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Antitumor studies. Part 3: Design, synthesis, antitumor activity, and molecular docking study of novel 2-methylthio-, 2-amino-, and 2-(*N*-substituted amino)-10-alkyl-2-deoxo-5-deazaflavins

Hamed I. Ali,[a] Noriyuki Ashida[b] and Tomohisa Nagamatsu[a,*]

[a]*Department of Drug Discovery and Development, Division of Pharmaceutical Sciences, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University 1-1-1, Tsushima-Naka, Okayama 700-8530, Japan*
[b]*Biology Laboratory, Research and Development Division, Yamasa Shoyu Co., Choshi, Chiba 288-0056, Japan*

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Abstract—Various novel 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins have been synthesized by reaction of 6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones with Vilsmeier reagent. The similar 2-(*N*-substituted amino) derivatives were prepared by nucleophilic replacement reaction of the 2-methylthio moiety by appropriate amines. The 2-oxo derivatives (i.e., 5-deazaflavins) were obtained by acidic hydrolysis of the 2-methylthio derivatives. The antitumor activities against CCRF-HSB-2 and KB cells and the antiviral activities against HSV-1 and HSV-2 have been investigated in vitro, and many compounds showed promising antitumor activities. Furthermore, AutoDock molecular docking into PTK has been done for lead optimization of these compounds as potential PTK inhibitors. Whereas, the designed 2-deoxo-5-deazaflavins connected with amino acids at the 2-position exhibited the good binding affinities into PTK with more hydrogen bonds.

1. Introduction

Design and synthesis of 5-deazaflavin and flavin analogs as potential cancer chemotherapeutic agents have been in progress in our laboratory.[1–6] Recently, we have reported the significant potential antitumor activities of 10-substituted 2-deoxo-2-phenyl-5-deazaflavins and flavin-5-oxides against NCI-H 460, HCT 116, A 431, CCRF-HSB-2, and KB cells (Scheme 1).[1] 5-Amino-5-deazaflavins also revealed antiproliferative activity against L1210 and KB cells.[7] 10-Aryl-5-deazaflavin derivatives were preliminarily reported to act as inhibitors against E3 activity of HMD2 in tumors that retain wild-type P53.[8] Actually, 5-deazaflavins have attracted great interest because of their homologues as potential riboflavin antagonists, bio-reductive cofactors for the naturally occurring flavins, for example, F_{420}, and potent antitumor agents.[9] The role of tyrosine kinase in the control of cellular 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.[10] Molecular docking has been a focus of attention for many years. Generally speaking, today's flexible docking programs such as AutoDock are able to predict protein-ligand complex structures with reasonable accuracy and speed.[11] One of the most reliable, robust, and popular energy-based docking packages is AutoGrid/AutoDock 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.).[12]

Despite the large number of publications on chemistry of 5-deazaflavin and related compounds, the information of 2-methylthio- and 2-(*N*-substituted amino)-2-deoxo-5-deazaflavins is none yet. The above circumstances led us to seek a convenient synthetic route to the 2-methylthio- and 2-(*N*-substituted amino)-2-deoxo-5-deazaflavins to search their potential antitumor

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* Corresponding author. Tel.: +81 86 251 7931; fax: +81 86 251 7926; e-mail: nagamatsu@pheasant.pharm.okayama-u.ac.jp

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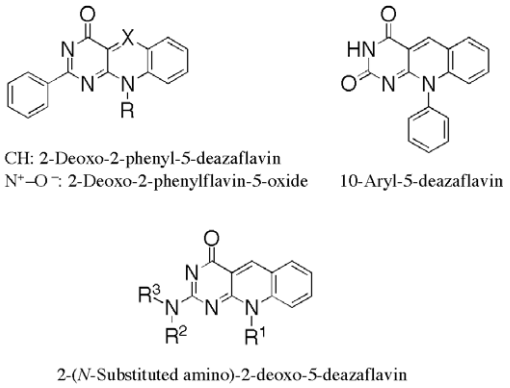


Scheme 1. 5-Deazaflavins, 2-deoxo-5-deazaflavins, and 2-deoxo-flavin-5-oxides.

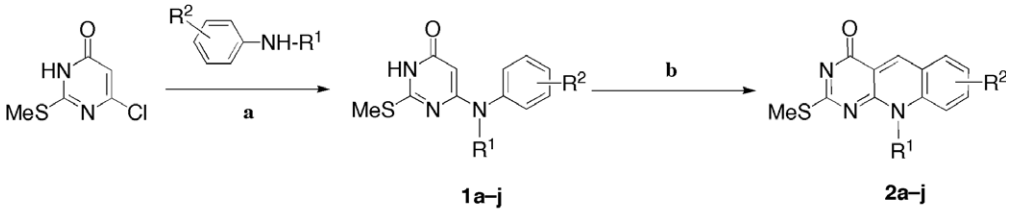
activities. In this study, we describe the facile synthesis of 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins as key compounds for antitumor active derivatives. These compounds were used as versatile intermediates for synthesis of the various amino coupling derivatives by nucleophilic substitution between 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins and suitable primary and secondary alkylamines. Furthermore, they were used in a novel route for the preparation of 5-deazaflavins {pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-diones} in good yields. These compounds were investigated for their in vitro antitumor and antiviral activities. In fact, many of the synthesized compounds showed promising antitumor activities against CCRF-HSB-2 and KB cells. Also the SAR study was carried out with the aid of molecular modeling using AutoDock for the aim to get designed compounds possessing higher binding affinities into c-kit receptor of PTK. The correlation between the growth inhibitory activities (IC_{50}, μg/mL) of the synthesized 5-deazaflavins against tumor cells and the AutoDock binding free energies was investigated, where a fairly good correlation for some compounds was obtained. Also the computationally designed 10-alkyl-2-deoxo-5-deazaflavins connected with amino acids at the 2-position exhibited preferential binding affinities into PTK with more hydrogen bonds.

