Bioorganic & Medicinal Chemistry 16 (2008) 9161–9170

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Antitumor studies. Part 5: Synthesis, antitumor activity, and molecular docking study of 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins

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ARTICLE INFO

Article history:
Received 28 July 2008
Revised 6 September 2008
Accepted 9 September 2008
Available online 12 September 2008

Keywords:
5-Deazaflavin
Antitumor activity
AutoDock
Protein tyrosine kinase

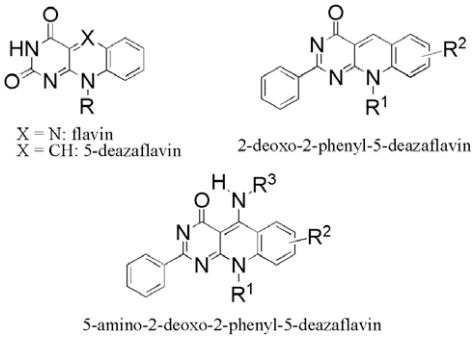
ABSTRACT

Various novel 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins derivatives have been synthesized by direct coupling of 5-deazaflavins and *N*-alkyl or aryl amines. The antitumor activities against human tumor cell lines CCRF-HSB-2 and KB cells have been investigated in vitro and many compounds showed promising potential antitumor activities with less cytotoxicities. AutoDock molecular docking into PTK (PDB code: 1t46) has been done for lead optimization of these compounds as potential PTK inhibitors. Some of the synthesized 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins at the 5-position exhibited reasonable binding affinities into PTK with the hydrogen bond through their C_5-NH moiety.

1. Introduction

Design, synthesis, and biological evaluation of 5-deazaflavins and flavin analogs have been greatly attracted our interest.[1–8] Our previous study on the selective protein kinase C (PKC) inhibitory activities of 5-deazaflavins, 10-substituted 2-deoxo-2-phenyl-5-deazaflavins {2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-ones}, and flavin-5-oxides have revealed that they can exhibit significant potential antitumor activities against NCI-H 460, HCT 116, A 431, CCRF-HSB-2, and KB cells.[2] The interest on 5-deazaflavins is by reason of their first synthesis as potential riboflavin antagonists or flavin models,[9] and the discovery that they can serve as the bioreductive cofactors for several flavin-catalyzed reactions.[10] (Scheme 1).

Moreover, it was found that the redox active coenzyme factor 420 (F_{420}), which has the 5-deazaflavin skeleton, was isolated from the methanogenic bacteria.[11] 5-Deazaflavins {5-deazaisoalloxazines, pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-diones} also have the potent broad-spectrum activity against coccidiosis.[12] 5-Deazaflavins have long been known for its strong oxidizing power which can oxidize alcohols and amines to the corresponding carbonyl compounds, and they behave as autorecycling turnover catalysts in the redox reaction.[13] The oxidizing power of 5-deazaflavin is due to the presence of high electron deficiency at the 5-position. The oxidizing property of 5-deazaflavin is effective especially in the case of the structure without a substituent at the 5-position,



Scheme 1. Flavin and its analogs.

whereas the compound with a substituent cannot perform as the redox catalyst. The 5-substituted 5-deazaflavins are, however, reported to have appreciable bioactivity and can act as potential antitumor agents. Namely, 5-amino-5-deazaflavins have been reported to have antiproliferative activity against L1210 and KB cells.[14] Also, 10-aryl-5-deazaflavin derivatives have been reported as inhibitors of the E3 activity of HMD2 in tumors that retain wild-type p53.[15]

Molecular modeling is a powerful tool in drug design which generously assists in investigating, interpreting, explaining and identification of molecular properties using three-dimensional structures. Many programs, including AutoDock,[16] LigandFit/Cerius2,[17] ICM,[18] FlexX,[19] GOLD,[20] Glide,[21] and DOCK,[22] are now available to quantify possible interactions between small molecules and proteins, in silico. The role of tyrosine kinase in the control of cel-

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doi:10.1016/j.bmc.2008.09.022

lular growth and differentiation is central to all organisms and the tyrosine kinase has been found to participate in human neoplastic diseases. Tyrosine kinase inhibitors and their potentials in the clinical applications are well documented by dramatic examples such as Gleevec, Iressa, and Herceptin. Several tyrosine kinase inhibitors are undergoing human trials and several are in the pipeline of drug discovery.[23] Molecular docking has been a focus of attention for many years. Generally speaking, today's flexible docking programs such as AutoDock can predict protein-ligand complex structures with reasonable accuracy and speed.[24] One of the most reliable, robust and popular energy-based docking packages is AutoGrid/AutoDock (Morris et al.)[16] because it allows a very efficient docking of flexible ligands (e.g., substrates, drug candidates, inhibitors, peptides, etc.) onto receptors (e.g., enzymes, antibodies, nucleic acids, etc.).[25]

In this study, our main objective is the development of potential inhibitors synthesized newly 5-(monosubstituted amino)-5-deazaflavins and their analogs as antitumor agents, based on molecular modeling and the investigation of SAR between new inhibitors and protein tyrosine kinase (PTK). As we described in our previous paper,[2] many of the synthesized, 2-deoxo-2-phenyl-5-deazaflavin derivatives showed promising antitumor activities against CRF-HSB-2 and KB cells as well as in vivo investigation against A 431 human adenocarcinoma cells transplanted subcutaneously into nude mouse exhibited effective antitumor activity. Moreover, molecular docking study revealed that the compounds possessed higher binding affinities into different PTKs. In the present paper, we describe the preparation of 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins, expecting to get better antitumor activities with lesser cytotoxicities. Also the AutoDock study for new inhibitors docked into the active sites of PTK has been carried out.

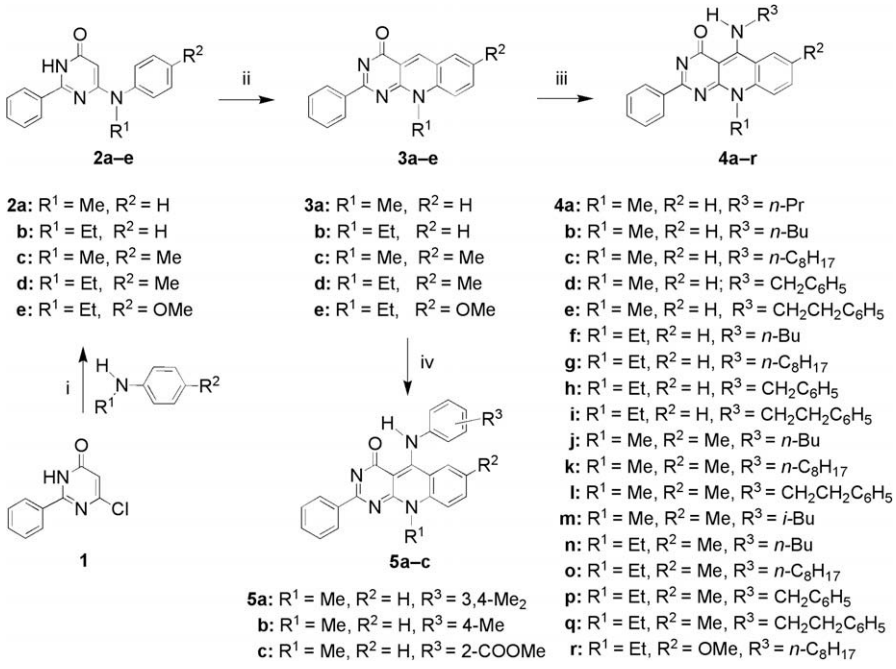
2. Results and discussion

2.1. Chemistry

The requisite precursors for the preparation of 2-deoxo-2-phenyl-5-deazaflavins (**3a–e**), 6-(*N*-monoalkylanilino)-2-phenylpyrimidin-4(3*H*)-ones (**2a–e**) were prepared by direct fusion of 6-chloro-2-phenylpyrimidin-4(3*H*)-one (**1**) with appropriate *N*-monoalkylanilines at 180–200 °C for 0.5–5 h according to the known procedure[26] as shown in Scheme 2.

The *N*-monomethylated aniline derivatives were synthesized from appropriate aniline derivatives by refluxing aniline derivatives with ethyl formate for 15 h to get the corresponding *N*-arylformamides, followed by reduction with $LiAlH_4$ in THF to afford the respective *N*-monomethylated anilines.[27] Similarly, the *N*-monoethylated aniline derivatives were synthesized by refluxing appropriate aniline derivatives with ethyl orthoformate in presence of concd sulfuric acid to get *N*-ethyl-formanilide derivatives and followed by refluxing with dilute hydrochloric acid and then aqueous potassium hydroxide.[28] The synthesized products were identified by comparing the IR spectra with authentic samples. The 10-alkyl-2-deoxo-2-phenyl-5-deazaflavins (**3a–e**) were prepared by following known procedure,[29] refluxing 6-(*N*-monoalkylanilino)-2-phenylpyrimidin-4(3*H*)-ones (**2a–e**) with Vilsmeier reagent (*N,N*-dimethylformamide-phosphoryl chloride) at 90 °C for 2–4 h. The target compounds, 5-(monosubstituted amino)-10-alkyl-2-deoxo-2-phenyl-5-deazaflavins (**4a–r** and **5a–c**) were then synthesized by treating the appropriate amines with **3a–e** and refluxing at 90–110 °C for 2–6 h. Particularly, alkyl amines were found to be easier to undergo the reaction. In case of aryl amines, more strenuous conditions were required and the reactions were carried out in the sealed glass tube at 120–135 °C for 10–15 h. This may be due to the steric hindrance of bulky aromatic ring in case of aryl amines. The reactions in general were carried out by direct fusion of the reactants, but in case of aryl amines, *n*-butanol as the reaction medium and triethylamine as a catalyst were used.

UV–vis, IR, NMR spectra, and elemental analyses were used for the identification and confirmation of the newly assigned structures. The structures of the products were confirmed in particularly by the disappearance of characteristic C_5-H in ^{1}H NMR spectra. In case of 2-deoxo-2-phenyl-5-deazaflavin derivatives showed a characteristic singlet signal at the lower field at 9.2–9.4 ppm due to the proton at the 5-position, implying that the



Scheme 2. General method of the preparation of 6-(*N*-monoalkylanilino)-2-phenylpyrimidin-4(3 H)-ones (**2a–e**), 2-deoxo-2-phenyl-5-deazaflavins (**3a–e**) and 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins (**4a–r**, **5a–c**). Reagents and conditions: (i) fusion at 180–200 °C, 0.5–5 h; (ii) Vilsmeier reagents (DMF-$POCl_3$), 90 °C, 2–4 h; (iii) Et_3 N, 90–110 °C, 2–6 h; (iv) Et_3 N, *n*-BuOH, sealed glass tube, 135 °C, 10–15 h.

