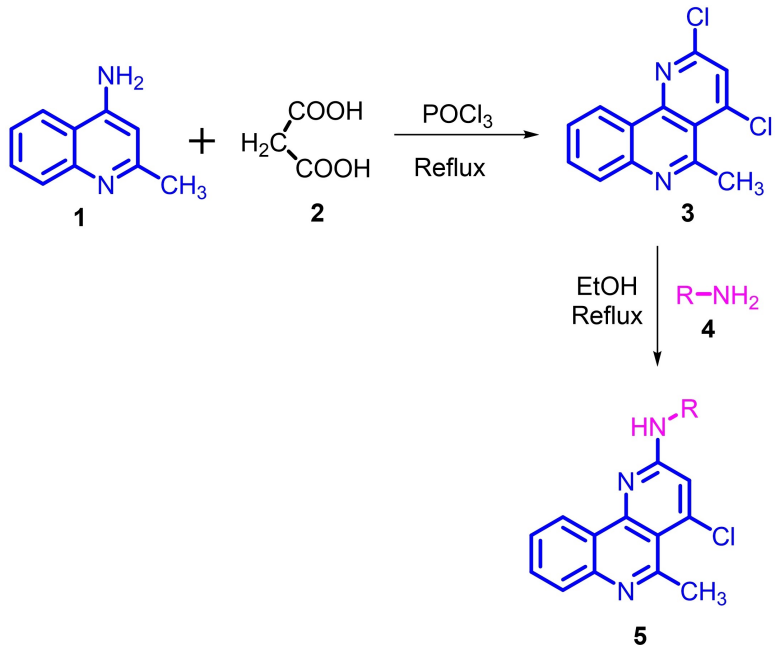
The precursor 2,4-dichlorobenzo[*h*]naphthyridine (**3**) was reacted with 3-amino-9-ethylcarbazole (**4**) in ethanol to afford a single product. The FT-IR spectrum of the product showed absorption bands at 3432 cm^{-1}, 1641 cm^{-1} and 1601 cm^{-1} which were due to one NH and two C=N key functional moieties. In its ^{1}H NMR spectrum C_5-methyl and C_2–NH of the naphthyridine moiety appeared as two singlets at δ 3.17 and δ 6.76 respectively. A broad singlet at δ 7.08 was due to C_3–H. A triplet at δ 1.48 (J = 7.20 Hz) and a quartet at δ 4.53 (J = 7.20 Hz) were assigned for methylene and methyl protons of aminocarbazole moiety. The other aromatic protons resonated in the region of δ 7.22-8.89. From its ^{13}C NMR spectrum 27 carbons were confirmed. All the spectral and analytical details assigned the structure of the compound as 4-chloro-*N*-(9-ethyl-9*H*-carbazol-3-yl)-5-methylbenzo[*h*][1,6]naphthyridin-2-amine (**5 a**) (Scheme 1).

The same reaction condition was extended to other aliphatic and heteroaromatic amines to get the corresponding amine substituted naphthyridines. The varieties of amines used reaction condition and their respective products were listed in Table 1. The structures of all compounds were confirmed by elemental and spectral analyses.

Biological activities

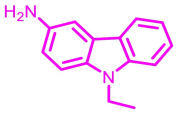
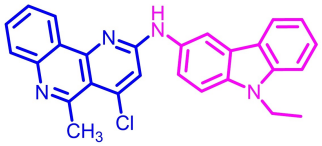
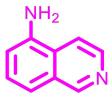
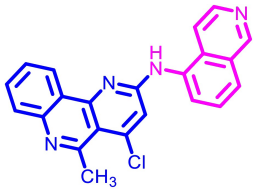
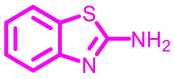
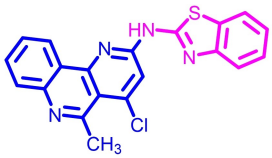
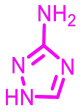
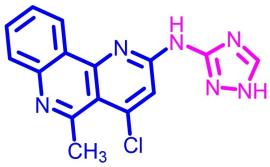
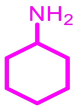
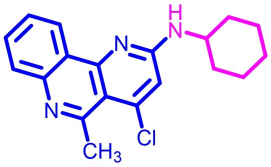
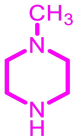
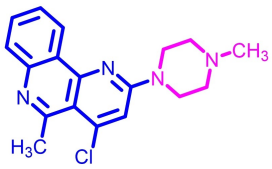
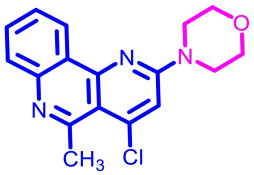
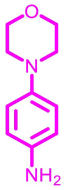
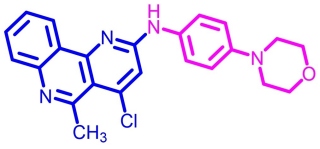
Cytotoxicity

All the synthesized and characterized compounds were then subjected to cytotoxic evaluation against a panel of human cancer cell lines by SRB (Sulforhodamine B) assay method.[48] The results were summarized in the Table 2. Cisplatin, a clinically used antitumor agent was taken as a reference



Scheme 1. Synthesis of 2-amino substituted benzonaphthyridines.

Table 1. Synthesis and reaction conditions of compounds **5a–h.**

Compounds	R-NH_2	Product	time (h)	Yield[a] (%)
5a			3	91
5b			3	89
5c			3	77
5d			3	76
5e			2	71
5f			2	83
5g			2.5	82
5h			2.5	80

[a] Isolated yield after purification by column chromatography.

material for the study. The anticancer potency of these compounds was indicated by their IC_{50} value.