2. Results and discussion

2.1. Chemistry

The requisite 6-chloro-2-methylthiopyrimidin-4(3*H*)-one was prepared by treatment of 4-amino-6-chloro-2-methylthiopyrimidine[13] with $NaNO_2$ in glacial acetic acid according to the method of Israel et al.[14] The 4-amino-6-chloro-2-methylthiopyrimidine was obtained by reaction of thiourea and ethyl cyanoacetate, followed by methylation of the produced 4-amino-6-hydroxy-2-mercaptopyrimidine with dimethylsulfate and by chlorination with phosphoryl chloride. 6-Chloro-2-methylthiopyrimidin-4(3*H*)-one was also prepared by alternative route from *S*-methylation of thiobarbituric acid with MeI,[15] followed by chlorination with phosphoryl chloride,[16] and partially basic hydrolysis of the obtained 4,6-dichloro-2-methylthiopyrimidine.[17] The key intermediate, 6-(*N*-monoalkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones (**1a**–**j**), was synthesized by the treatment of 6-chloro-2-methylthiopyrimidin-4(3*H*)-one[14,16] with appropriate *N*-alkylanilines in *n*-butanol under reflux for 12–72 h in 55–83% yields as shown in Scheme 2. The commercially unavailable *N*-monomethylated anilines were prepared from the suitable aniline derivatives in two steps. Thus, the *N*-formylation of anilines with ethyl formate under reflux for 15 h gave the corresponding *N*-arylformamides, followed by reduction of the *N*-arylformamides using $LiAlH_4$ in dry THF to afford the corresponding *N*-monomethylated anilines.[18] The intended 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2a**–**j**) were prepared by the reaction of **1a**–**j** with Vilsmeier reagent (*N*,*N*-dimethylformamide–phosphoryl chloride) at 90 °C for 0.5–2 h to afford the products as yellow needles in 71–99% yields. Since the direct cyclization of 6-(*N*-methylanilino)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidines was not successful, the 2-thioxo moiety should be protected by methylation before the cyclization with Vilsmeier reagent.

The preparation for such *N*-substituted amino derivatives by reaction of alkylthio derivatives with appropriate primary or secondary amines has long been utilized in heterocyclic chemistry. Many of these reactions require rather strenuous conditions and are usually carried out in a stainless steel sealed vessel except when amines of high boiling point are used.[19] The pyrimidine moieties of 5-deazaflavins to undergo nucleophilic substitution reactions on the carbons adjacent to ring nitrogens are well authenticated and readily explicable in view of the π electron-deficient nature. Therefore, the various aminations were applied for the replacement of 2-methylthio group by appropriate amines for the purpose of syntheses of 2-amino- and 2-(substituted amino)-10-alkyl-2-deoxo-5-deazaflavins {2-amino- and 2-(substituted amino)-10-alkylpyrimido[4,5-*b*]quinolin-4(10*H*)-ones} (**3a**–**e**, **4a**–**c**, **6**, and **7a**–**q**) as shown in Scheme 3. Namely, 10-alkyl-2-deoxo-2-dimethylamino-5-deazaflavins (**3a**–**e**) were prepared by heating the suitable 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2**) with aqueous dimethylamine in a stainless steel sealed vessel at 135 °C for 4 h to afford the corresponding products as yellow needles in 59–96% yields. Whereas, the 10-alkyl-2-amino-2-deoxo-5-deazaflavins (**4a**–**c**) were prepared by fusion of the suitable **2** with ammonium acetate at 160–165 °C for 0.5–3 h to afford the corresponding products as yellow needles in 57–77% yields. A similar treatment of 2-deoxo-10-methyl-2-methylthio-5-deazaflavin (**2a**) with *n*-butylammonium acetate (prepared in situ) at 150–165 °C for 1–3 h resulted in the formation of 2-*n*-butylamino-2-deoxo-10-methyl-5-deazaflavin (**6**) as a yellow crystalline solid in 91% yield. Moreover, a variety of 2-(substituted amino)-10-alkyl-2-deoxo-5-deazaflavins (**7a**–**q**) were prepared by the facile method involving reflux of compound **2** with an appropriate morpholine, piperidine, *N*-methylpiperazine, *N*-methyl-*N*-ethanolamine, ethanolamine, or *n*-octylamine in *n*-butanol for 2–5 h. All products **7a**–**q** were obtained as bright yellow needles in quite good yields of 70–99%. For all the above-mentioned reactions which involve treatment



a: R^1 = Me, R^2 = H
b: R^1 = Et, R^2 = H
c: R^1 = Me, R^2 = 4-Me
d: R^1 = Me, R^2 = 2-Me
e: R^1 = Me, R^2 = 4-OMe
f: R^1 = Me, R^2 = 3-OMe
g: R^1 = Me, R^2 = 4-Cl
h: R^1 = Et, R^2 = 4-Me
i: R^1 = Me, R^2 = 3,4-di-Me
j: R^1 = Me, R^2 = 2,5-di-OMe

a: R^1 = Me, R^2 = H
b: R^1 = Et, R^2 = H
c: R^1 = Me, R^2 = 7-Me
d: R^1 = Me, R^2 = 9-Me
e: R^1 = Me, R^2 = 7-OMe
f: R^1 = Me, R^2 = 8-OMe
g: R^1 = Me, R^2 = 7-Cl
h: R^1 = Et, R^2 = 7-Me
i: R^1 = Me, R^2 = 7,8-di-Me
j: R^1 = Me, R^2 = 6,9-di-OMe