5-position of these compounds is the most electron-deficient methine and very reactive towards nucleophiles.[2] This explains the nucleophilic substitution of the C_5 proton by alkyl or aryl amines, producing 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins (**4a–r** and **5a–c**). Hence, the disappearance of signal at the 9.2–9.4 ppm in ^{1}H NMR spectra confirmed the substitution of amines at 5-position. In ^{13}C NMR, the C_5 carbon appeared at more deshielded region around 153–156 ppm due to the presence of the more electronegative nitrogen atom. UV absorption spectra of the 5-(monoalkylamino)-2-deoxo-2-phenyl-5-deazaflavins (**4a–r**) showed two absorption maxima at 270–277 and 342–351 nm together with three absorption maxima as shoulders at 229–238, 251–260, and 380–410 nm. Comparing these data with those of the corresponding 2-deoxo-2-phenyl-5-deazaflavin,[2] which showed four absorption maxima at 283–297, 336–355, 414–431, and 433–456 nm together with an absorption maximum as shoulder at 262–288 nm, it has been revealed the hypsochromic shift at 283–297 nm with loss of two absorption maxima at 414–431 and 433–456 nm and addition of two shoulders at 229–238 and 380–410 nm. In contrast with the aforementioned UV absorption spectra, the compound with aryl amino substituent at the 5-position (**5a** and **b**) exhibited hypsochromic shift in the region of longer wavelength with two absorption maxima at 231 and 282–283 nm along with an absorption maximum as shoulder at 329–337 nm. In case of compound **5c**, the rather different pattern of UV absorption spectra was gained with four absorption maxima at 229, 299, 337, and 452 nm along with one absorption maximum as shoulder at 434 nm. This may due to the presence of ester moiety in the aryl group at the 5-position. In general, due to the presence of absorption maximum at 452 nm, compound **5c** was reddish in color and other compounds showed yellow or colorless crystals due to the absence of absorption maxima in the region of longer wavelength.

2.2. In vitro antitumor activities of 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins against human tumor cell lines

The in vitro tests of the synthesized compounds were carried out for the growth inhibitory activities against various cultured tumor cell lines. Two human tumor cell lines viz, human T-cell acute lymphoblastoid leukemia cell line (CCRF-HSB-2) and human oral epidermoid carcinoma cell line (KB) were used for the test and the antitumor agent, cytosine arabinoside (Ara-C), was used as positive control in the study. The IC_{50} values [the concentration (μg/mL) required for 50% inhibition of cell growth] for these compounds are shown in Table 1.

As can be seen in Table 1, many 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins (**4a–k**, **n–p**, and **5a–c**) have been found to show fairly good antitumor activities. Although the tested compounds exhibited less antitumor activities than that of Ara-C (IC_{50}: 0.017 μg/mL) as the control, most of the compounds showed the 4.7–8.9 μg/mL potential growth inhibitory activities against CCRF-HSB-2 cell line appreciably revealing their promising potential inhibitory activities and less cytotoxicities. It was also noteworthy that these compounds exhibited less potential growth inhibitory activities than the corresponding 2-deoxo-2-phenyl-5-deazaflavin,[2] also supporting the fact that substitution at 5-position leads to reduce the cytotoxicities of the compounds. Among them, the compounds **5a**, **5b**, and **4j** were found to be better inhibitors with less than 2.5 μg/mL (IC_{50}). Similarly, in case of KB cell line, the compounds **4j**, **4o**, **5a**, and **5b** exhibited good growth inhibitory activities of about one-fifth antitumor potency of Ara-C (IC_{50}: 0.42 μg/mL), that is, the IC_{50} values of them were 2.7, 2.0, 1.6, and 1.8 μg/mL, respectively. From this study, it has been generalized that the arylamino substituents at the 5-position increased the potency in the case of **5a** and **5b**, while the longer chained alkylamino substituents reduced the potency in the case of **4c, 4g**, and **4k** comparing with that of **4b, 4f**, and **4j.** Substituents at the 7-position such as the cases of **4j**, **4k**, and **4o** were found to improve the potential antitumor activities of the compounds than their corresponding 7-unsubstituted derivatives.

2.3. Molecular docking study

Both pharmaceutical companies and university laboratories have been active to develop compounds which can inhibit tyrosine kinase activity in the expectation that the potent and selective inhibitors would represent a new class of therapeutics for cancers as well as other proliferative diseases. Therefore, PTK inhibitors can be applied aptly as a new mode of cancer therapy. Depending on the above mentioned idea, herein, we investigated the AutoDock binding affinities of the synthesized 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins into PTK. Towards optimization of the aforementioned lead compounds of the promising antitumor activities, the advanced docking program AutoDock 3.05[16] was

Table 1
Growth inhibitory activities of 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins (**4a–k**, **n–p**, and **5a–c**) against human tumor cell lines of CCRF-HSB-2 and KB cells

Compound	R^1	R^2	R^3	Inhibitory activity against tumor cell lines [IC_{50} (μg/mL)]	
				CCRF-HSB-2	KB
4a	Me	H	*n*-Pr	>100.00	18.00
4b	Me	H	*n*-Bu	4.70	6.60
4c	Me	H	*n*-C_8H_{17}	>100.00	32.90
4tJ	Me	H	$CH_2C_6H_5$	>100.00	66.90
4e	Me	H	$CH_2CH_2C_6H_5$	17.70	22.00
4f	Et	H	*n*-Bu	8.30	7.60
4g	Et	H	*n*-C_8H_{17}	20.40	12.00
4h	Et	H	$CH_2C_6H_5$	6.10	4.70
4i	Et	H	$CH_2CH_2C_6H_5$	63.30	53.40
4j	Me	Me	*n*-Bu	2.10	2.70
4k	Me	Me	*n*-C_8H_{17}	8.60	4.70
4n	Et	Me	*n*-Bu	8.90	8.60
4o	Et	Me	*n*-C_8H_{17}	5.70	2.00
4p	Et	Me	$CH_2C_6H_5$	6.90	17.20
5a	Me	H	3,4-Me_2-C_6H_3	0.75	1.60
5b	Me	H	4-Me-C_6H_4	1.10	1.80
5c	Me	H	2-COOMe-C_6H_4	>100.00	>100.00
AraC				0.02	0.42

used to evaluate the binding free energies as potential inhibitors into the target PTK macromolecule.

2.3.1. Validation of the accuracy and performance of AutoDock

According to the method of validation cited in literature,[23] where if the RMSD (root mean square deviation) of the best docked conformation is ⩽2.0 Å from the experimental one, the used scoring function is successful. The obtained success rates of AutoDock (Morris et al)[16] was highly excellent as illustrated in Table 2 for the native ligand STI ligand (Imatinib® or Gleevec®), 4-(4-methylpiperazin-1-ylmethyl)-*n*-[4-methyl-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)phenyl]-benzamide, which was docked into its c-Kit receptor PTK (PDB code: 1t46). The RMSD of the docked ligand was 0.25 Å. The validation results were discussed in details in our previous publication.[3] The results indicated the high accuracy of the AutoDock simulation in comparison with the biological methods.[30]

2.3.2. AutoDock binding affinities of the synthesized and designed compounds into PTK

The binding affinity was evaluated by the binding free energies (ΔG_b, kcal/mol), inhibition constants (K_i), hydrogen bonding, and RMSD values. The compounds which revealed the highest binding affinities, that is, lowest binding free energies, within PTK and the hydrogen bond interactions into the target macromolecule are represented in Table 2. These compounds include 5-alkylamino-2-deoxo-10-methyl-5-deazaflavins (**4a–e** and **4j–l**), 5-alkylamino-2-deoxo-10-ethyl-5-deazaflavins (**4f–i**, and **4n–p**), and 5-arylamino-2-deoxo-10-methyl-5-deazaflavins (**5a–c**). Many of these derivatives exhibited one or two hydrogen bonds between C_5-NH of the docked 5-deazaflavins and different amino acids of the target PTK including Leu 595, Ala 636, Glu 640, Thr 670, Ilu 789, His 790, and Asp 810 as cited in Table 2.

The molecular docking study revealed that the majority of the compounds docked into the c-Kit receptor of PTK (PDB code: 1t46) with its bound ligand STI exhibited hydrogen bonds via their C_5-NH group as illustrated for compounds **4b** and **4g** in Figure 1 and for compounds **4a** and **5b** in Figure 2.

The correlation between the growth inhibitory activities (IC_{50}, μg/mL) of the synthesized 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins against tumor cells and the binding affinities predicted by AutoDock was highly good for some compounds. Considering the growth inhibition against CCRF-HSB-2 cells, it was noticed that the correlation between IC_{50} of **4b–g**, **i**, **n,** and **5a–c** and their AutoDock binding free energies revealed an excellent correlation coefficient (R^2) of 0.7871 as represented in Figure 3.

Moreover, the growth inhibition against KB cells revealed a better correlation with AutoDock binding free energies for compounds **4b–g**, **i**, **n** and **5a–c** of correlation coefficient (R^2) value of 0.7753 as shown in Figure 4.

3. Conclusions

In this study, various 5-alkylamino and 5-arylamino derivatives (**4a–r** and **5a–c**) of 2-deoxo-2-phenyl-5-deazaflavin have been synthesized by direct coupling of 2-deoxo-2-phenyl-5-deazaflavins and appropriate *N*-alkyl or aryl amines to investigate their potential antitumor activities. In vitro growth inhibitory activities of the compounds **4a–k**, **n–p**, and **5a–c** against two human tumor cell lines viz, human T-cell acute lymphoblastoid leukemia cell line (CCRF-HSB-2) and human oral epidermoid carcinoma cell line (KB) have been investigated. From the study, it has been revealed that the alkylamino substituents at the 5-position decreased the antitumor activities, whereas arylamino substituents at the 5-position increased it. Especially, compounds 5a and **5b** were found to exhibit better antitumor activities with IC50 values less than 2.0 μg/mL. Also, it has been generalized that the substitution of 7-H by a methyl group tends to increase the antitumor activities. The overall antitumor activities of compounds **4a–k**, **n–p**, and **5a–c** were found to be lesser than corre-

Table 2
The best docking results based on the binding free energies (ΔG_b) and inhibition constants (K_i) of compounds docked into PTK, the distances and angles of hydrogen bonds between compounds and amino acids involved in PTK, and RMSD from the co-crystallized STI ligand

Compound	ΔG_b[a] (kcal/mol)	K_i[b]	Hydrogen bonds between atoms of compounds and amino acids				RMSD[c](A)
			Atom of compound	Amino acid	Distance (A)	Angle (°)	
4a	−10.28	2.90E−08	N_1-N	HN of Asp 810	1.68	158.5	2 24
			C_5-NH	OH of Thr 670	2.11	143.6	
4b	−8.74	3.91E−07	C_5-NH	CO of Leu 595	2.26	137.4	7.32
4c	−8.17	1.02E−06	C_5-NH	CO of His 790	1.94	177.1	8.49
4d	−7.68	2.33E−06	C_5-NH	O of His 790	2.33	149.4	9.33
4e	−8.49	5.96E−07	C_5-NH	O of Asp 810	1.71	130.0	8.80
4f	−9.07	2.26E−07	C_5-NH	CO of Ile 789	2.10	124.5	7.61
4g	−9.18	1.88E−07	C_5-NH	Co of Glu 640	2.44	113.7	4.98
4h	−7.84	1.80E−06	C_5-NH	O of Asp 810	1.94	114.4	9.06
4i	−7.82	1.86E−06	C_5-NH	O of Asp 810	2.06	125.5	9.01
4j	−7.34	4.18E−06	—	—	—	—[d]	7.85
4k	−10.39	2.43E−08	C_5-NH	CO of Leu 595	1.85	145.7	7.98
4l	−7.95	1.49E−06	C_5-NH	O of Ala 636	2.08	113.4	8.76
4n	−8.90	2.99E−07	C_5-NH	O of His 790	2.45	163.5	7.09
			C_5-NH	O of Ile 789	2.37	106.7	
4o	−7.94	1.52E−06	C_5-NH	O of Ala 636	2.13	146.7	8.88
5a	−9.20	1.79 E−07	C_5-NH	NH of Glu 640	2.18	163.4	8.75
5b	−9.35	1.39 E−07	C_5-NH	NH of Glu 640	2.08	168.2	8.09
			C_5-NH	O of Ala 636	2.45	110.1	
5c	−7.85	1.75 E−06	C_5-NH	O of Ala 636	2.17	133.0	8.91
STI[e]	−18.43	3.08E−14	N_3-N	HN of Cys 673	1.66	161.0	0.25
			NH (H79)	OH of Thr 670	1.86	147.2	
			O29	HN of Asp 810	2.01	133.3	

[a] Binding free energy.
[b] Inhibition constant.
[c] Root mean square deviation.
[d] No hydrogen bond.
[e] The native co-crystallized bound ligand (STI-571) of PTK (PDB code: 1t46).