In this juncture compounds **5a** and **5d** were found to show promising activity when compared to reference drug cisplatin against six human cancer cell lines. Further compound **5a** was considered to be more potent against A549, HCT-15, T47D and C6 human cancer cell lines having growth inhibitory property (IC_{50}) value of 4.72 ± 0.22, 2.73 ± 0.26, 2.36 ± 0.13 and 10.11 ± 0.31 μg/mL (Table 2) followed by compound **5d** which depicted strong cytotoxicity against A549, HCT-15, T47D and C6 human cancer cell lines with the IC_{50} values of 7.22 ± 0.32, 2.65 ± 0.18, 7.12 ± 0.27 and 2.74 ± 0.16 μg/mL, respectively. Both compounds outranged the positive control which has IC_{50} values of 10.56 ± 2.1, 7.98 ± 0.62, 11.16 ± 1.23 and 16.75 ± 2.20.

Table 2. *in vitro* cytotoxicity and IC_{50} (μg/ml).

Compounds	A549[a]	HCT-15[b]	T47D[c]	C6[d]	HepG2[e]	HEp-2[f]
5a	4.72 ± 0.22	2.73 ± 0.26	2.36 ± 0.13	10.11 ± 0.31	54.85 ± 0.28	High value*
5b	13.56 ± 0.32	2.83 ± 0.17	18.21 ± 0.33	2.77 ± 0.25	6.89 ± 0.22	103.62 ± 0.38
5c	17.520 ± 2.7	31.07 ± 1.72	30.63 ± 2.51	1.28 ± 0.84	High Value*	10.86 ± 1.64
5d	7.22 ± 0.32	2.65 ± 0.18	7.12 ± 0.27	2.74 ± 0.16	High Value*	High Value*
5e	High Value*	99.57 ± 4.2	High Value*	106.45 ± 3.18	High Value*	70.76 ± 2.54
5f	50.38 ± 0.17	41.22 ± 0.34	25.16 ± 0.53	93.96 ± 0.46	17.77 ± 0.42	34.98 ± 0.52
5g	66.12 ± 0.41	2.26 ± 0.18	33.06 ± 0.14	1.85 ± 0.25	High Value*	41.94 ± 0.15
5h	56.70 ± 0.73	5.42 ± 1.30	7.57 ± 1.70	1.71 ± 0.28	High Value*	26.97 ± 2.18
Cisplatin	10.56 ± 2.1	7.98 ± 0.62	11.16 ± 1.23	16.75 ± 2.20	3.72 ± 1.1	2.28 ± 0.8

[a] carcinomia human alveolar basal epithelial cell line, [b] human colon adenocarcinoma cell line, [c] Human ductal breast epithelial tumor cell line, [d] Rat glioma cells, [e] Human hepatocellular carcinoma cell line, [f] human laryngeal Epidermoid carcinoma cell line. High Value* represents values above 250 μg/ml. Each data point represents mean ± SD from three different experiments performed in triplicate.

Both the compounds **5a** and **5d** did not show much activity against HepG2 and Hep-2 human cancer cell lines. Further compound **5h** displayed selectively strong cytotoxicity against HCT-15, T47D and C6 human cancer cell lines with the IC_{50} values 5.42 ± 1.30, 7.57 ± 1.70 and 1.71 ± 0.28 and weak activity towards A549, HepG2 and Hep-2 human cancer cell lines. Compound **5b** showed very good cytotoxicity against HCT-15 and C6 human cancer cell lines having IC_{50} values of 2.83 ± 0.17 and 2.77 ± 0.25 and showed significant activity against A549, T47D and HepG2 with IC_{50} values of 13.56 ± 0.32, 18.21 ± 0.33 and 6.89 ± 0.22 and weak activity against Hep-2 cancer cell line. Compound **5g** selectively showed stronger activity against HCT-15 and C6 cancer cell lines with the IC_{50} values of 2.26 ± 0.18 and 1.85 ± 0.25 and showed weak activity against the other cell lines. Compound **5c** exhibited stronger activity against C6 cancer cell line with the IC_{50} value 1.28 ± 0.84 and showed moderate activity against A549, HCT-15, T47D, and Hep-2 and weak activity against HepG2 cancer cell lines. Furthermore, compounds **5e** and **5f** portrayed moderate to weak activity against all the six human cancer cell lines.

The present study investigated the effect of several substituents at 2nd position of the naphthyridine moiety and from the results of cytotoxicity of the synthesized compounds; the following structure-activity relationships can be derived.

- In general, the 2nd position of the naphthyridine moiety was substituted with 3-amino-9-ethylcarbazole (*i.e.*, Compound **5a**) and 1*H*-1,2,4-triazol-3-amine (*i.e.*, Compound **5d**) showed excellent cytotoxicity when compared to all the other compounds. This might be due to the presence of carbazole and triazole moiety which played the important role in the cytotoxicity of the compounds.
- In the compounds **5h** and **5b** holding aminophenyl substituted morpholine and amino isoquinoline moiety at C_2 position showed selective activity against HCT-15 and C6 human cancer lines.
- The substitution at C_2 position contains aliphatic amine groups (Compounds **5e** and **5f**) showed moderate to weak activity against a panel of human cancer cell lines.
- In general, we observed that the substitution at C_2 position with aromatic amines showed excellent activity when compared to that of the substitution at C_2 position with aliphatic amines. The observations are pictorially represented in Figure 1.