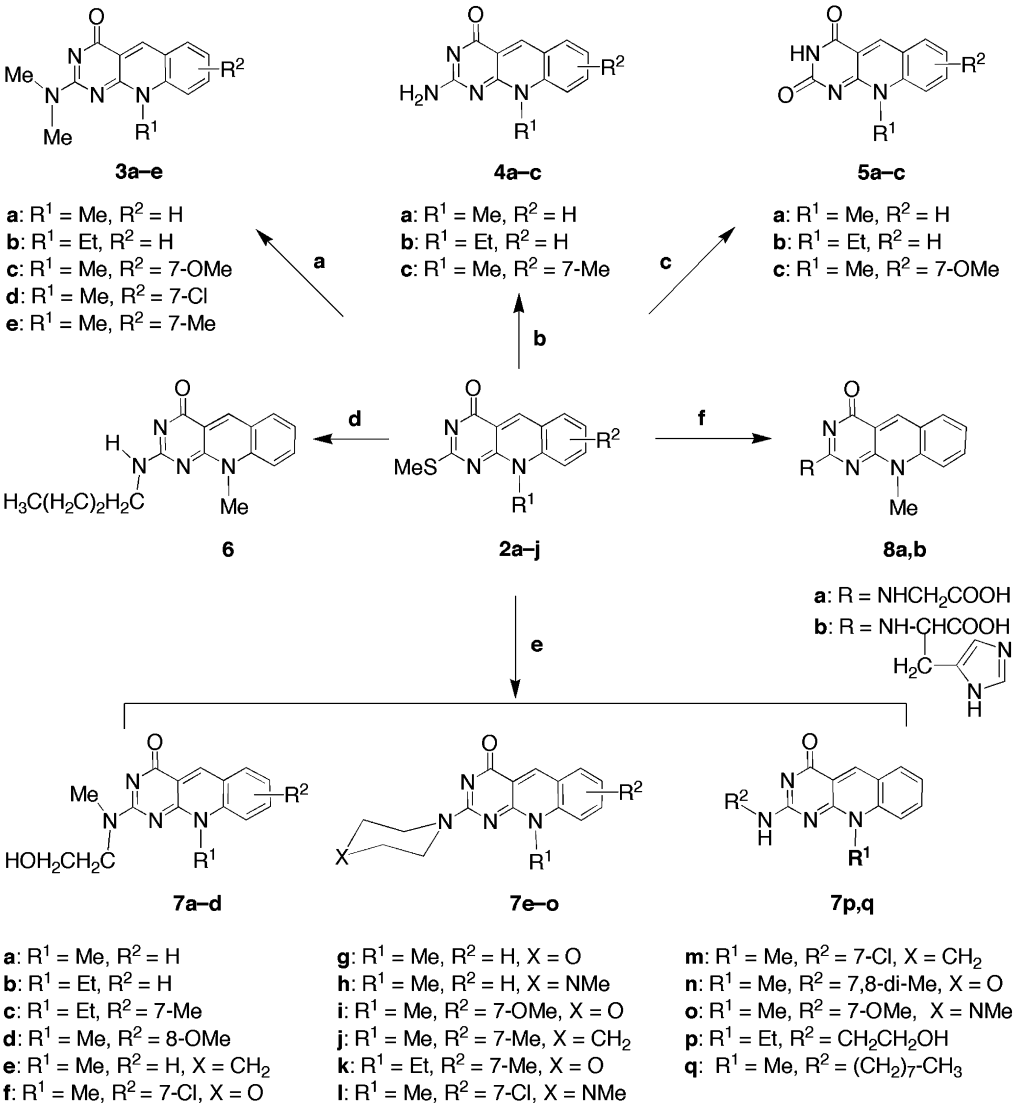
Scheme 2. General methods for the preparation of 6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones (**1a**–**j**) and 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2a**–**j**). Reagents and conditions: (a) *n*-Butanol, reflux 12–72 h; commercially unavailable *N*-monoalkylanilines were prepared by refluxing with HCOOEt for 15 h, followed by reduction with $LiAlH_4$ in THF at 0 °C for 5–12 h; (b) Vilsmeier reagent (DMF-$POCl_3$), 90 °C, 1–3 h.

with different amines, the excess volatile amines were removed by concentration in vacuo. Whereas, the amines of high boiling point were removed by leaching from the residue with water or ethanol for piperidine, morpholine, *N*-(2-hydroxyethyl)-*N*-methylamine, and *N*-methylpiperazine, and petroleum ether for *n*-octylamine to get the amine-free products. Acidic hydrolysis of some 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2**) in 5 N hydrochloric acid by heating under reflux for 1–2 h gave the corresponding 10-alkyl-5-deazaflavins (**5a**–**c**) as yellow needles in 78–91% yields as shown in Scheme 3.

UV–vis, IR, and NMR spectra and elemental analyses were used for determination and identification of the newly assigned structures. The structures of the key intermediates **1a**–**j** were confirmed in particular by the presence of a proton resonance at the 5-position as a singlet signal at 4.62–5.08 ppm in ^{1}H NMR spectra. The cyclized 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2a**–**j**) showed a characteristic singlet signal due to the C_5-proton in the lower field at 9.09–9.63 ppm. It is implying that the methine at the 5-position of **2a**–**j** is more electron-deficient than the other methines. Any substituent at the benzene ring led this singlet signal of the C_5-proton to the slight upfield, except for **2j** (2-deoxo-6,9-dimethoxy-10-methyl-2-methylthio-5-deazaflavin), which exhibits this signal in more downfield at 9.63 ppm. This can be explained by the possible formation of a hydrogen bond between the oxygen atom of 6-OMe moiety and the proton at the 5-position. This suggestion is supported by the similar effect between 9-OMe group and the N_{10}-methyl moiety of **2j**, that is, the chemical shift of methyl group at the 10-position shown in more downfield region at 4.48 ppm in comparison with N_{10}-methyl moieties of other derivatives appeared in the range of 4.21–4.35 ppm. This effect was also clearly observed in the UV spectrum of **2j**, which indicated some hypsochromic shift, compared with other spectra of **2**. Considering the ^{1}H NMR spectra, the 2-deoxo-5-deazaflavin derivatives (**3**–**7**) are characterized by disappearance of the strong characteristic singlet signal of the 2-methylthio group, which were assigned for compounds **2a**–**j** at 2.56–2.63 ppm. The 2-(substituted amino) derivatives (**3**, **4**, **6**, and **7**) and 5-deazaflavins (**5**) showed a characteristic singlet signal of the C_5-proton in the lower field at 8.77–9.12 and 8.93–9.0 ppm, respectively. The methine protons (8.77–9.12 ppm) at 5-position for **3**, **4**, **6**, and **7** appeared in upper field than those (9.09–9.63 ppm) of **2a**–**j**, due to the higher electron donating properties of the 2-(substituted amine) groups in comparison with that of the 2-methylthio group.