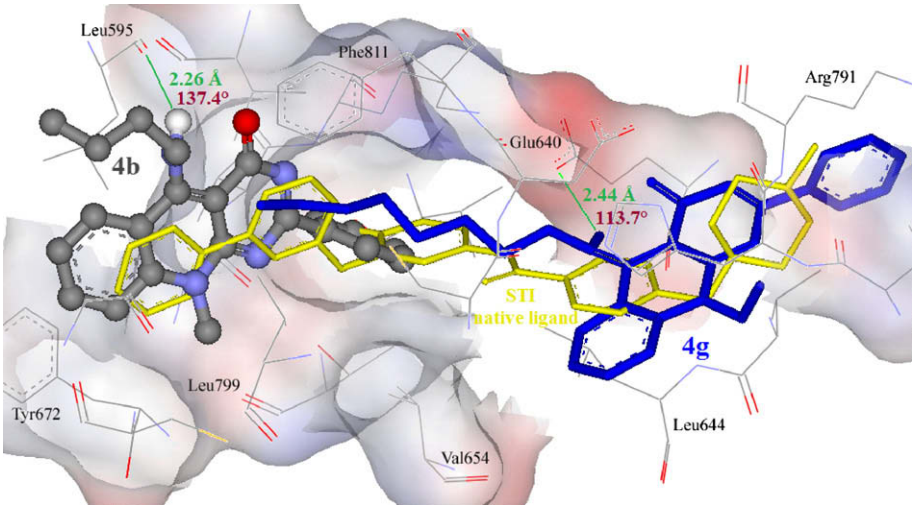


Figure 1. Compound **4b** (colored by element, ball and stick) is bound into PTK receptor site via hydrophobic interactions with Leu 595, Tyr 672, Leu 799, and Phe 811 in addition to hydrophilic binding by hydrogen bond between its C_5-NH and C═O of Leu 595. While **4g** (blue, stick) is extended through the whole groove of the pocket forming one hydrogen bond with Glu 640. The binding pocket is shown in the solid surface with labeled amino acids and the STI ligand is shown as yellow stick.

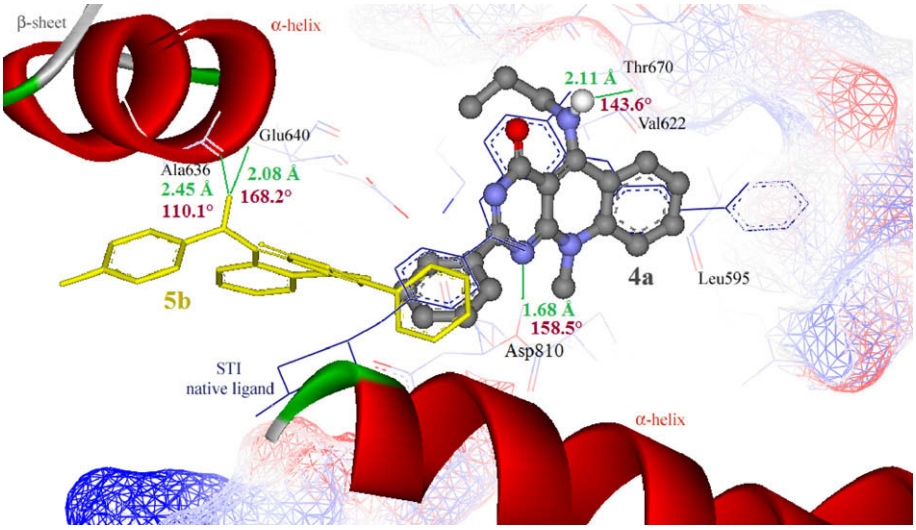


Figure 2. Differential binding affinities of 2-deoxo-10-methyl-2-phenyl-5-(*n*-propylamino)-5-deazaflavins (**4a**; colored by element, ball and stick) and 2-deoxo-10-methyl-2-phenyl-5-(*p*-toluidino)-5-deazaflavins (**5b**: yellow, stick) into PTK. Each of the docked compounds exhibited two hydrogen bonds shown as green lines. The binding pocket of PTK is shown in the wire mesh surface with labeled amino acids, the PTK protein structure is shown in solid ribbon view, and the STI ligand is shown as a blue line.

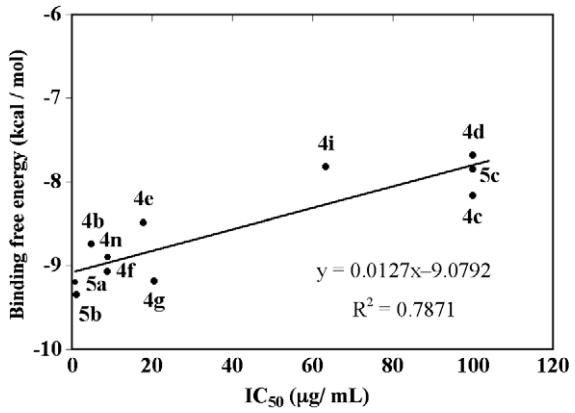


Figure 3. Correlation between the binding free energy (ΔG_b) and antitumor activity (IC_{50}) of 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins (4 b**–g**, **i**, **n** and **5a**–**c**) against human T-cell acute lymphoblastoid leukemia cell line (CCRF-HSB-2).

sponding 2-deoxo-2-phenyl-5-deazaflavins, which reveals the potential antitumor activities of the compounds with lesser cytotoxicities.

The AutoDock investigation of the synthesized 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins (**4a–l**, **n**, **o**, and **5a–c**) was carried out by docking into the c-kit of protein tyrosine kinase (PDB code: 1t46). The correlation between the growth inhibitory activities (IC_{50}, μg/mL) of the synthesized 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins (**4b–g**, **i**, **n**, and **5a–c**) against tumor cells namely CCRF-HSB-2 and KB cell lines and the binding affinities predicted by AutoDock was highly good, being of the correlation coefficients (R^2) of 0.7871 and 0.7753, respectively. The docked analogs exhibited preferential binding affinities into PTK with one or two hydrogen bonds and reasonably low binding free energies. Thus, the studies of the 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins by synthesis, biological investigation and in silico study revealed the less cytotoxicities with reasonably low binding free energies and promising potential antitumor activities. Therefore, these compounds

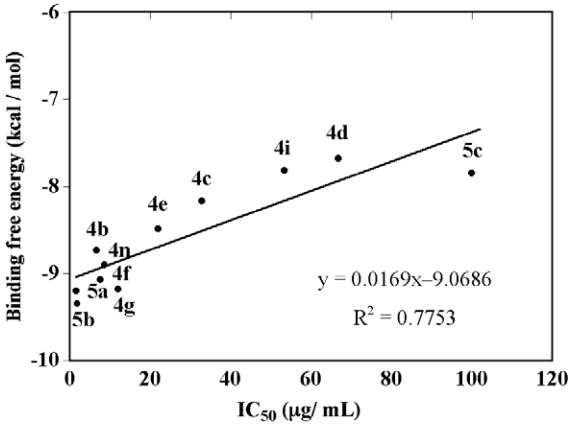


Figure 4. Correlation between the binding free energy (ΔG_b) and antitumor activity (IC_{50}) of 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins (4 b–**g**, **i**, **n** and **5a**–**c**) against human oral epidermoid carcinoma cell line (KB).

may be considered as promising candidates for further antitumor investigations.

4. Experimental

4.1. Chemistry

Mps were obtained on a Yanagimoto micro melting point apparatus and were uncorrected. Microanalyses were measured by Yanaco CHN corder MT-5 apparatus. IR spectra were recorded on a JASCO FT/IR-200 spectrophotometer as Nujol mulls. ^{1}H and ^{13}C NMR spectra were obtained using a Varian VXR 300 and 75 MHz spectrophotometer, respectively, and chemical shift values are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as internal standard. Coupling constants are given in Hz and signals are quoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; sex, sextet; sept, septet; br, broad; m, multiplet. UV spectra were measured in absolute EtOH using Beckman DU-68S UV spectrophotometers and absorption values in italics refer to wave lengths at which shoulders or inflexions occur in the absorption. All reagents were of commercial quality and were used without further purification. Organic solvents were dried in the presence of appropriate drying agents and were stored over suitable molecular sieves. Reaction progress was monitored by analytical thin-layer chromatography (TLC) on pre-coated glass plates (Silica gel 60 F_{254}-plate-Merck) and the products were visualized by UV light.

4.1.1. Preparation of 10-ethyl-7-methoxy-2-phenylpyrimido-[4,5-*b*]quinolin-4(10*H*)-one (3e)

A mixture of 6-(*N*-ethylanisidino)-2-phenylpyrimidin-4(3*H*)-one (**2e**)[2] (14.5 g, 0.05 mol) and phosphoryl chloride (77 g, 0.5 mol) in *N*,*N*-dimethylformamide (100 mL) was heated under stirring at 90 °C for 3 h. Then, the reaction mixture was poured onto ice and neutralized (pH 7) with aqueous ammonia. The separated yellow crystals were filtered off, washed with water, dried, and recrystallized from ethanol to get yellow needles.

Yield, 15.1 g (94%); mp 238–240 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 235 (3.99), 293 (4.04), *318* (3.72), *445* (3.36), 470 (3.39); IR (ν_{max}/cm^{-1}): 1621 (C=O); ^{1}H NMR ($CDCl_3$): δ_H 1.63 (3H, t, J = 7.2 Hz, 10-NCH$_2$*CH$_3$*), 3.96 (3H, s, 7-O*CH$_3$*), 5.17 (2H, q, J = 7.2 Hz,, 10-N*CH$_2$*CH$_3$), 7.34 (1H, d, $J_{6,8}$ = 3.0 Hz, 6-H), 7.44–7.53 (3H, m, Ph-*m, p*H), 7.64 (1H, dd, $J_{6,8}$ = 3.0 Hz, $J_{8,9}$ = 9.6Hz, 8-H), 7.84 (1H, d, $J_{8,9}$ = 9.6 Hz, 9-H), 8.63–8.66 (2H, m, Ph-*o*H), 9.28 (1H, s, 5-H). Anal. Calcd for $C_{20}H_{17}N_3O_2{\cdot}0.66H_2O$: C, 71.20; H, 5.28; N, 12.46. Found: C, 71.33; H, 5.52; N, 12.76.