Molecular Docking studies

Molecular docking is a method used to predict the preferred orientation of one molecule to a second when bound to each other to form a stable complex. It is of extreme relevance in

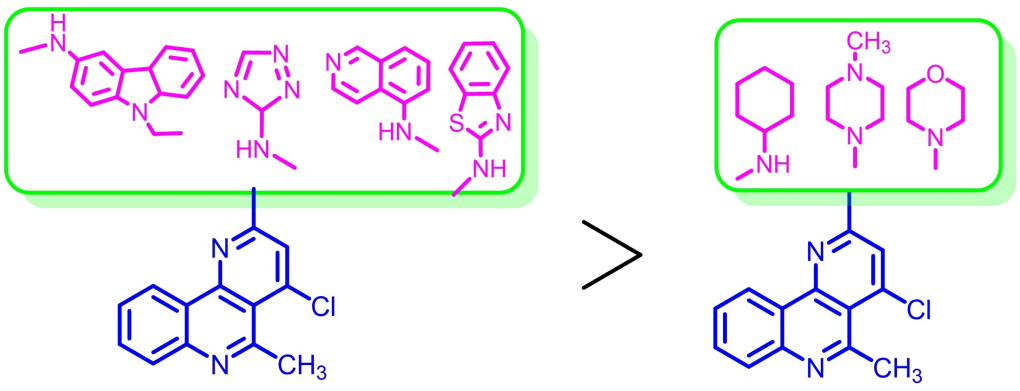


Figure 1. Selective activity against human cancer cell lines.

cellular biology, where the function of protein is accomplished by proteins interacting with themselves and with other molecular components.[49] The results of docking can be used to find inhibitors for specific target proteins and thus to design new drugs. The goal of protein-ligand docking is to predict the position and orientation of a ligand (a small molecule) when it is bound to a protein receptor or enzyme.[50] Protein-ligand docking studies determines the pose(s) and conformation(s) minimizing the total energy of the protein-ligand complex.[51] Here we have performed molecular docking studies to analyze the binding conformation of the synthesized compounds. Extensive molecular docking studies were performed to investigate the protein-ligand interactions and analyze the possible binding conformations for this class of compounds, which may give an idea about the proposed mechanism of action.[52] The synthesized compounds (**5a**–**h**) summarized in Table 3 were docked with PDK 1(PDB ID: 3H9O), using the docking program FlexX and the ligand-receptor interactions were analyzed using LeadIT.[53] All the molecules show the interaction with the amino acids of the target protein, which is compared with standard cisplatin drugs. The docking interactions of the synthesized compounds with the receptor PDK 1(PDB ID: 3H9O) imply that the compound **5d** has the highest binding energy of −29.6348 Kcal/mol and compound **5g** showed the lowest binding energy of −19.1617 kcal/mol. The docking results are given in Table 3 The amino acids of the receptor protein that interacted with chemical molecules were found to be LYS207, ALA162, TYR161, THR222, GLU166, LYS111 and GLY225.

Table 3. Docking interactions of amino acid residues of PDK1 inhibitors and compounds (**5a**–**h**) with binding energy values.

Ligand	Binding energy kcal/mol	No. of hydrogen bonds formed	Amino acid residues involved in binding
5a	−29.3200	1	ALA162
5b	−25.1614	2	LYS111, GLY225
5c	−27.2109	2	TYR161, ALA162
5d	−29.6348	2	TYR161, ALA162
5e	−24.3627	1	ALA162
5f	−23.6146	2	GLU166, ALA162
5g	−19.1617	1	THR222
5h	−27.8707	2	LYS207, ALA162
Cisplatin	−13.814	1	GLU166

In the predicted binding orientation, visual inspection of the pose of the compound **5a** indicates that it forms hydrogen bond by the interaction of NH group of naphthyridin-2-aminocarbazole substituted moiety with oxygen atom of ALA162 and it showed favorable binding mode with energy of −29.32 kcal/mol (Table 3) with active site of the amino acids. Compound **5d** forms hydrogen bond by the interaction of NH group of naphthyridine-2-triazole substituted moiety with oxygen atom of ALA162 and forms one more hydrogen bond interaction between triazole –N=N– and –OH group of the TRY161 residue and it showed good binding energy of −29.63 kcal/mol. Theoretically Compounds **5d** and **5a** showed the highest binding energy in the series of compounds with the binding energy of −29.63 and −29.32 kcal/mol respectively. Compound **5g** has the lowest binding energy in the series of compounds having the binding energy of −19.31 kcal/mol and forms hydrogen bond interaction of 'N' atom of the naphthyridine ring nucleus with the -OH group of the THR222. Compound **7** holds 2-aminobenzothiazole moiety forms hydrogen bong interaction with TYR161, ALA162 and Compound **5h** which contains aminophenyl morpholine substitution forms hydrogen bond with LYS207, ALA162. Both the compounds have the binding energies of −27.21 kcal/mol and −27.87 kcal/mol respectively. The other compounds showed the binding energy varying from −25.16 kcal/mol and −23.61 kcal/mol. The docking interaction results of the protein PDK1 with the synthesized compounds (**5a**–**h**) are showed in Figures 2–9.

The proposed binding mode can help to better understand the nature of interactions of this series of compounds. The structural analysis revealed that 2-substitution containing aminotriazole moiety (**5d**) showed better binding interaction