Regarding the ^{1}H NMR of 10-alkyl-2-deoxo-2-dimethylamino-5-deazaflavins (**3a**–**e**), it was noticed that the N_{10}-methyl derivatives (**3a,c**–**e**) exhibited the equivalent N_2-dimethyl groups as an equivalent singlet signal of 6H integral almost at 3.33 ppm. Whereas, the N_{10}-ethyl derivative (**3b**) exhibited the nonequivalent N_2-dimethyl groups as two singlet signals at 3.34 and 3.48 ppm due to the steric hindrance of longer chain of ethyl group at the N-10 position.

Interestingly, the phenomenon of reversible interconversion of two isomers at 27 °C in case of the 2-monoalkylamino (secondary amino) derivatives (**6**, and **7p,q**) was observed as tautomerism in ^{1}H NMR spectra. The twin overlapped spectra of approximately 1:2 or 2:1 ratio of the 2-monoalkylamino and 2-monoalkylimino tautomers in $(CD_3)_2SO$ were obtained for the two resonance species (tautomers). Especially, such duplicated spectra were observed in the range of 4.0–9.0 ppm based on the tautomers of the guanidine adjacent to carbonyl group. At higher temperature of 90–100 °C, the coalescence of the duplicated spectrum was observed to produce the single spectrum. As indicated in Figure 1(A) for compound **6**, the ^{1}H NMR spectrum at 27 °C exhibited two singlet signals of C_5-H and N_{10}-Me at 8.81 and 8.87 ppm and at 4.05 and 4.14 ppm, respectively, and other protons at 6, 7, 8, and 9-positions were also duplicated. On the other hand, in the case of 100 °C, the singlet signal at 8.80 and 4.07 ppm, which was attributable to the C_5-H and N_{10}-Me, respectively, appeared as



Scheme 3. General methods for the preparation of 10-alkyl-2-deoxo-2-(substituted amino)-5-deazaflavins (**3a**–**e**, **6**, and **7a**–**q**), 10-alkyl-2-amino-2-deoxo-5-deazaflavins (**4a**–**c**), and 10-alkyl-5-deazaflavins (**5a**–**c**). Reagents and conditions: (a) 50% aqueous $NHMe_2$, steel sealed tube, 135 °C, 4 h; (b) CH_3COONH_4, 160–165 °C, 0.5–3 h; (c) 5 N HCl, reflux, 3–5 h; (d) *n*-Butylammonium acetate, fusion, 150–165 °C, 1–3 h; (e) appropriate amines, *n*-butanol, reflux, 2–5 h; (f) computationally designed structures.

shown Figure 1(B) and the duplicated spectral signals for other protons also coalesced. The 2-*n*-butylimino tautomer is proposed predominantly at higher temperature because it has a heat of formation (PM3-Mozyme) of 36.23 kcal/mol which is higher than that of 2-butylamino tautomer (16.95 kcal/mol). This phenomenon is mainly attributed to the presence of a secondary amine at the 2-position, whereas it does not take place in case of 2-substituted primary and teritiary amines, for example, compounds **4a**–**c** and **7a**–**o**, respectively.

The structural identity of the prepared compounds was verified by UV–vis absorption spectra. The spectra for 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2a**–**j**) located in shorter wavelength region in comparison with those for previously reported 2-deoxo-2-phenyl-5-deazaflavin derivatives,[1] which have more conjugated ring system. The UV absorption spectra of the 6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones (**1a**–**j**) showed one absorption maximum at 239–246 nm together with two absorption shoulders at 259–275 and 287–293 nm. Whereas, the tricyclic 5-deazaflavins (**2a**–**j**) showed five absorption maxima at 213–231, 259–275, 278–291, 311–351, and 403–450 nm. The absorption band at 259–275 nm was shown as a maximum absorption peak or a shoulder. Compound **2j** indicated the characteristic blue shift (hypsochromic shift) in all wavelength regions in comparison with those of **2a**–**i**. This may be attributed to the hydrogen bond formation between the oxygen atom of 6-OMe moiety and C_5-proton of **2j**. This hinders the delocalization of the lone pair on oxygen of 6-OMe to the ring system by the hydrogen bond. The UV spectra of 2-(substituted amino)-10-alkyl-2-deoxo-5-deazaflavins (**3**, **4**, **6**, and **7**) showed five absorption maxima at 220–225, 268–288, 318–326, 407–428, and 424–454 nm, together with an absorption shoulder at 383–404 nm, except for compound **7n**. All 5-deazaflavin analogs (**2**–**7**) showed yellow color owing to the presence of absorption maximum at 400–440 nm in the longest wavelength. Generally, the UV spectra