4.2. General procedure for the preparation of 5-alkyl amino-2-deoxo-5-deazaflavins (4a–r)

A mixture of 10-alkyl-2-deoxo-2-phenyl-5-deazaflavins (**3a–e**)[2] (1.5 g) and *N*-alkylamines (15 equiv.) was refluxed at 90–110 °C for 2–6 h. After cooling, the solution was diluted with diethyl ether to get the precipitate, which was filtered off, washed with diethyl ether, dried and recrystallized from ethanol to afford the corresponding products as colorless or pale yellow needles.

4.2.1. 10-Methyl-5-(*n*-propylamino)-2-phenylpyrimido[4,5-*b*]-quinolin-4(10*H*)-one (4a)

Yield, (1.1 g, 61%); mp 233–235 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1}cm^{-1}): *231* (4.09), *251* (4.21), 271 (4.28), 346 (4.02), *380* (3.76); IR (ν_{max}/cm^{-1}): 1648 (C=O), 3140 (NH); ^{1}H NMR ($CDCl_3$): δ_H 1.17 (3H, t, J = 7.5 Hz, 5-CH$_2$*CH$_3$*), 1.95 (2H, m, 5-CH$_2$*CH$_2$* CH$_3$), 3.93 (2H, m, 5-N*CH$_2$*CH$_2$CH$_3$), 4.14 (3H, s, 10-N*CH$_3$*), 7.34 (1H, m, 7-H), 7.43–7.46 (3H, m, 2-Ph-*m, p*H), 7.63 (1H, d, J = 9.0 Hz, 9-H), 7.78 (1H, m, 8-H), 8.27 (1H, d, J = 8.7 Hz, 6-H), 8.57–8.60 (2H, m, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 11.51 (5-NCH$_2$CH$_2$*CH$_3$*), 23.75 (5-NCH$_2$*CH$_2$*CH$_3$), 31.62 (10-N*CH$_3$*), 50.45 (5-N*CH$_2$*CH$_2$CH$_3$), 97.59 (C_{4a}), 115.51 (C_7), 116.22 (C_9), 121.85 (C_8), 127.94 ($C_{3'}$ and $C_{5'}$), 128.27 (C_6), 128.97 ($C_{2'}$ and $C_{6'}$), 130.78 ($C_{4'}$), 134.03 ($C_{1'}$), 138.25 (C_{5a}), 141.90 (C_{9a}), 155.73 (C_5), 158.72 (C_{10a}), 166.30 (C_2), 175.62 (C=O). Anal. Calcd for $C_{21}H_{20}N_4O{\cdot}0.16H_2O$: C, 72.60; H, 5.90; N, 16.13. Found: C, 72.84; H, 6.17; N, 15.73.

4.2.2. 5-(*n*-Butylamino)-10-methyl-2-phenylpyrimido[4,5-*b*]-quinolin-4(10*H*)-one (4b)

Yield, (1.05 g, 56%); mp 249–251 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1}cm^{-1}): *229* (4.38), *252* (4.45), 272 (4.56), 346 (4.26), *384* (3.99); IR (ν_{max}/cm^{-1}): 1651 (C=O), 3140 (NH); ^{1}H NMR ($CDCl_3$): δ_H 1.10 [3H, t, J = 7.2 Hz, 5-N(CH$_2$)$_3$*CH$_3$*], 1.61 [2H, m, 5-N(CH$_2$)$_2$*CH$_2$*CH$_3$], 1.92 [2H, m, 5-NCH$_2$*CH$_2$*CH$_2$CH$_3$], 3.97 [2H, m, 5-N*CH$_2$*CH$_2$CH$_2$CH$_3$], 4.15 (3H, s, 10-N*CH$_3$*), 7.33 (1H, m, 7-H),7.45–7.47 (3H, m, 2-Ph-*m*, *p*H), 7.66 (1H, d, J = 8.7 Hz, 9-H), 7.79 (1H, m, 8-H), 8.33 (1H, d, J = 8.4 Hz, 6-H), 8.57–8.61 (2H, m, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 13.74 (5-NCH$_2$CH$_2$CH$_2$*CH$_3$*), 20.08 (5-NCH$_2$CH$_2$*CH$_2$*CH$_3$), 31.61 (10-N*CH$_3$*), 32.38 (5-NCH$_2$*CH$_2$*CH$_2$CH$_3$), 48.47 (5-N*CH$_2$*CH$_2$CH$_2$CH$_3$), 97.61 (C_{4a}), 115.52 (C_7), 116.21 (C_9), 121.84 (C_8), 127.95 ($C_{3'}$ and $C_{5'}$), 128.27 (C_6), 128.99 ($C_{2'}$ and $C_{6'}$), 130.78 ($C_{4'}$), 134.02 ($C_{1'}$), 138.28 (C_{5a}), 141.92 (C_{9a}), 155.75 (C_5), 158.70 (C_{10a}), 166.33 (C_2), 175.62 (C=O); Anal. Calcd for $C_{22}H_{22}N_4O{\cdot}0.16H_2O$: C, 73.11; H, 6.23; N, 15.50. Found: C, 73.11; H, 5.99; N, 15.37.

4.2.3. 10-Methyl-5-(*n*-octylamino)-2-phenylpyrimido[4,5-*b*]-quinolin-4(10*H*)-one (4c)

Yield, (0.97 g, 45%); mp 205–207 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *230* (4.51), *252* (4.66), 271 (4.72), 345 (4.51), *383* (4.25); IR (ν_{max}/cm^{-1}): 1680 (C=O), 3180 (NH); ^{1}H NMR ($CDCl_3$): δ_H 0.8 8 [3H, t, J = 6.9 Hz, 5-NCH$_2$(CH$_2$)$_6$*CH$_3$*], 1.22–1.41 [8H, m, 5-NCH$_2$CH$_2$CH$_2$(*CH$_2$*)$_4$CH$_3$], 1.50–1.61 [2H, m, 5-NCH$_2$CH$_2$*CH$_2$*(CH$_2$)$_4$CH$_3$], 1.87–1.98 [2H, m, 5-NCH$_2$*CH$_2$*(CH$_2$)$_5$CH$_3$], 3.96 [2H, m, 5-N*CH$_2$*(CH$_2$)$_6$CH$_3$], 4.15 (3H, s, 10-N*CH$_3$*), 7.32 (1H, m, 7-H), 7.44–7.47 (2H, m, 2-Ph-*m*, *p*H), 7.63 (1H, d, J = 8.7 Hz, 9-H), 7.78 (1H, m, 8-H), 8.30 (1H, d, J = 8.7 Hz, 6-H), 8.57–8.60 (2H, m, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 14.25 [5-NCH$_2$(CH$_2$)$_6$*CH$_3$*], 22.80 [5-NCH$_2$(CH$_2$)$_5$*CH$_2$*CH$_3$], 27.07 [5-NCH$_2$CH$_2$*CH$_2$*(CH$_2$)$_4$CH$_3$], 29.41 [5-NCH$_2$(CH$_2$)$_3$*CH$_2$*(CH$_2$)$_2$CH$_3$], 30.56 [5-NCH$_2$(CH$_2$)$_2$*CH$_2$*(CH$_2$)$_3$CH$_3$], 31.08 [5-NCH$_2$(CH$_2$)$_4$*CH$_2$*CH$_2$CH$_3$], 31.75 (10-N*CH$_3$*), 31.95 [5-NCH$_2$*CH$_2$*(CH$_2$)$_5$CH$_3$], 48.93 [5-N*CH$_2$*(CH$_2$)$_6$CH$_3$], 97.74 (C_{4a}), 115.67 (C_7), 116.35 (C_9), 121.97 (C_8), 128.09 ($C_{3'}$ and $C_{5'}$), 128.43 (C_6),

129.14 ($C_{2'}$ and $C_{6'}$), 130.92 ($C_{4'}$), 134.16 ($C_{1'}$), 138.45 (C_{5a}), 142.06 (C_{9a}), 155.90 (C_5), 158.80 (C_{10a}), 166.47 (C_2), 175.78 (C=O). Anal. Calcd for $C_{26}H_{30}N_4O{\cdot}0.12H_2O$: C, 74.92; H, 7.32; N, 13.44. Found: C, 74.94; H, 7.04; N, 13.26.

4.2.4. 5-Benzylamino-10-methyl-2-phenylpyrimidio[4,5-*b*]-quinolin-4(10*H*)-one (4d)

Yield, (1.12 g, 55%); mp 238–240 °C (lit.,[26] 228 °C); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *229* (3.92), *252* (4.04), 271 (4.11), 351 (3.92), *381* (3.64); IR (ν_{max}/cm^{-1}): 1670 (C=O), 3120 (NH); ^{1}H NMR ($CDCl_3$): δ_H 4.18 (3 H, s, 10-NCH_3), 5.18 (2H, d, J = 6.0 Hz, 5-NHCH_2Ph), 7.25–7.30 (1H, m, 7-H), 7.34–7.49 (9H, m, 5-Ph-H, 2-Ph-*m*, *p*H), 7.65 (1H, d, J = 8.1 Hz, 9-H), 7.78 (1H, m, 8-H), 8.23 (1H, d, J = 8.4 Hz, 6-H), 8.57–8.59 (2H, m, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 31.81 (10-NCH_3), 51.78 (5-NCH_2Ph), 98.19 (C_{4a}), 115.21 (C_7), 116.35 (C_9), 122.10 (C_8), 126.93 ($C_{3''}$ and $C_{5''}$), 128.06 ($C_{3'}$, $C_{5'}$ and C_6), 129.04 ($C_{2''}$ and $C_{6''}$), 129.18 ($C_{2'}$ and $C_{6'}$), 130.96 ($C_{4'}$), 134.30 ($C_{1'}$), 136.41 ($C_{1''}$), 138.25 (C_{5a}), 142.01 (C_{9a}), 155.98 (C_5), 159.6 (C_{10a}), 166.78 (C_2), 175.71 (C=O). Anal. Calcd for $C_{25}H_{20}N_4O{\cdot}0.60H_2O$: C, 74.09; H, 5.77; N, 13.82. Found: C, 73.97; H, 6.00; N, 13.69.