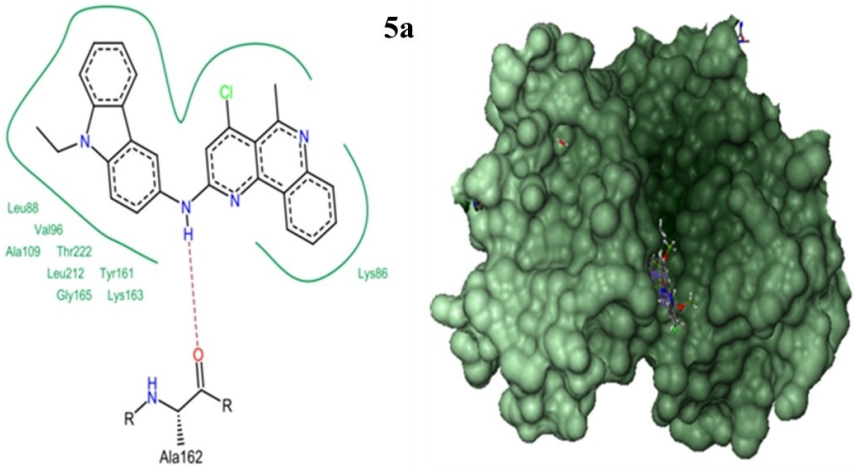


Figure 2. The docking results of the protein PDK1 with compounds **5a.**

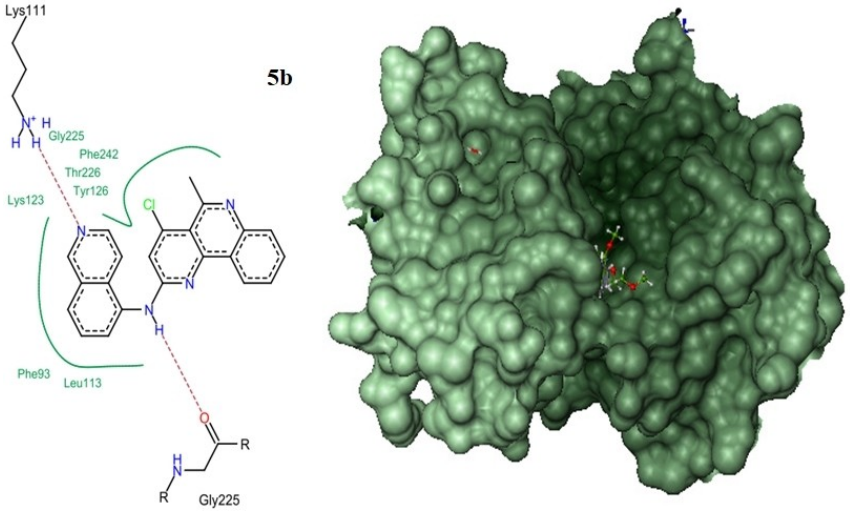


Figure 3. The docking results of the protein PDK1 with compounds **5 b.**

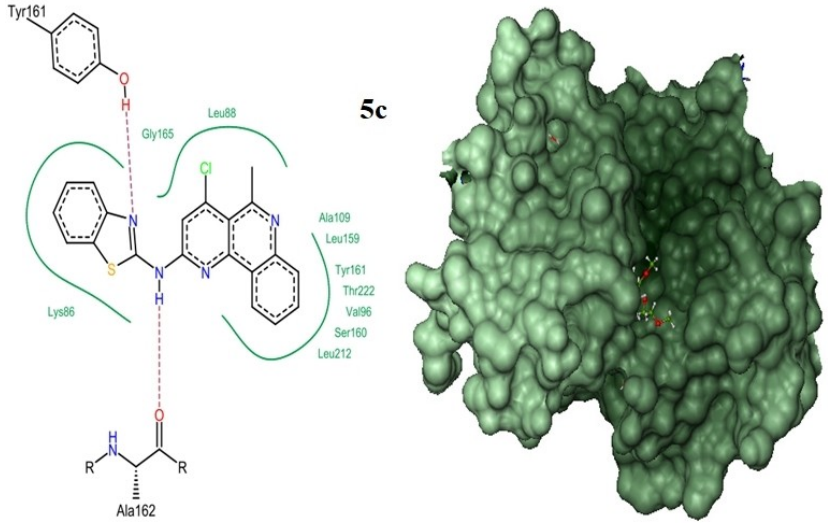


Figure 4. The docking results of the protein PDK1 with compounds **5 c.**

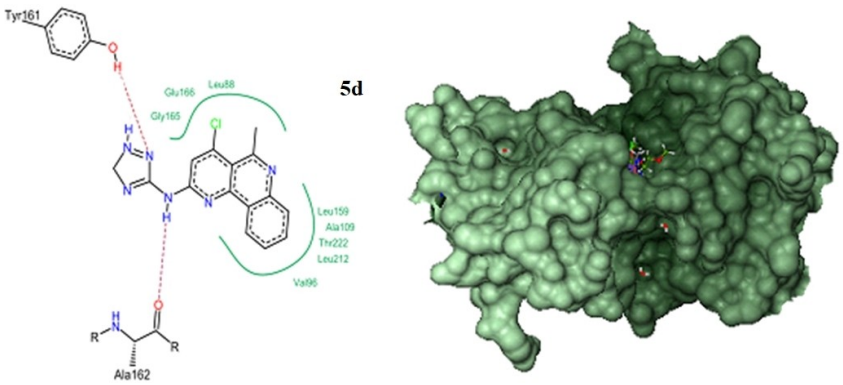


Figure 5. The docking results of the protein PDK1 with compounds **5 d.**

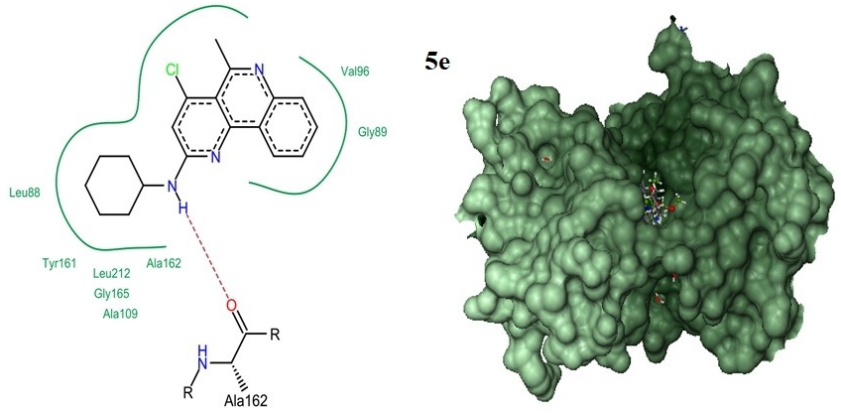


Figure 6. The docking results of the protein PDK1 with compounds **5e.**

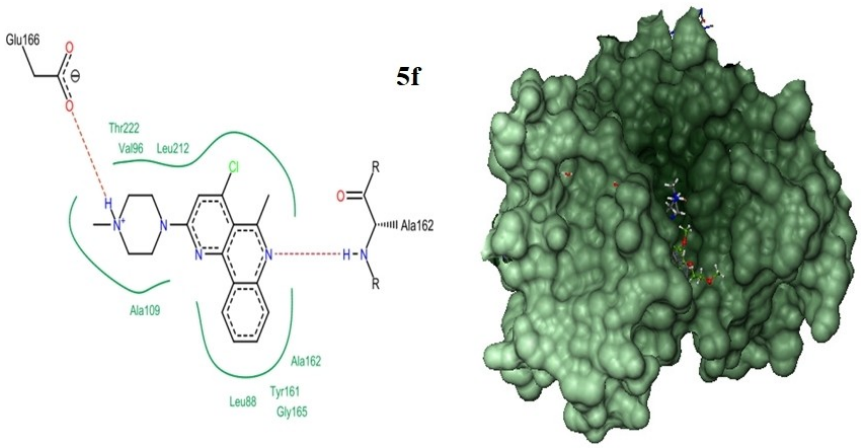


Figure 7. The docking results of the protein PDK1 with compounds **5f.**

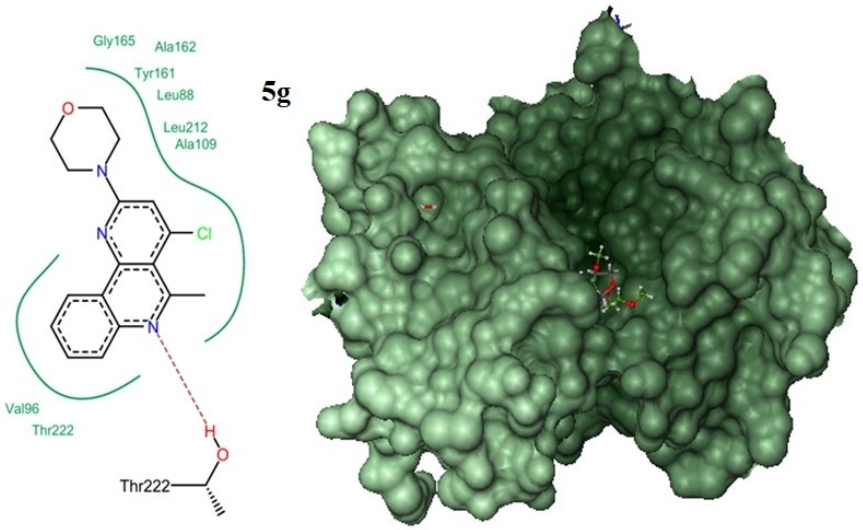


Figure 8. The docking results of the protein PDK1 with compounds **5g.**

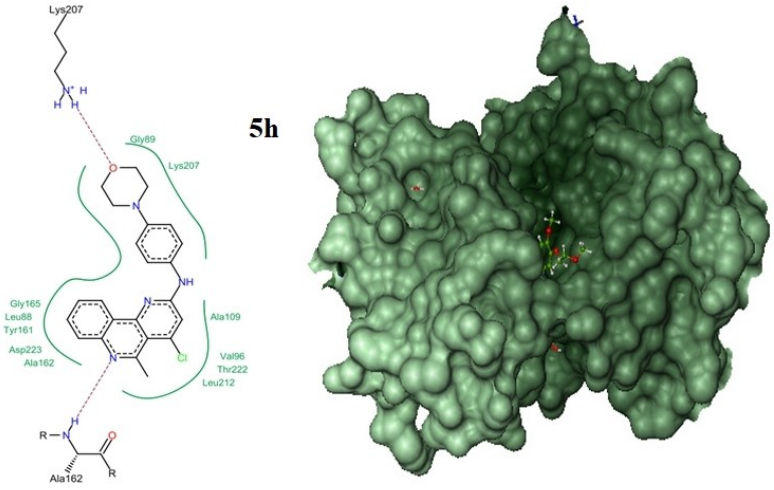


Figure 9. The docking results of the protein PDK1 with compounds **5 h.**

followed by the compound **5 a** which contains 3-aminocarbazole moiety than all the other compounds.

Computational analysis

The computational analyses were performed using Gaussian programs.[44] The synthesized compounds (**5 a**–**h**) were optimized at B3LYP-D3/6-31G(d) level in the water, and optimized structures are represented in Figure 10.

Spectral analysis is essential for the characterization of structures. The most fundamental spectrophotometric analysis that has been used to study the above compounds by IR analysis. The calculated IR frequencies are consistent values, while frequencies gained through empirical techniques are inconsistent values. Due to this fact, it is maybe some differences between calculated and empirical frequencies. As experimental, N–H stretching frequencies has been calculated in the ambit of 3500–3700 cm^{-1}, 1400–1575 cm^{-1} for C=C