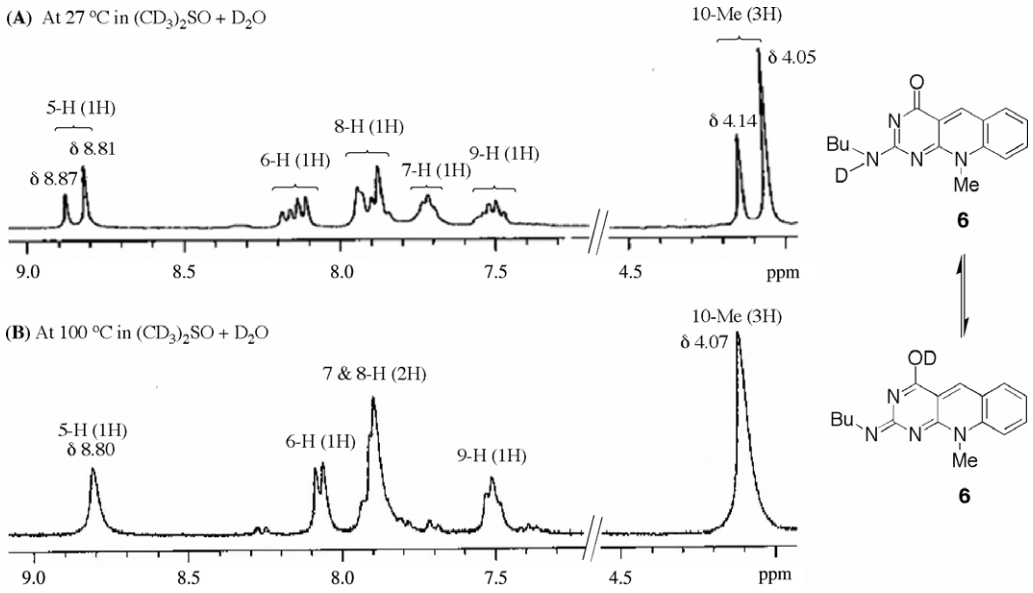


Figure 1. The ^{1}H NMR spectra of compound 6 at 27 °C (A) and at 100 °C (B) in $(CD_3)_2SO$.

of 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2**) exhibited longer wavelength than those of 2-(substituted amino)-10-alkyl-2-deoxo-5-deazaflavins and 5-deazaflavins (**3**–**7**) as represented in Figure 2 for compounds **1a, 2a**, **5a**, and **7a**. This is attributed to the S-atom which causes a generally red shift (bathochromic shift) in the spectrum due to its easier polarizability.[20] Thus, the spectrum of 2-deoxo-10-methyl-2-methylthio-5-deazaflavin (**2a**) exhibited the bathochromic shift of ca. 20 nm longer wavelength than that of 5-deazaflavin (**5a**) in all UV and visible regions.

2.2. In vitro antitumor activities of 5-deazaflavins and their 2-(*N*-substituted amino) derivatives against human tumor cell lines

The compounds (**2**–**5** and **7**) synthesized in this study were tested in vitro for their growth inhibitory activities against two human cultured tumor cell lines, that is, human T-cell acute lymphoblastoid leukemia cell line (CCRF-HSB-2) and human oral epidermoid carcinoma cell line (KB). The antitumor agent, cytosine arabinoside (Ara-C), was used as positive control.

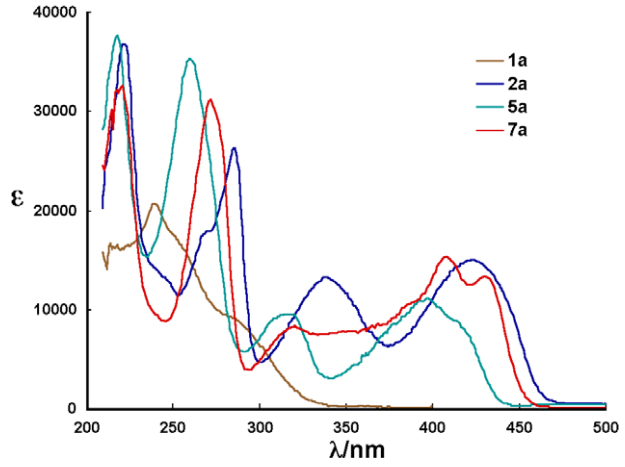


Figure 2. UV–vis spectra of 6-(*N*-methylanilino)-2-methylthiopyrimidin-4(3*H*)-one (**1a**), 2-deoxo-10-methyl-2-methylthio-5-deazaflavin (**2a**), 10-methyl-5-deazaflavin (**5a**), and 2-deoxo-2-[*N*-(2-hydroxyethyl)-*N*-methylamino]-10-methyl-5-deazaflavin (**7a**).

As can be seen in Table 1, although all compounds of 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2**) and 10-alkyl-2-deoxo-2-dimethylamino-5-deazaflavins (**3**) inferior antitumor activities compared to Ara-C, they have been found to show significant antitumor activities against CCRF-HSB-2 cell line. Among them, the compounds **2a,h**, **3b**, and **4b** indicated better activities of less than 2.0 μg/mL (IC_{50}). Against KB cell line they exhibited good growth inhibitory activities of about one-tenth antitumor potency of Ara-C (IC_{50}: 0.23 μg/mL), that is, the IC_{50} of compounds **2a,h**, **3b**, and **4b** were 1.97, 1.73, 3.96, and 1.00 μg/mL, respectively. The 2-deoxo-5-deazaflavins may reveal fairly good and less toxic antitumor activities in comparison with Ara-C. Moreover, compounds **2b,f,i,j**, **6**, and **7h,l,n** exhibited reasonable potential growth inhibitory activities of ca. 5.0–7.0 μg/mL (IC_{50}) against CCRF-HSB-2 cell line. Also compounds **2b,f**, **6**, and **7q** exhibited prospective growth inhibitory activities of ca. 5.0–8.0 μg/mL (IC_{50}) against KB cell line.