4.2.5. 10-Methyl-5-phenethylamino-2-phenylpyrimido[4,5-*b*]-quinolin-4(10*H*)-one (4e)

Yield, (1.22 g, 57%); mp 271–273 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1}cm^{-1}): *229* (4.18), *253* (4.30), 271 (4.38), 346 (4.15), *383* (3.92); IR (ν_{max}/cm^{-1}): 1650 (C=O), 3120 (NH); ^{1}H NMR ($CDCl_3$): δ_H 3.2 2.2H, t, J = 7.2 Hz, 5-NCH$_2$$CH_2$Ph), 4.19 (3 H, s, 10-N$CH_3$), 4.23 (2H, m, 5-N$CH_2CH_2$Ph), 7.25–7.40 (6H, m, 5-Ph-H and 7-H), 7.44 (3H, m, 2-Ph-*m*, *p*H), 7.61 (1H, d, J = 9.0 Hz, 9-H), 7.75 (1H, m, 8-H), 8.24 (1H, d, J = 8.4 Hz, 6-H), 8.57–8.60 (2H, m, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 31.63 (10-NCH_3), 36.86 (5-NCH$_2$$CH_2$Ph), 50.62 (5-N$CH_2CH_2$Ph), 97.71 ($C_{4a}$), 115.46 ($C_7$), 116.26 ($C_9$), 121.94 ($C_8$), 126.99 ($C_{4''}$), 127.97 ($C_{3'}$ and $C_{5'}$), 128.18 (C_6), 128.83 ($C_{3''}$ and $C_{5''}$), 129.02 ($C_{2''}$ and $C_{6''}$), 129.06 ($C_{2'}$ and $C_{6'}$), 130.86 ($C_{4'}$), 134.09 ($C_{1'}$), 137.72 ($C_{1''}$), 138.19 (C_{5a}), 141.86 (C_{9a}), 155.72 (C_5), 158.78 (C_{10a}), 166.41 (C_2), 175.61 (C=O). Anal. Calcd for $C_{26}H_{22}N_4O{\cdot}0.33H_2O$: C, 75.71; H, 5.54; N, 13.58. Found: C, 75.69; H, 5.89; N, 13.35.

4.2.6. 5-(*n*-Butylamino)-10-ethyl-2-phenylpyrimido[4,5-*b*]-quinolin-4(10*H*)-one (4f)

Yield, (1.04 g, 56%); mp 186–188 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *229* (4.47), *253* (4.58), 271 (4.38), 346 (4.15), *382* (4.18); IR (ν_{max}/cm^{-1}): 1650 (C=O), 3150 (NH); ^{1}H NMR ($CDCl_3$): δ_H 1.01 [3H, t, J = 7.5 Hz, 5-N(CH$_2$)$_3$$CH_3$], 1.52 [3H, t, J = 7.2 Hz, 10-NCH$_2$$CH_3$], 1.56–1.64 (2H, m, 5-NCH$_2$CH$_2$$CH_2CH_3$), 1.83–1.96 (2H, m, 5-NCH$_2$$CH_2CH_2CH_3$), 3.95–4.02 (2H, m, 5-N$CH_2CH_2CH_2CH_3$), 4.85 (2H, br s, 10-N$CH_2CH_3$), 7.30–7.36 (1H, m, 7-H), 7.44–7.48 (3H, m, 2-Ph-*m*, *p*H), 7.65 (1H, d, J = 8.7 Hz, 9-H), 7.57–7.81 (1H, m, 8-H), 8.31 (1H, d, J = 8.4 Hz, 6-H), 8.58–8.61 (3H, m, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 12.73 [5-N(CH$_2$)$_3$$CH_3$], 13.72 (10-NCH$_2$$CH_3$), 20.07 (5-NCH$_2CH_2$$CH_2CH_3$), 32.44 (5-NCH$_2$$CH_2CH_2CH_3$), 39.54 (5-N$CH_2CH_2CH_2CH_3$), 48.51 (10-N$CH_2CH_3$), 97.66 ($C_{4a}$), 115.79 ($C_7$), 116.02 ($C_9$), 121.61 ($C_8$), 127.97 ($C_{3'}$ and $C_{5'}$), 128.56 (C_6), 129.05 ($C_{2'}$ and $C_{6'}$), 130.77 ($C_{4'}$), 133.99 ($C_{1'}$), 138.47 (C_{5a}), 140.97 (C_{9a}), 155.20 (C_{5a}), 158.82 (C_{10a}), 166.60 (C_2), 175.68 (C=O). Anal. Calcd for $C_{23}H_{24}N_4O$: C, 74.17; H, 6.49; N, 15.04. Found: C, 74.26; H, 6.44; N, 14.91.

4.2.7. 10-Ethyl-5-(*n*-octylamino)-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4g)

Yield, (1.05 g, 49%); mp 163–165 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *229* (4.15), *254* (4.21), 270 (4.27), 346 (4.04), *384* (3.79); IR (ν_{max}/cm^{-1}): 1680 (C=O), 3120 (NH); ^{1}H NMR ($CDCl_3$): δ_H 0.87 [3H, t, J = 6.9 Hz, 5-NCH$_2$(CH$_2$)$_6$$CH_3$], 1.22–1.26 (10H, m, NCH$_2$CH$_2$(CH_2)$_5$CH$_3$), 1.40–1.51 [2H, m, 5-NCH$_2$$CH_2$(CH$_2$)$_5CH_3$], 1.56 (3H, t, J = 7.2 Hz, 10-NCH$_2$$CH_3$), 2.70 [2H, m, 5-N$CH_2CH_2$(CH$_2$)$_5CH_3$], 4.91 (2H, br s, 10-N$CH_2CH_3$), 7.38–7.56 (4H, m, 7-H, 2-Ph-*m*, *p*H), 7.70 (1H, d, J = 7.8 Hz, 9-H), 7.84–7.89 (1H, m, 8-H), 8.52–8.61 (3H, m, 6-H, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 12.89 [5-NCH$_2$(CH$_2$)$_6$$CH_3$], 14.26 (10-NCH$_2$$CH_3$), 22.80 [5-N(CH$_2$)$_6$$CH_2CH_3$], 27.08 [5-N(CH$_2$)$_2$$CH_2$(CH$_2$)$_4CH_3$], 29.40 [5-N(CH$_2$)$_4$$CH_2$(CH$_2$)$_2CH_3$], 30.63 [5-N(CH$_2$)$_3$$CH_2$(CH$_2$)$_3CH_3$], 31.09 [5-N(CH$_2$)$_5$$CH_2CH_2CH_3$], 31.95 [5-NCH$_2$$CH_2$(CH$_2$)$_5CH_3$], 39.71 [5-N$CH_2CH_2$(CH$_2$)$_5CH_3$], 48.98 [10-N$CH_2CH_3$], 97.81 ($C_{4a}$), 115.96 ($C_7$), 116.17 ($C_9$), 121.74 ($C_8$), 128.12 ($C_{3'}$ and $C_{5'}$), 128.73 (C_6), 129.22 ($C_{2'}$ and $C_{6'}$), 130.93 ($C_{4'}$), 134.13 ($C_{1'}$), 138.65 (C_{5a}), 141.15 (C_{9a}), 156.37 (C_5), 158.95 (C_{10a}), 166.77 (C_2), 175.85 (C=O). Anal. Calcd for $C_{27}H_{32}N_4O$: C, 75.67; H, 7.53; N, 13.07. Found: C, 75.32; H, 7.24; N, 12.90.

4.2.8. 5-Benzylamino-10-ethyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4h)

Yield, (0.88 g, 44%); mp 171–173 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *229* (3.89), *252* (3.99), 271 (4.09), 340 (3.79), *372* (3.55), 381 (3.57); IR (ν_{max}/cm^{-1}): 1670 (C=O), 3180 (NH); ^{1}H NMR ($CDCl_3$): δ_H 1.54 (3H, t, J = 6.9 Hz, 10-NCH$_2$$CH_3$), 4.90 (2H, br s, 10-N$CH_2CH_3$), 5.20 (2H, d, J = 4.5 Hz, 5-NHCH_2Ph), 7.27–7.40 (6H, m, 7-H, 5-Ph-H), 7.42–7.49 (3H, m, 2-Ph-*m*, *p*H), 7.70 (1H, d, J = 9.0 Hz, 9-H), 7.79 (1H, m, 8-H), 8.26 (1H, d, J = 8.7 Hz, 6-H), 8.56–8.58 (2H, m, 2-Ph-*o*H)); ^{13}C NMR ($CDCl_3$): δ_C 13.08 (10-NCH$_2$$CH_3$), 39.42 (10-N$CH_2CH_3$), 55.14 (5-N$CH_2$Ph), 95.82 ($C_{4a}$), 114.94 ($C_7$), 117.11 ($C_9$), 123.37 ($C_8$), 125.37 ($C_{4''}$), 128.42 ($C_{3'}$, $C_{3''}$, $C_{5'}$ and $C_{5''}$), 128.54 (C_6), 128.64 ($C_{2'}$, $C_{2''}$, $C_{5'}$ and $C_{5''}$), 131.17 ($C_{4'}$), 131.78 ($C_{1'}$), 135.20 ($C_{1''}$), 138.65 (C_{5a}), 139.32 (C_{9a}), 156.07 (C_5), 158.57 (C_{10a}), 166.43 (C_2), 174.87 (C=O). Anal. Calcd for $C_{26}H_{24}N_4O_2{\cdot}H_2O$: C, 73.56; H, 5.70; N, 13.20. Found: C, 73.78; H, 5.73; N, 12.96.

4.2.9. 10-Ethyl-5-phenethylamino-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4i)

Yield, (0.80 g, 38%); mp 258–260 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *233* (4.02), *254* (4.13), 273 (4.21), 350 (3.97), *381* (3.71); IR (ν_{max}/cm^{-1}): 1650 (C=O), 3150 (NH); ^{1}H NMR ($CDCl_3$): δ_H 1.51 (3H, t, J = 7.2 Hz, 10-NCH$_2$$CH_3$), 3.23 (2H, t, J = 7.2 Hz, 5-NCH$_2$$CH_2$Ph), 4.23 (2H, m, 5-N$CH_2CH_2$Ph), 4.91 (2H, br s, 10-NCH_2CH$_3$), 7.27–7.39 (6H, m, 5-Ph-H), 7.43–7.56 (3 H, m, 2-Ph-*m*, *p*H), 7.65 (1H, d, J = 9.0 Hz, 9-H), 7.77 (1H, m, 8-H), 8.28 (1H, d, J = 8.4 Hz, 6-H), 8.58–8.63 (2H, m, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 12.76 (10-NCH$_2$$CH_3$), 36.24 (5-NCH$_2$$CH_2$Ph), 39.42 (10-N$CH_2CH_3$), 49.90 (5-N$CH_2CH_2$Ph), 97.08 ($C_{4a}$), 115.29 ($C_7$), 116.65 ($C_9$), 122.34 ($C_8$), 126.66 ($C_{4''}$), 128.12 ($C_{3''}$ and $C_{5''}$), 128.51 ($C_{3'}$, $C_{5'}$ and C_6), 129.07 ($C_{2''}$ and $C_{6''}$), 129.90 ($C_{2'}$ and $C_{6'}$), 130.89 ($C_{4'}$), 134.69 ($C_{1'}$), 138.06 ($C_{1''}$), 138.21 (C_{5a}), 140.48 (C_{9a}), 154.84 (C_5), 158.61 (C_{10a}), 165.27 (C_2), 174.39 (C=O). Anal. Calcd for $C_{27}H_{24}N_4O{\cdot}0.50H_2O$: C, 75.50; H, 5.87; N, 13.04. Found: C, 75.76; H, 6.06; N, 12.94.