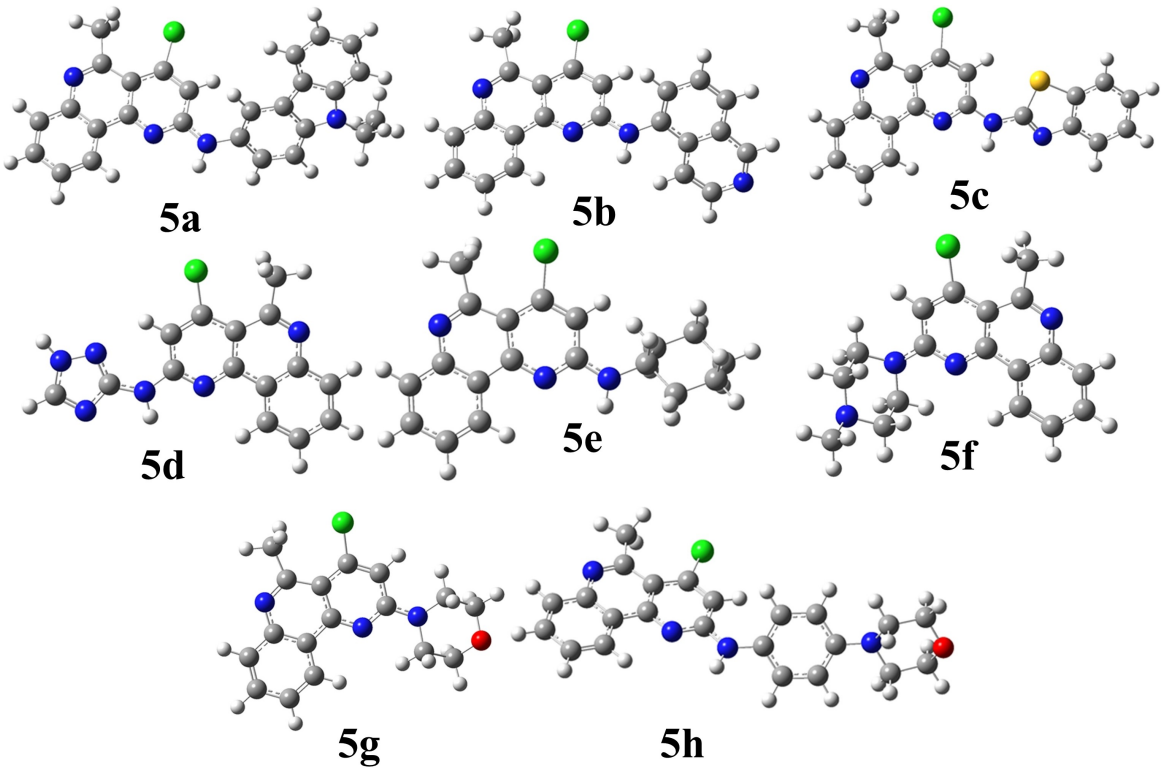


Figure 10. Optimized structures of synthesized compounds (**5 a**–**h**).

stretching frequencies, and 1251–1614 cm^{-1} for C=N stretching frequencies. As stated by this information, the calculated frequencies and experimental frequencies were shown in Table. S1. which are in the convention with spread frequencies.

The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), called frontier molecular orbital, are considerable to determine the active regions on the synthesized molecules (**5a–h**). Therefore, the contour plot of these orbitals can be used to predict the active regions. The contour plots of the synthesized compounds (**5a–h**) are represented in Figure 11.

According to Figure 11, there are two colors, red and green. The green region means electron poor, while the red zone implies electron-rich regions. According to the HOMO diagram, the whole structure in each studied compound (**5a–h**) seems active, and the molecule can easily interact with the appropriate. As for the LUMO diagram (Figure 11), the naphthyridine region on the molecule is mainly more active than the other aliphatic / aromatic amines regions. The electronic properties of synthesized compounds (**5a–h**) are investigated using MEP map. These maps are calculated at the same level of theory, and which are represented in Figure 12.

According to the MEP maps, there are many coloured surfaces on the structures. Every colour has a sense in conditions of activity. These colours are composed according to the deployment of electronic charges on the surface. The red zones are electron-rich and known as convenient to nucleophilic assault. Conversely, the blue regions are electron-poor spaces and appropriate to electrophilic attack.

Quantum chemical descriptors are important to determine the biological activity ranking. In this study some quantum chemical descriptors which are given in "Material and Method" section are calculated and given in Table 4. The quantum chemical parameters which are the energy of the highest occupied molecular orbital (E_{HOMO}), the energy of the lowest unoccupied molecular orbital (E_{LUMO}), the energy gap between LUMO and HOMO (E_{gap}), absolute hardness (η), absolute softness (σ), absolute electronegativity (χ), chemical potential (μ), electrophilicity index (N), electrophilicity index (ω), nucleophilicity index (N) additional electronic charges (ΔN_{max}) and global softness (S), are calculated using Eq. (1)–(11):