On the other hand, the anti-herpes simplex virus activities of these flavin analogs were investigated in vitro against HSV-1 and HSV-2 according to the known procedure,[21,22] using acyclovir as a positive control. The potency of antiviral activity of each compound is expressed as a minimum inhibitory concentration required to reduce virus plaque formation by 50% under experimental conditions (ED_{50}). The 2-deoxo-5-deazaflavin derivatives, namely **2a,b,h,j**, and **4b**, exhibited some antiviral activities of 0.8, 20, 4, 20, and 4 μg/mL (ED_{50}), respectively, against HSV-1. The obvious antiviral effects are not their actual antiviral potencies but mainly attributed to the pronounced cytotoxicities of these compounds.

Table 1. Growth inhibitory activities of 2-methylthio-, 2-(substituted amino)-, and 2-amino-10-alkyl-2-deoxo-5-deazaflavins (**2–4**, **6**, and **7**) and 5-deazaflavin (**5**) against CCRF-HSB-2 and KB tumor cell lines

Compound	Inhibitorty activity [IC_{50} (μg/mL)]		Compound	Inhibitorty activity [IC_{50} (μg/mL)]		Compound	Inhibitorty activity [IC_{50} (μg/mL)]	
	CCRF-HSB-2	KB		CCRF-HSB-2	KB		CCRF-HSB-2	KB
2a	1.91	1.97	**3d**	46.4	58.5	**7g**	>100.0	68.1
2b	7.07	5.42	**3e**	16.4	24.8	**7h**	5.9	8.44
2c	9.57	9.27	**4a**	13.7	17.9	**7i**	24.5	38.3
2d	15.3	12.0	**4b**	1.64	1.0	**7j**	25.2	32.7
2e	9.27	9.82	**4c**	14.3	44.5	**7k**	27.7	50.8
2f	5.92	7.69	**5c**	37.1	10.0	**71**	7.4	16.6
2g	9.87	21.5	**6**	6.04	7.31	**7m**	69.4	82.6
2h	1.82	1.73	**7a**	34.9	34.3	**7n**	6.0	8.8
2i	7.06	11.4	**7b**	26.3	23.5	**7o**	12.6	27.0
2j	4.84	8.75	**7c**	9.5	25.1	**7p**	11.3	14.0
3a	22.6	20.1	**7d**	39.0	36.0	**7q**	8.9	7.9
3b	1.96	3.96	**7e**	24.7	32.0	Ara-C	0.047	0.23
3c	35.6	23.6	**7f**	96.1	52.7			

The previously reported potent 2-deoxo-2-phenyl-5-deazaflavins,[1] which revealed minimum IC_{50} in the range of 0.15–0.68 and 0.16–0.72 μg/mL against CCRF-HSB-2 and KB cell lines, respectively, exhibited ca. 3–10 times more efficient antitumor activities than those of the aforementioned 2-methylthio-, 2-amino- and 2-(substituted amino)-2-deoxo-5-deazaflavins (**2–4** and **7**), which revealed the minimum IC_{50} in the range of 1.64–1.96 and 1.00–1.97 μg/mL against the same cell lines. This can be explained that the phenyl group at the 2-position provided better affinity to the enzyme on account of the force of electrostatic attraction between the planar phenyl and the target site pocket of the tumor cells. Hence, the phenyl derivatives exhibit a good fitting within the active site and better antitumor activity. The SAR revealed that the highest antitumor activities (ca. 2.0 μg/mL) for 2-deoxo-5-deazaflavins were obtained with the structural features on the pyrimidoquinoline skeleton; SMe, $N(Me)_2$, or NH_2 group at the C-2 position, Et group at the N-10 position, and Me group at the 7-position, and the moderate antitumor activities (ca. 5.0 μg/mL) were obtained with the structural features: NHC_4H_9, morpholino or 4-methylpiperazinyl substituent at the C-2 position, Me group at the N-10 position, and 7-OMe, 6,9-$(OMe)_2$, or 7,8-$(Me)_2$ substituent on the benzene ring. However, the interaction of electrostatic van der Waals attraction for these substituents with the active site of enzyme is less than planar phenyl substituent. Therefore, they showed less potential affinities and less antitumor activities than the 2-phenyl derivatives. It is noteworthy that the 2-amino and 2-methylthio derivatives (**2–4** and **7**) exhibited promising antitumor potencies in comparison with the less potent 2,4-dioxo-5-deazaflavins, which revealed lower activity (IC_{50} > 10 μg/mL) against KB cell line.[23]

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 2-deoxo-5-deazaflavins (**2–7**) and computationally designed 2-deoxo-5-deazaflavins (**8a,b**) connected with amino acid at the 2-position into PTK. Toward optimization of the aforementioned lead compounds of the promising antitumor activities, the advanced docking program AutoDock 3.05[24] was 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. The most straightforward method for validation of the used scoring function is to inspect how closely the best-docked conformation resembles the bound ligand in the experimental crystal structure. As cited in the literature,[10] if the RMSD (root mean square deviation) of the best docked conformation is $\leqslant$2.0 Å from the experimental one, the used scoring function is successful. Therefore, the validation of the function implemented in AutoDock was done by docking of the native ligand into its binding site. The docked results were compared to the crystal structure of the bound ligand–protein complex. The obtained success rates of AutoDock[24] were highly excellent as shown in Figure 3. The STI-571 ligand (Imatinib or Gleevec), 4-(4-methylpiperazin-1-ylmethyl)-*n*-[4-methyl-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)phenyl]-benzamide, was docked into its c-Kit receptor PTK (pdb code: 1t46).