4.2.10. 5-(*n*-Butylamino)-7,10-dimethyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4j)

Yield, (1.15 g, 60%); mp 220–222 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *236* (4.26), *255* (4.42), 271 (4.49), 347 (4.19), *368* (3.96), 390 (3.90); IR (ν_{max}/cm^{-1}): 1634 (C=O), 3185 (NH); ^{1}H NMR ($CDCl_3$): δ_H 1.01 [3H, t, J = 7.5 Hz, 5-N(CH$_2$)$_3$$CH_3$], 1.56–1.65 (2H, sex, J = 7.5 Hz, 5-NCH$_2$CH$_2$$CH_2$ CH$_3$), 1.91 (2H, quin, J = 7.5 Hz, 5-NCH$_2$$CH_2CH_2CH_3$), 2.46 (3H, s, 7-$CH_3$), 3.95 (2H, m, 5-N$CH_2CH_2CH_2CH_3$), 4.09 (3 H, s, 10-N$CH_3$), 7.41–7.48 (3H, m, 2-Ph-*m*, *p*H), 7.49 (1H, d, J = 8.7 Hz, 9-H), 7.56 (1H, d, J = 8.7 Hz, 8-H), 8.01 (1H, s, 6-H), 8.53–8.57 (2H, m, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$):

δ_C 13.76 [5-N(CH$_2$)$_3$*C*H$_3$], 20.08 (5-NCH$_2$CH$_2$*C*H$_2$CH$_3$), 21.16 (7-NC*H*$_3$), 31.56 (5-NCH$_2$*C*H$_2$CH$_2$CH$_3$), 32.38 (10-NC*H*$_3$), 48.39 (5-NC*H*$_2$CH$_2$CH$_2$CH$_3$), 97.61 (C_{4a}), 115.48 (C_8), 116.12 (C_9), 127.72 (C_6), 127.93 ($C_{3'}$ and $C_{5'}$), 128.96 ($C_{2'}$ and $C_{6'}$), 130.71 ($C_{4'}$), 131.55 (C_7), 135.46 ($C_{1'}$), 138.36 (C_{5a}), 139.96 (C_{9a}), 155.46 (C_5), 158.56 (C_{10a}), 166.15 (C_2), 175.65 (C=O). Anal. Calcd for $C_{23}H_{24}N_4O\cdot 0.66H_2O$: C, 71.85; H, 6.64; N, 14.57. Found: C, 71.88; H, 6.68; N, 14.45.

4.2.11. 7,10-Dimethyl-5-(*n*-octylamino)-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4k)

Yield, (1.32 g, 62%); mp 192–194 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *232* (4.32), *257* (4.46), 271 (4.51), 346 (4.21), *371* (3.97), *392* (3.90); IR (ν_{max}/cm^{-1}): 1630 (C=O), 3170 (NH); ^{1}H NMR (CDCl$_3$): δ_H 0.88 [3H, t, *J* = 6.9 Hz, 5-NCH$_2$(CH$_2$)$_6$*CH*$_3$], 1.25–1.28 (10H, m, NCH$_2$CH$_2$(*CH*$_2$)$_5$CH$_3$), 1.40–1.46 [2H, m, 5-NCH$_2$*CH*$_2$(CH$_2$)$_5$CH$_3$], 2.47 (3H, s, 7-*CH*$_3$), 2.69 [2H, t, *J* = 6.9 Hz, 5-NC*H*$_2$CH$_2$(CH$_2$)$_5$CH$_3$], 4.18 (3H, s, 10-NC*H*$_3$), 7.48–7.51 (3H, m, 2-Ph-*m*, *p*H), 7.56 (1H, d, *J* = 9.0 Hz, 9-H), 7.64 (1H, d, *J* = 9.0 Hz, 8-H), 8.3 (1H, s, 6-H), 8.54–8.57 (2H, m, 2-Ph-*o*H); ^{13}C NMR (CDCl$_3$): δ_C 14.11 [5-NCH$_2$(CH$_2$)$_6$*CH*$_3$], 21.16 (7-*CH*$_3$), 22.66 [5-NCH$_2$(CH$_2$)$_5$*CH*$_2$CH$_3$], 26.95 [5-N(CH$_2$)$_2$*CH*$_2$(CH$_2$)$_4$CH$_3$], 29.31 [5-N(CH$_2$)$_4$*CH*$_2$(CH$_2$)$_2$CH$_3$], 30.44 [5-N(CH$_2$)$_3$*CH*$_2$(CH$_2$)$_3$CH$_3$], 30.94 [5-N(CH$_2$)$_5$*CH*$_2$CH$_2$CH$_3$], 31.54 (10-*NCH*$_3$), 31.81 [5-NCH$_2$*CH*$_2$(CH$_2$)$_5$CH$_3$], 48.69 [5-NC*H*$_2$(CH$_2$)$_6$CH$_3$], 97.55 (C_{4a}), 115.47 (C_8), 116.10 (C_9), 127.70 (C_6), 127.91 ($C_{3'}$ and $C_{5'}$), 128.96 ($C_{2'}$ and $C_{6'}$), 130.68 ($C_{4'}$), 131.55 (C_7), 135.44 ($C_{1'}$), 138.37 (C_{5a}), 139.92 (C_{9a}), 155.42 (C_5), 158.45 (C_{10a}), 166.10 (C_2), 175.63 (C=O). Anal. Calcd for $C_{27}H_{32}N_4O\cdot 0.33H_2O$: C, 74.62; H, 7.58; N, 12.89. Found: C, 74.50; H, 7.58; N, 12.89.

4.2.12. 7,10-Dimethyl-5-phenethylamino-2-phenylpyrimido-[4,5-*b*]quinolin-4(10*H*)-one (4l)

Yield, (1.35 g, 65%); mp 138–140 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *233* (4.03), *260* (4.15), 270 (4.17), 345 (3.74), *394* (3.43); IR (ν_{max}/cm^{-1}): 1634 (C=O), 3150 (NH); ^{1}H NMR (CDCl$_3$): δ_H 2.43 (3H, s, 7-*CH*$_3$), 3.22 (2H, t, *J* = 6.9 Hz, 5-NCH$_2$*CH*$_2$Ph), 4.08 (3H, s, 10-NC*H*$_3$), 4.20 (2H, m, 5-NC*H*$_2$CH$_2$Ph), 7.32–7.41 (5H, m, 5-Ph-H), 7.43–7.46 (3H, m, 2-Ph-*m*, *p*H), 7.51 (1H, d, *J* = 9.0 Hz, 8-H), 7.57 (1H, d, *J* = 9.0 Hz, 9-H), 7.98 (1H, s, 6-H), 8.55–8.58 (2H, m, 2-Ph-*o*H); ^{13}C NMR (CDCl$_3$): δ_C 21.15 (7-*CH*$_3$), 31.55 (10-*NCH*$_3$), 36.88 (5-NCH$_2$*CH*$_2$Ph), 50.41 (5-NC*H*$_2$CH$_2$Ph), 97.66 (C_{4a}), 115.37 (C_8), 116.16 (C_9), 126.97 ($C_{4''}$), 127.44 (C_6), 127.92 ($C_{3''}$ and $C_{5''}$), 128.82 ($C_{3'}$, $C_{5'}$ and C_6), 128.97 ($C_{2''}$ and $C_{6''}$), 129.11 ($C_{2'}$ and $C_{6'}$), 130.75 ($C_{4'}$), 131.71 (C_7), 135.57 ($C_{1'}$), 137.81 ($C_{1''}$), 138.22 (C_{5a}), 139.84 (C_{9a}), 155.31 (C_5), 158.57 (C_{10a}), 166.10 (C_2), 175.58 (C=O). Anal. Calcd for $C_{27}H_{24}N_4O\cdot 0.66H_2O$: C, 74.98; H, 5.90; N, 12.95. Found: C, 75.05; H, 6.13; N, 12.63.

4.2.13. 5-(iso-Butylamino)-7,10-dimethyl-2-phenylpyrimido-[4,5-*b*]quinolin-4(10*H*)-one (4m)

Yield, (1.15 g, 62%); mp 236–238 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *258* (4.10), 273 (4.17), 344 (3.78), *370* (3.51), *392* (3.41); IR (ν_{max}/cm^{-1}): 1635 (C=O), 3120 (NH); ^{1}H NMR (CDCl$_3$): δ_H 1.16 [6H, d, *J* = 6.6 Hz, 5-NCH$_2$CH(*CH*$_3$)$_2$], 2.15-2.24 [1H, sept, *J* = 6.6 Hz, 5-NCH$_2$*CH*(CH$_3$)$_2$], 2.48 (3H, s, 7-*CH*$_3$), 3.79 [2H, t, *J* = 6.6 Hz, 5-NC*H*$_2$CH(CH$_3$)$_2$], 4.12 (3H, s, 10-NC*H*$_3$), 7.44–7.46 (3H, m, 2-Ph-*m*, *p*H), 7.51–7.61 (2 H, m, 8 and 9-H), 8.02 (1 H, s, 6-H), 8.57–8.61 (2 H, m, 2-Ph-*o*H); ^{13}C NMR (CDCl$_3$): δ_C 20.24 [5-NCH$_2$CH(*CH*$_3$)$_2$], 21.18 (7-*CH*$_3$), 29.69 [5-NCH$_2$*CH*(CH$_3$)$_2$], 31.53 (10-NC*H*$_3$), 56.37 [5-NC*H*$_2$CH(CH$_3$)$_2$], 97.55 (C_{4a}), 115.45 (C_8), 116.11 (C_9), 127.73 (C_6), 127.89 ($C_{3'}$ and $C_{5'}$), 128.92 ($C_{2'}$ and $C_{6'}$), 130.67 ($C_{4'}$), 131.54 (C_7), 135.48 ($C_{1'}$), 138.27 (C_{5a}), 139.92 (C_{9a}), 155.36 (C_5), 158.67 (C_{10a}), 166.02 (C_2), 175.59 (C=O). Anal. Calcd for $C_{23}H_{24}N_4O\cdot 0.14H_2O$: C, 73.66; H, 6.53; N, 14.94. Found: C, 73.63; H, 6.28; N, 15.13.