$$I = -E_{HOMO} \quad (1)$$

$$A = -E_{LUMO} \quad (2)$$

$$E_{GAP} = E_{LUMO} - E_{HOMO} \quad (3)$$

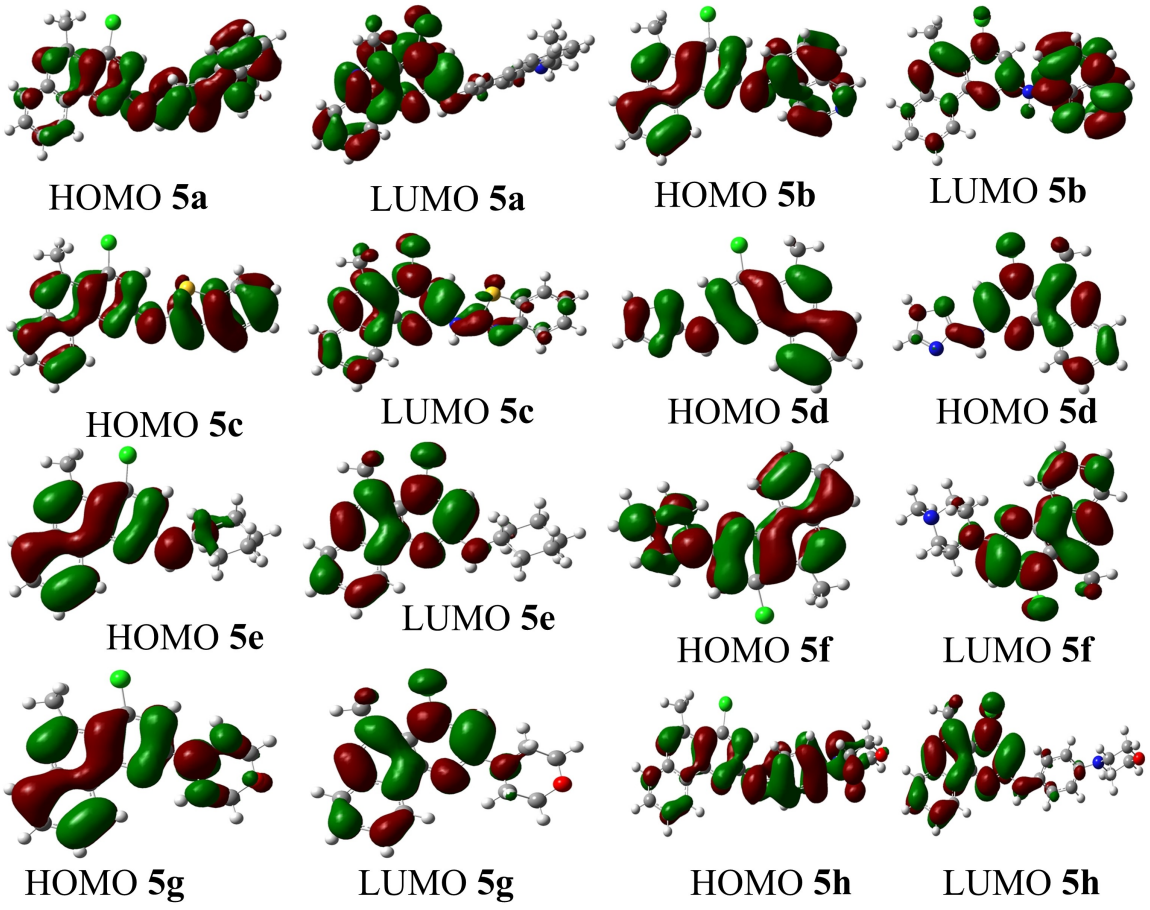


Figure 11. Contour plots of frontier molecular orbitals compounds **5a–h.**

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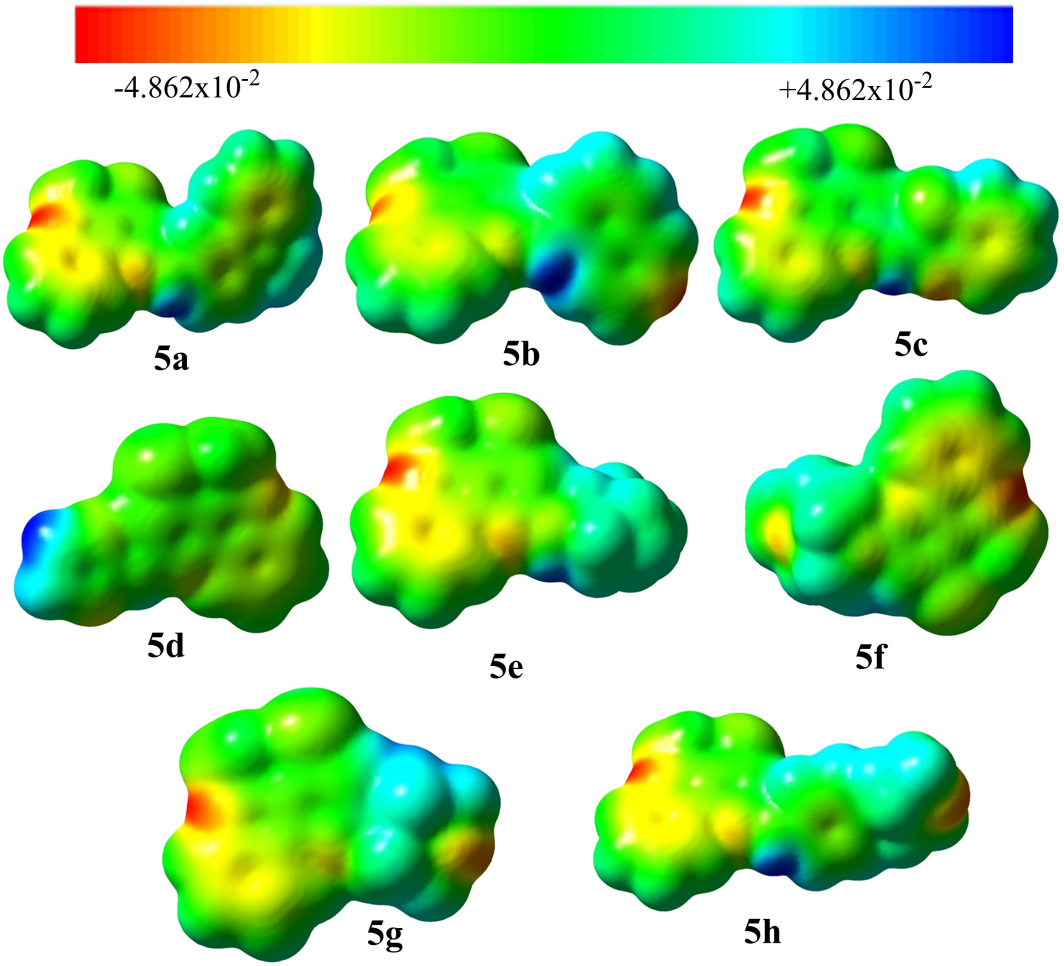

Figure 12. MEP maps of related structures in the gas phase.