The RMSD of the docked ligand was 0.25 Å as it seems exactly superimposed on the native bound one. Moreover, the obtained binding free energy (ΔG_b) was quite low being −18.43 kcal/mol. The docked ligand (yellow stick) exhibited hydrogen bonds with almost same atoms of amino acids involved with the native ligand (ball and stick, colored by element). The docked STI ligand exhibited three hydrogen bonds between Cys 673, Thr 670, and Asp 810 amino acids. Whereas, the native ligand exhibited four hydrogen bonds between same three amino acids involved in the docked ligand and

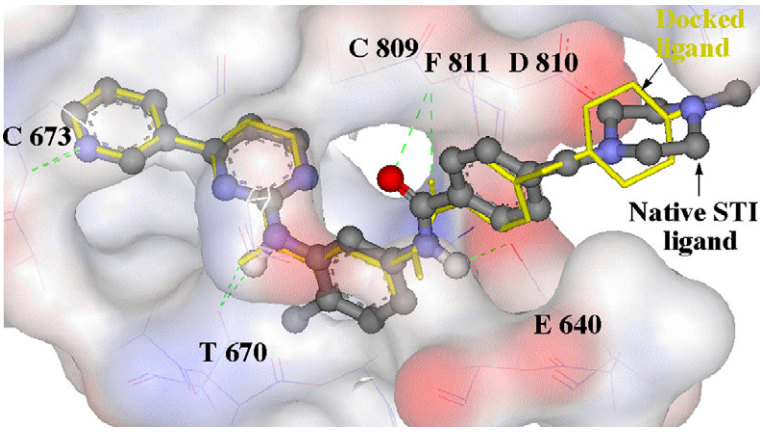


Figure 3. The docked STI ligand into PTK seems superimposed on the native STI ligand (ball and stick, colored by elements). The docked ligand (yellow stick) exhibited hydrogen bonds (green dotted lines) with almost same amino acids involved by the native one, the ΔG_b: −18.43 kcal/mol, and RMSD: 0.25 Å.

another amino acid of Glu 640. These results indicated the high accuracy of the AutoDock simulation in comparison with the biological methods.[25]

2.3.2. AutoDock binding affinities of the synthesized and the 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 7-substituted 2-deoxo-10-methyl-2-dimethylamino-5-deazaflavins (**3d,e**), 10-alkyl-2-amino-2-deoxo-5-deazaflavins (**4a–c**), 2-(substituted amino)-10-alkyl-2-deoxo-5-deazaflavins (**7c,e,n,p,q**), and the computationally designed 2-deoxo-5-deazaflavins connected with amino acids (**8a,b**). The latter derivatives exhibited many hydrogen bonds (4–6) between the target PTK. As shown in Table 2, compound **8a** (ΔG_b: −8.88 kcal/mol) exhibited four hydrogen bonds with Thr 670 and RMSD: 5.67 Å, compound **8b**, (ΔG_b: −10.63 kcal/mol) exhibited six hydrogen bonds with Lys 623, Val 668, Thr 670, and Asp 810 and RMSD: 4.29 Å, compound **7c** (ΔG_b: −13.37 kcal/mol) exhibited two hydrogen bonds with Ala 621 and Thr 670 and RMSD: 4.78 Å, compound **7n** (ΔG_b: −14.50 kcal/mol) exhibited one hydrogen bond with Cys 673 and RMSD: 7.93 Å, and compound **7q** (ΔG_b: −15.05 kcal/mol) exhibited one hydrogen bond with Thr 670 and RMSD: 3.80 Å.

To investigate the potential PTK inhibition, the comparatively antitumor active 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2a–j**) were also docked into PTK (1t46). Unfortunately, the docking of most compounds (**2**) was too far for the distance (>2.50 Å) between the C_4-oxo group and HO of Thr 670 and/or HN of Lys 623 (as common hydrogen bond donors) to form the hydrogen bond into the binding site. However, the planar pyrimidoquinoline ring was involved in hydrophobic electrostatic surface interaction, where it was sandwiched between the phenyl moieties of Phe 811 and Tyr 672 and the terminal hydrocarbon chain of Leu 595 within distance of 3.46, 4.53, and 3.36 Å, respectively. Also the 2-methylthio group interacts with lys 623, Val 668, and Cys 809 by hydrophobic attraction within distance of 3.22, 3.90, and 3.47 Å, respectively. The interactions keep these derivatives in the binding site, providing better RMSD values than other derivatives (3.80–5.41 Å). Exceptionally, compounds **2c** and **2 e** showed one hydrogen bond between the C_4-oxo and HO of Thr 670. This aforementioned docking results of **2a–j** indicate that these compounds are not expected to be a reasonable candidate for PTK inhibition.

The molecular docking study revealed that compounds **8a,b** prepared by replacement of 2-methylthio moiety of 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2**) by amino acids such as glycine and histidine exhibited reasonable binding energies and more important higher number of hydrogen bonds. The comparative docking modes of **8a,b** and **7n** into c-Kit receptor PTK with its bound ligand STI are shown in detail in Figure 4. The amino acid coupled to 5-deazaflavins has been shown to enhance remarkably the binding potentials in PTK. The higher affinity of these designed derivatives is presumably attributed to the formation of more hydrogen bonds (4–6 hydrogen bonds) and/or tighter hydrogen bonds between their 2-amino acid moieties and the binding site. While 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2**) and other 2-(substituted amino)-2-deoxo-5-deazaflavins (**3, 6**, and **7**) exhibited less binding affinities into the binding sites of PTK. This may be explained by missing of substituents at C_2-position which provide tight hydrogen bond interaction or surface electrostatic attraction such as 2-phenyl group as cited in our previous publication.[1]

Figure 5 illustrates docking of compound **4b** (ΔG_b: −12.16 kcal/mol) in another region of the binding site along with STI-ligand and **4b** exhibited one hydrogen bond. Whereas, compounds **7c** and **7q** were docked in another groove with two and one hydrogen bonds, respectively. This different binding mode of these com-