4.2.14. 5-(*n*-Butylamino)-10-ethyl-7-methyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4n)

Yield, (0.90 g, 49%); mp 231–233 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *259* (4.50), 271 (4.55), *308* (4.09), 346 (4.31), *372* (4.06), 390 (4.04); IR (ν_{max}/cm^{-1}): 1637 (C=O), 3150 (NH); ^{1}H NMR (CDCl$_3$): δ_H 1.03 [3H, t, *J* = 7.2 Hz, 5-N(CH$_2$)$_3$*CH*$_3$], 1.45 (3H, t, *J* = 7.2 Hz, 10-NCH$_2$*CH*$_3$), 1.58 (2H, m, 5-NCH$_2$CH$_2$*CH*$_2$CH$_3$), 1.85 (2H, m, 5-NCH$_2$*CH*$_2$CH$_2$CH$_3$), 2.48 (3H, s, 7-*CH*$_3$), 4.04 (2H, m, 5-NC*H*$_2$CH$_2$CH$_2$CH$_3$), 4.81 (2H, br s, 10-NC*H*$_2$CH$_3$), 7.42–7.45 (3H, 2-Ph-*m*, *p*H), 7.65 (1H, d, *J* = 8.1 Hz, 9-H), 7.78 (1H, d, *J* = 9.0 Hz, 8-H), 8.16 (1H, s, 6-H), 8.40–8.42 (2H, m, 2-Ph-*o*H); ^{13}C NMR (CDCl$_3$): δ_C 12.86 [5-N(CH$_2$)$_3$*CH*$_3$], 13.86 (10-NCH$_2$*CH*$_3$), 19.82 (5-NCH$_2$CH$_2$*CH*$_2$CH$_3$), 20.85 (7-*CH*$_3$), 32.22 (5-NCH$_2$*CH*$_2$CH$_2$CH$_3$), 39.42 (5-NC*H*$_2$CH$_2$CH$_2$CH$_3$), 47.92 (10-NC*H*$_2$CH$_3$), 97.16 (C_{4a}), 115.36 (C_8), 116.51 (C_9), 128.04 ($C_{3'}$, $C_{5'}$ and C_6), 128.57 ($C_{2'}$ and $C_{6'}$), 130.71 ($C_{4'}$), 131.54 ($C_{1'}$), 136.01 (C_7), 138.58 (C_{5a}), 138.67 (C_{9a}), 154.65 (C_{5a}), 158.39 (C_{10a}), 165.41 (C_2), 174.84 (C=O). Anal. Calcd for $C_{24}H_{26}N_4O\cdot 0.33H_2O$: C, 73.44; H, 6.85; N, 14.27. Found: C, 73.55; H, 7.06; N, 13.95.

4.2.15. 10-Ethyl-7-methyl-5-(*n*-octylamino)-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4o)

Yield, (0.98 g, 47%); mp 179–181 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *238* (3.82), *258* (4.02), 270 (4.05), *283* (3.98), 345 (3.72), 370 (3.52), *390* (3.41); IR (ν_{max}/cm^{-1}): 1634 (C=O), 3150 (NH); ^{1}H NMR (CDCl$_3$): δ_H 0.87 [3H, t, *J* = 6.6 Hz, 5-NCH$_2$(CH$_2$)$_6$*CH*$_3$], 1.22–1.38 (10 H, m, NCH$_2$CH$_2$(*CH*$_2$)$_5$CH$_3$), 1.49 (3H, t, *J* = 7.2 Hz, 10-NCH$_2$*CH*$_3$), 1.50–1.57 [2H, m, 5-N(CH$_2$)$_6$*CH*$_2$CH$_3$], 1.86–1.96 [2H, m, 5-NCH$_2$*CH*$_2$(CH$_2$)$_5$CH$_3$], 2.48 (3H, s, 7-CH$_3$), 3.95 [2H, m, 5-NC*H*$_2$CH$_2$(CH$_2$)$_5$CH$_3$], 4.87 (2H, br s, 10-NC*H*$_2$CH$_3$), 7.44–7.47 (3H, m, 2-Ph-*m*, *p*H), 7.51 (1H, d, *J* = 7.8 Hz, 9-H), 7.59 (1H, d, *J* = 7.8 Hz, 8-H), 8.06 (1H, s, 6-H), 8.58–8.61 (2H, m, 2-Ph-*o*H); ^{13}C NMR (CDCl$_3$): δ_C 12.89 [5-NCH$_2$(CH$_2$)$_6$*CH*$_3$], 14.26 (10-NCH$_2$*CH*$_3$), 21.14 (7-*CH*$_3$), 22.64 [5-N(CH$_2$)$_6$*CH*$_2$CH$_3$], 26.93 [5-N(CH$_2$)$_2$*CH*$_2$(CH$_2$)$_4$CH$_3$], 29.15 [5-N(CH$_2$)$_4$*CH*$_2$(CH$_2$)$_2$CH$_3$], 29.29 [5-N(CH$_2$)$_3$*CH*$_2$(CH$_2$)$_3$CH$_3$], 30.50 [5-N(CH$_2$)$_5$*CH*$_2$CH$_2$CH$_3$], 31.80 [5-NCH$_2$*CH*$_2$(CH$_2$)$_5$CH$_3$], 39.44 [5-NC*H*$_2$CH$_2$(CH$_2$)$_5$CH$_3$], 48.75 [10-NC*H*$_2$CH$_3$], 97.66 (C_{4a}), 115.75 (C_8), 115.92 (C_9), 127.93 ($C_{3'}$ and $C_{5'}$), 128.40 (C_6), 129.02 ($C_{2'}$ and $C_{6'}$), 130.68 ($C_{4'}$), 135.44 ($C_{1'}$), 136.69 (C_7), 138.54 (C_5), 138.98 (C_{9a}), 154.90 (C_5), 158.60 (C_{10a}), 166.41 (C_2), 175.71 (C=O). Anal. Calcd for $C_{28}H_{34}N_4O\cdot 0.33H_2O$: C, 74.97; H, 7.79; N, 12.49. Found: C, 75.09; H, 7.56; N, 12.43.

4.2.16. 5-Benzylamino-10-ethyl-7-methyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4p)

Yield, (0.85 g, 42%); mp 243–245 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): *256* (3.96), 272 (3.99), *309* (3.48), 347 (3.71), *375* (3.45), 390 (3.42); IR (ν_{max}/cm^{-1}): 1638 (C=O), 3180 (NH); ^{1}H NMR (CDCl$_3$): δ_H 1.37 (3H, t, *J* = 6.9 Hz, 10-NCH$_2$*CH*$_3$), 2.40 (3H, s, 7-*CH*$_3$), 4.82 (2H, br s, 10-NC*H*$_2$CH$_3$), 5.27 (2H, d, *J* = 4.8 Hz, 5-NC*H*$_2$Ph), 7.20–7.46 (5H, m, 5-Ph-H), 7.47–7.52 (3H, m, 2-Ph-*m*, *p*H), 7.73 (1H, d, *J* = 8.7 Hz, 9-H), 7.85 (1H, d, *J* = 8.7 Hz, 8-H), 8.24 (1H, s, 6-H), 8.38–8.42 (2H, m, 2-Ph-*o*H); ^{13}C NMR (CDCl$_3$): δ_C 12.79 (10-NCH$_2$*CH*$_3$), 20.54 (7-*CH*$_3$), 39.39 (10-NC*H*$_2$CH$_3$), 51.38 (5-NC*H*$_2$Ph), 97.30 (C_{4a}), 115.03 (C_7), 116.59 (C_9), 126.67 ($C_{4''}$), 127.77 ($C_{3''}$ and $C_{5''}$), 127.97 (C_6), 128.14 ($C_{3'}$ and $C_{5'}$), 128.41 ($C_{2''}$ and $C_{5''}$), 128.93 ($C_{2'}$ and $C_{5'}$), 130.82 ($C_{4'}$), 131.63 ($C_{1'}$), 136.11 (C_7), 137.42 ($C_{1''}$), 138.45 (C_{5a}), 139.09 (C_{9a}), 154.60 (C_5), 158.47 (C_{10a}), 165.34 (C_2), 174.71 (C=O). Anal. Calcd for $C_{27}H_{24}N_4O$: C, 77.12; H, 5.75; N, 13.32. Found: C, 77.23; H, 5.97; N, 13.32.

4.2.17. 10-Ethyl-7-methyl-5-phenethylamino-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4q)

Yield, (0.90 g, 44%); mp 215–217 °C; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1}cm^{-1}): *233* (3.59), *272* (3.78), 282 (3.78), 351

(3.36), *373* (3.12), *390* (2.95); IR (ν_{max}/cm^{-1}): 1640 (C=O), 3150 (NH); ^{1}H NMR ($CDCl_3$): δ_H 1.38 (3 H, t, J = 6.9 Hz, 10-NCH$_2$*CH$_3$*), 2.45 (3 H, s, 7-*CH$_3$*), 3.10 (2H, t, J = 6.6 Hz, 5-NCH$_2$*CH$_2$*Ph), 4.31 (2H, m, 5-NCH$_2$CH$_2$Ph), 4.79 (2H, br s, 10-NCH$_2$CH$_3$), 7.20–7.42 (5H, m, 5-Ph-H), 7.47–7.49 (3H, m, 2-Ph-*m*, *p*H), 7.70 (1H, d, J = 9.0 Hz, 9-H), 7.80 (1H, d, J = 9.0 Hz, 8-H), 8.19 (1H, s, 6-H), 8.39–8.43 (2H, m, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 12.90 (10-NCH$_2$*CH$_3$*), 36.21 (5-NCH$_2$*CH$_2$*Ph), 39.42 (10-NCH$_2$CH$_3$), 49.69 (5-NCH$_2$CH$_2$Ph), 97.08 (C_{4a}), 114.00 (C_8), 116.79 (C_9), 126.82 ($C_{4''}$), 128.52 ($C_{3''}$ and $C_{5''}$), 128.67 ($C_{3'}$, $C_{5'}$ and C_6), 128.86 ($C_{2''}$ and $C_{6''}$), 129.29 ($C_{2'}$ and $C_{6'}$), 131.08 ($C_{4'}$), 131.84 ($C_{1'}$), 136.28 (C_7), 138.46 ($C_{1''}$ and C_{5a}), 139.64 (C_{9a}), 154.60 (C_5), 158.52 (C_{10a}), 165.33 (C_2), 174.81 (C=O). Anal. Calcd for $C_{28}H_{26}N_4O{\cdot}0.5H_2O$: C, 75.82; H, 6.14; N, 12.63. Found: C, 75.88; H, 6.38; N, 12.87.

4.2.18. 10-Ethyl-7-methoxy-5-(n-octylamino)-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4r)

Yield, (1.35 g, 65%); mp 144–146 °C; UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1}cm^{-1}): *257* (4.44), 277 (4.48), *282* (4.47), 342 (4.12), 387 (3.94), *410* (3.84); IR (ν_{max}/cm^{-1}): 1635 (C=O), 3140 (NH); ^{1}H NMR ($CDCl_3$): δ_H 0.88 [3H, t, J = 7.2 Hz, 5-NCH$_2$(CH$_2$)$_6$*CH$_3$*], 1.22–1.32 (8H, m, 5-NCH$_2$CH$_2$CH$_2$(*CH$_2$*)$_4$CH$_3$), 1.50 (3H, t, J = 7.2 Hz, 10-NCH$_2$*CH$_3$*), 1.52–1.56 [2H, m, 5-NCH$_2$CH$_2$*CH$_2$*(CH$_2$)$_4$CH$_3$], 1.88–1.97 [2H, m, 5-NCH$_2$*CH$_2$*(CH$_2$)$_5$CH$_3$], 3.91 (3H, s, 7-O*CH$_3$*), 3.91–4.03 [2H, m, 5-N*CH$_2$*CH$_2$(CH$_2$)$_5$CH$_3$], 4.91 (2H, br s, 10-N*CH$_2$*CH$_3$), 7.38–7.56 (4H, m, 7-H, 2-Ph-*m*, *p*H), 7.60–7.75 (2H, m, 8 and 9-H), 8.52–8.61 (3H, m, 6-H, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 12.89 [5-NCH$_2$(CH$_2$)$_6$,*CH$_3$*], 14.10 (10-NCH$_2$*CH$_3$*), 22.27 [5-N(CH$_2$)$_6$*CH$_2$*CH$_3$], 26.42 [5-NCH$_2$CH$_2$*CH$_2$*(CH$_2$)$_4$CH$_3$], 28.83 [5-N(CH$_2$)$_3$*CH$_2$CH$_2$*(CH$_2$)$_2$CH$_3$], 30.13 [5-N(CH$_2$)$_5$*CH$_2$*CH$_2$CH$_3$], 31.39 [5-NCH$_2$*CH$_2$*(CH$_2$)$_5$CH$_3$], 39.71 [5-N*CH$_2$*CH$_2$(CH$_2$)$_5$CH$_3$], 48.76 [10-N*CH$_2$*CH$_3$], 54.85 (7-O*CH$_3$*), 96.97 (C_{4a}), 115.96 (C_8), 118.24 (C_9), 128.11 ($C_{3'}$ and $C_{5'}$), 128.42 ($C_{2'}$ and $C_{6'}$), 128.52 (C_6), 130.73 ($C_{4'}$), 131.23 (C_7), 134.46 ($C_{1'}$), 135.06 (C_{5a}), 138.56 (C_{9a}), 153.87 (C_5), 158.05 (C_{10a}), 166.41 (C_2), 174.79 (C=O). Anal. Calcd for $C_{28}H_{34}N_4O_2{\cdot}0.5H_2O$: C, 71.92; H, 7.54; N, 11.98. Found: C, 72.14; H, 7.61; N, 11.68.