Table 4. Calculated quantum chemical descriptors of synthesized compounds **5a–h.**

Compounds	5a	5b	5c	5d	5e	5f	5g	5h
E_{HOMO}[a]	−5.287	−5.794	−5.873	−5.802	−5.688	−5.631	−5.778	−5.342
E_{LUMO}[a]	−1.357	−1.817	−1.887	−1.502	−1.362	−1.391	−1.476	−1.430
E_{GAP}[a]	3.930	3.977	3.986	4.301	4.325	4.240	4.303	3.911
η[a]	1.965	1.988	1.993	2.150	2.163	2.120	2.151	1.956
Σ[b]	0.509	0.503	0.502	0.465	0.462	0.472	0.465	0.511
χ[a]	3.322	3.806	3.880	3.652	3.525	3.511	3.627	3.386
μ[a]	−3.322	−3.806	−3.880	−3.652	−3.525	−3.511	−3.627	−3.386
ΔN_{Max}	2.808	3.642	3.777	3.101	2.873	2.907	3.057	2.931
ω[a]	0.356	0.275	0.265	0.322	0.348	0.344	0.327	0.341
N[b]	1.690	1.914	1.947	1.698	1.630	1.656	1.686	1.731
S[a]	0.254	0.251	0.251	0.233	0.231	0.236	0.232	0.256

[a] in eV. [b] in eV^{-1}

$$\eta = \frac{I - A}{2} = \frac{E_{LUMO} - E_{HOMO}}{2} \quad (4)$$

$$\sigma = \frac{1}{\eta} \quad (5)$$

$$\chi = \frac{|I + A|}{2} = \frac{|-E_{HOMO} - E_{LUMO}|}{2} \quad (6)$$

$$CP = -\chi \quad (7)$$

$$\Delta N_{Max} = -\frac{CP}{\eta} \quad (8)$$

$$\omega = \frac{CP^2}{2\eta} \quad (9)$$

$$N = \frac{1}{\omega} \quad (10)$$

$$S = \frac{1}{2\eta} \quad (11)$$

Quantum chemical descriptors can be useful in the determination of the biological activity ranking. These parameters only give suggestion about the synthesized compounds (**5a**–**h**) and the further analyses should be done absolutely. First descriptor is the energy of HOMO (E_{HOMO}). If the energy level of HOMO is high, electrons in HOMO move more easily can pass to upper levels. Since the biological reactivity of molecules increases with the increasing of electron mobility, second parameter is the energy of LUMO (E_{LUMO}). The lower energy level of LUMO mean that electrons can be easily attained in LUMO. Therefore, biological activity of molecules increases with decreasing of LUMO energy level. The other parameter is energy gap between HOMO and LUMO (E_{GAP}). If the value of the energy gap between the occupied and empty molecular orbitals decreases, the reactivity of the molecules electronically is expected to increase as well. The absolute chemical hardness and softness are other important parameters. The increasing of chemical softness (σ) or decreasing of chemical hardness (η) means increasing of polarizability of molecules. There is direct correlation between polarizability and biological activity. The absolute electronegativity (χ) and chemical potential (μ) are other descriptors. Electron delocalization increases with decreasing of the absolute electronegativity. Additionally, there is direct correlation between biological activity and chemical potential. The other descriptor is additional electronic charge. Electronic charge is related to polarizability of molecule. The higher value is the more active in biological applications. The general ranking is given as follow:

5a > **5h** > **5f** > **5e** > **5g** > **5b** > **5d** > **5c** (in E_{HOMO})

5c > **5b** > **5d** > **5g** > **5h** > **5f** > **5e** > **5a** (in E_{LUMO})

5h > **5a** > **5b** > **5c** > **5f** > **5d** > **5g** > **5e** (in E_{GAP})

5h > **5a** > **5b** > **5c** > **5f** > **5g** > **5d** > **5e** (in η and σ)

5a > **5h** > **5f** > **5e** > **5g** > **5b** > **5d** > **5c** (in χ and μ)

5a > **5e** > **5f** > **5h** > **5g** > **5d** > **5b** > **5c** (in ω and N)

5c > **5b** > **5h** > **5d** > **5a** > **5g** > **5f** > **5e** (in ΔN_{Max})

5h > **5a** > **5b** > **5c** > **5f** > **5d** > **5g** > **5e** (in S)

According to above rankings, it can be said that **5a** and **5h** can be used in biological investigations. Their reactivities are better than those of others.

Conclusion

The reaction of 2,4-dichloro-5-methylbenzo[*h*][1,6]naphthyridine (**3**) with a variety of aliphatic and aromatic amines (**4a**–**h**) to obtain 2-amino substituted 2,4-dichlorobenzo[*h*] naphthyridine (**5a**–**h**), where the substituents hold alkyl, aryl and hetero moieties were successfully attained. All the compounds were examined for their *in vitro* anticancer activity against six human cancer lines (A549, HCT-15, T47D, C6, Hep-G2, and Hep-2) compared with cisplatin as reference drugs and the compounds (**5a**–**h**) were subjected to molecular docking studies with PDK1 inhibitors. The structure-activity relationship analysis revealed that the compounds (**5a**–**h**) holding aminocarbazole moiety (**5a**) and triazole amine (**5d**) analogue strengthen the activity profile. Quantum chemical calculations of compounds (**5a**–**h**) were optimized at B3LYP-D3/6-31G(d) level in the water. Moreover, the active aspects are determined by calculating the contour diagrams of the molecular orbitals (FMO), the MEP maps and the biological interrogations said that aminocarbazole containing naphthyridine molecule (**5a**) showed potent activity.

Supporting information

General procedure for synthesis, analytical details, and copies of FT-NMR spectral data of the synthesized compounds associated with this article will be available as supporting information.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that supports the findings of this study are available in the supporting information of this article.

Keywords: Cytotoxicity · DFT calculations · 1,6-Naphthyridin-2-amines · PDK 1 inhibitors

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