4.3. General procedure for the preparation of 5-anilino-2-deoxo-2-phenyl-5-deazaflavins (5a–c)

A mixture of 10-methyl-2-deoxo-2-phenyl-5-deazaflavins (**3a–e**) (1.5 g) and *N*-aryl-amines (15 equiv.) in *n*-butanol was refluxed in sealed glass tube at 135 °C for 10–15 h. After cooling, the solution was diluted with diethyl ether to get the precipitate, which was filtered off, washed with diethyl ether, dried, and recrystallized from ethanol to afford the corresponding products as red needles.

4.3.1. 10-Methyl-2-phenyl-5-(3,4-xylidino)pyrimido[4,5-*b*]-quinolin-4(10*H*)-one (5a)

Yield, (0.98 g, 47%); mp 207–209 °C; UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 231 (3.96), 282 (4.14), *337* (3.20); IR (ν_{max}/cm^{-1}): 1646 (C=O), 3150 (NH); ^{1}H NMR ($CDCl_3$): δ_H 2.16 [6H, d, J = 8.4 Hz, 5-Ar(*CH$_3$*)$_2$], 3.61 (3H, s,10-N*CH$_3$*), 6.46 (1H, d, J = 7.2 Hz, $C_{6''}$), 6.52 (1H, s, $C_{2''}$), 6.91 (1H, d, J = 7.8 Hz, $C_{5''}$), 6.95–7.03 (2H, m, 7 and 9-H), 7.16–7.25 (2H, m, 6 and 8-H), 7.54 (3H, m, 2-Ar-*m*, *p*-H), 8.19–8.22 (2H, m, 2-Ar-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 24.51 [5-Ar-(*CH$_3$*)$_2$], 30.57 (10-N*CH$_3$*), 94.02 (C_{4a}), 114.02 (C_7), 121.75 (C_9), 122.57 (C_8), 127.48 ($C_{2''}$ and C_6), 127.75 ($C_{3'}$ and $C_{5'}$), 128.54 ($C_{5''}$), 128.83 ($C_{2'}$ and $C_{6'}$), 129.00 ($C_{6''}$), 131.71 ($C_{4'}$), 132.13 (C_{5a}), 132.83 ($C_{4''}$), 140.46 (C_{9a} and $C_{3''}$), 142.12 ($C_{1''}$), 154.50 (C_5), 155.51 (C_{10a}), 162.47 (C_2), 169.79 (C=O). Anal. Calcd for $C_{26}H_{22}N_4O{\cdot}0.33H_2O$: C, 75.98; H, 5.52; N, 13.63. Found: C, 75.68; H, 5.50; N, 13.97.

4.3.2. 10-Methyl-2-phenyl-5-(*p*-toluidino)pyrimido[4,5-*b*]-quinolin-4(10*H*)-one (5 b)

Yield, (1.20 g, 59%); mp 234-236 °C; UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 231 (3.88), 283 (4.09), *329* (3.07); IR (ν_{max}/cm^{-1}): 1649 (C=O), 3150 (NH); ^{1}H NMR ($CDCl_3$): δ_H 3.08 (3 H, s, 5-Ph-*CH$_3$*), 3.83 (3H, s, 10-N*CH$_3$*), 6.92 (2H, d, J = 7.5 Hz, 5-Ph-*m*H), 6.91–6.98 (1H, m, 7-H), 7.10–7.18 (1H, m, 8-H), 7.14 (2H, d, J = 7.5 Hz, 5-Ph-*o*H), 7.45–7.52 (4H, m, 9-H, 2-Ph-*m*, *p*H), 8.16–8.19 (3H, m, 6-H and 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 24.59 [5-Ar-*CH$_3$*], 30.67 (10-N*CH$_3$*), 94.11 (C_{4a}), 114.12 (C_7), 121.83 (C_9), 122.69 (C_8), 127.59 ($C_{3''}$ and $C_{5''}$), 127.84 ($C_{3'}$ and $C_{5'}$), 128.95 ($C_{2'}$ and $C_{6'}$), 129.10 ($C_{2''}$ and $C_{6''}$), 131.82 ($C_{4'}$), 132.33 (C_{5a}), 133.02 ($C_{4''}$), 140.52 (C_{9a}), 150.85 ($C_{1''}$), 153.16 (C_5), 155.61 (C_{10a}), 162.59 (C_2), 175.58 (C=O). Anal. Calcd for $C_{25}H_{20}N_4O{\cdot}0.66H_2O$: C, 74.24; H, 5.32; N, 13.85. Found: C, 74.16; H, 5.60; N, 14.07.

4.3.3. 5-(2-Methoxycarbonylanilino)-10-methyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (5c)

Yield, (1.02 g, 45%); mp >300 °C; UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1}cm^{-1}): 229 (4.23), 299 (4.02), 337 (3.80), *434* (3.66), 452 (3.72); IR (ν_{max}/cm^{-1}): 1692, 1645 (C=O), 3180 (NH); ^{1}H NMR ($CDCl_3$): δ_H 3.74 (3H, s, 10-N*CH$_3$*), 4.50 (3H, s, 5-COO*CH$_3$*), 6.91–6.97 (3H, m, 4′, 5′ and 6′-H of 5-Ph), 7.19–7.22 (1H, m, 3′-H of 5-Ph), 7.49–7.59 (4H, m, 7-H, 2-Ph-*m*, *p*H), 7.70 (1H, d, J = 8.4 Hz, 9-H), 8.08 (1H, m, 8-H), 8.18 (1H, d, J = 9.0 Hz, 6-H), 8.49–8.51 (2H, m, 2-Ph-*o*H); ^{13}C NMR ($CDCl_3$): δ_C 30.56 (10-N*CH$_3$*), 51.80 (–COO*CH$_3$*), 102.25 (C_{4a}), 108.30 (C_9), 115.42 (C_7 and $C_{6''}$), 122.73 ($C_{2'}$ and C_6), 124.88 ($C_{4''}$), 125.34 ($C_{2''}$ and $C_{6'}$), 128.56 ($C_{3'}$, $C_{5'}$ and C_8), 129.08 ($C_{4'}$), 130.81 ($C_{3''}$), 132.07 ($C_{5''}$), 136.10 ($C_{1'}$), 137.84 (C_{5a}), 140.34 (C_{9a}), 151.62 ($C_{1''}$), 157.18 (C_5), 158.24 (C_{10a}), 162.60 (C_2), 167.89 (C=O). Anal. Calcd for $C_{26}H_{20}N_4O_3{\cdot}H_2O$: C, 68.71; H, 4.88; N, 12.33. Found: C, 68.63; H, 4.92; N, 12.29.

4.4. Growth inhibitory activities of test compounds against human tumor cell lines

The procedure was carried out using the modified 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay[31] to determine the inhibitory effects of test compounds, namely, 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins (**4** and **5**), on cell growth in vitro as mentioned in detail in our previous paper.[2] Two human tumor cell lines CCRF-HSB-2 (human T-cell acute lymphoblastoid leukemia) and KB (human oral epidermoid carcinoma) were used in this study. AraC was used as a positive control, where the IC_{50} was determined from the dose-response curve.

4.5. Experimental protocol of docking study

The automated docking studies were carried out using AutoDock version 3.0.5. First, AutoGrid component of the program pre-calculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. The cubic grid box of 60 Å size (x, y, z) with a spacing of 0.375 Å and grid maps were created representing the catalytic active target site region where the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitors within the macromolecules. The GA-LS search algorithm (genetic algorithm with local search) was chosen to search for the best conformers. The parameters were set using the software ADT (Auto-Dock Tool Kit) on PC which is associated with Auto-Dock 3.0.5. For all docking parameters, default values were used with 10 independent docking runs for each docking case. The AutoDock performs the task of the docking, where the

ligand moves randomly in any one of six degrees of freedom, and the energy of the new ligand 'state' is calculated. If the energy of the new state is lower than that of the old state, the new one is automatically accepted as the next step in docking.

4.5.1. Preparation of ligands and target tyrosine kinase

The compounds involved in this study as ligands including **4a–l, n**, **o** and **5a–c** were studied for their binding activities into protein tyrosine kinase. The three-dimensional structures of the aforementioned compounds were constructed using Chem 3D ultra 8.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2003)], then they were energetically minimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The Gasteiger-Hückel charges of ligands were assigned. The crystal structures of protein tyrosine kinase (1t46) complex with (STI) were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) (PDB code: 1t46). All bound waters and ligands were eliminated from the protein and the polar hydrogens and the Kollman-united charges were added to the proteins. The amino acids of the ligand-target binding site were defined using data in pdbsum (http://www.ebi.ac.uk/pdbsum/).

4.5.2. Molecular modeling and analysis of the docked results

The predicted binding free energy that includes the intermolecular energy and torsional free energy was used as the criterion for ranking. Furthermore, the intermolecular hydrogen bonds, whose effect has already been counted in the binding energy, were also investigated in order to find useful information for drug design. A comparison of the results suggests that the binding free energy is more reliable as a criterion for the virtual screening via molecular docking. Cluster analysis was performed on the docked results using a root mean square (RMS) tolerance of 0.5 Å. Each of the clusters that exhibited significant negative interaction energies was examined by Accelrys, DS visualizer 2.0 [Accelrys Inc., San Diego CA (2007)] to determine their binding orientations, molecular modeling, evaluation of the hydrogen bonds. Being the native ligand (STI) and the docked inhibitors have different chemical structures, the RMSD values were measured as distance between the centroids of the docked inhibitor and the native ligand. The mode of interaction of the native ligand (STI) within the crystal structure of PTK was used as a standard docked model as well as for RMSD calculation. Regarding the hydrogen bond interaction, the more linear hydrogen bond is likely to be stronger. Therefore, in our modeling results we consider the hydrogen bond angle more than 100° to be of a reasonable strength.

Acknowledgments

The authors are indebted to the SC NMR Laboratory of Okayama University for the NMR spectral measurements.

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