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 crystallized STI ligand

Compound	ΔG_b[a] (kcal/mol)	K_i[b]	Hydrogen bonds between atoms of compounds and amino acids				RMSD[c] (Å)
			Atom of compound	Amino acid	Distance (Å)	Angle (°)	
3d	−12.23	1.09E-09	C_4-Oxo	HN of Cys673	2.34	150.0	9.37
3e	−12.87	3.66E-10	C_4-Oxo	HN of Thr670	2.40	127.1	3.73
			C_4-Oxo	HO of Thr670	1.88	153.6	
4a	−12.71	4.82E-10	C_2-NH_a	N of Lys623	1.91	163.1	3.02
			C_2-NH_b	N of Thr670	2.49	153.0	
4b	−12.16	1.21E-09	C_2-NH	OH of Glu640	1.91	120.2	4.61
4c	−12.97	3.11E-10	C_2-NH	O of Thr670	1.96	139.6	6.19
7c	−13.37	1.59E-10	C_2-OH	O of Ala 621	2.45	121.8	4.78
			C_2-O	HO of Thr670	1.72	140.1	
7e	−14.27	3.49E-11	C_4-Oxo	—	—	—[d]	6.51
7n	−14.50	1.59E-10	C_4-Oxo	HN of Cys673	2.15	124.0	7.93
7p	−13.47	1.35E-10	C_2-OH	OH of Thr670	2.13	143.1	5.73
			C_2-O	HO of Thr670	2.04	144.9	
7q	−15.05	9.34E-12	C_2-NH	OH of Thr670	2.03	157.2	3.80
			C_2-NH	OH of Thr670	2.07	131.8	
			COOH	OH of Thr670	2.37	139.2	
8a	−8.88	3.11E-07	COOH	N of Thr670	2.24	127.7	5.67
			COO	HO of Thr670	2.23	147.0	
			C_2-NH	OH of Thr670	2.11	121.3	
			CO	HNl of Thr670	2.35	119.5	
8b	−10.63	1.63E-08	COO	HN of Lys623	1.33	128.1	4.29
			COOH	O of Val668	1.71	131.2	
			CO	HO of Thr670	2.01	148.6	
			NH (imidazole)	O of Asp 810	2.18	121.5	
			N3	HN of Cys673	1.66	161.0	
STI[e]	−18.43	3.08E-14	NH (H79)	OH of Thr670	1.86	147.2	0.25
			029	HN of Asp 810	2.01	133.3	

[a] Binding free energy.
[b] Inhibition constant.
[c] Root mean square deviation.
[d] The angle of the detected H-bond (91.8°) does not fit with the angle requirements.
[e] The native co-crystalline ligand (STI-571) bound to PTK (PDB code: It46).

pounds may explain their different antiproliferative activity against CCRF-HSB-2 and KB tumor cell lines as cited in Table 1.

The overall correlation between the growth inhibitory activities (IC_{50}, μg/mL) of the synthesized 5-deazaflavins against tumor cells and the binding affinities predicted

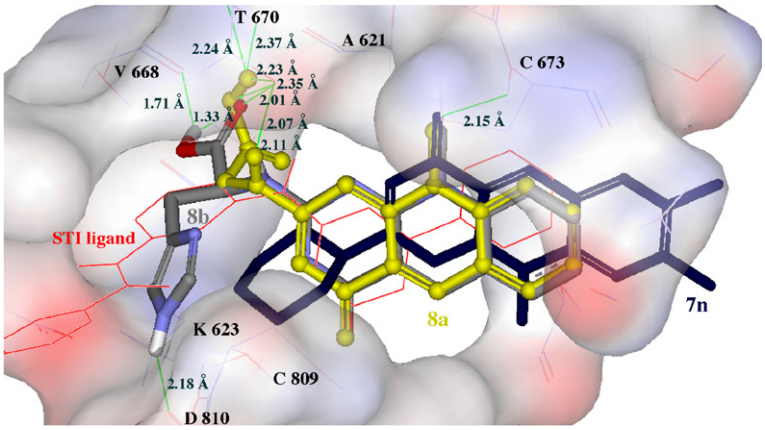


Figure 4. Differential binding affinities of the synthesized 2-deoxo-5-deazaflavin (**7n**; indigo, stick) and the designed 2-deoxo-5-deazaflavins connected with amino acids (**8a**: yellow, ball and stick; **8b**: colored by element stick) into PTK. Compounds **8a,b** exhibited the higher binding with 4–6 hydrogen bonds. The binding pocket of PTK is shown in transparent solid surface with labeled amino acids and the STI ligand is shown as red line. The settled hydrogen bonds are shown as green lines.

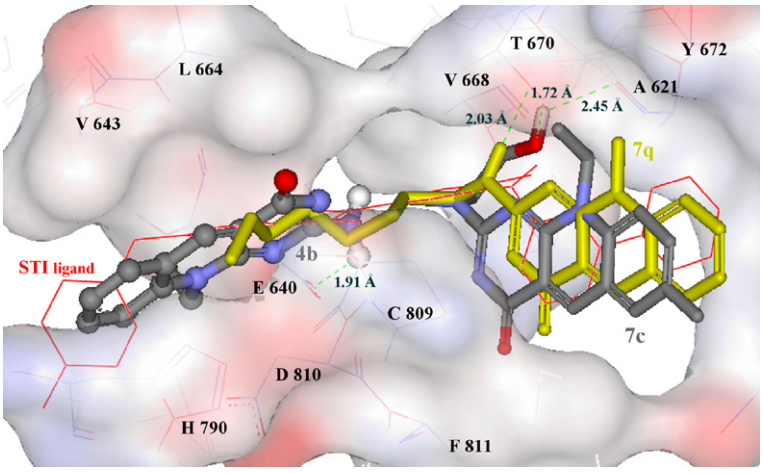


Figure 5. Inhibitor **4b** (colored by element, ball and stick) is docked horizontally into the plane of the groove of the binding site of PTK, where its C_2-NH_2 group was involved in hydrogen bond with Glu 640, while **7c** (colored by elememt, stick) is embedded perpendicularly into the groove exhibiting two hydrogen bonds. Compound **7q** (yellow, stick) is extended through the whole groove of the pocket forming one hydrogen bond with Thr 670. The binding pocket is shown in solid surface and the STI ligand is shown as red line.

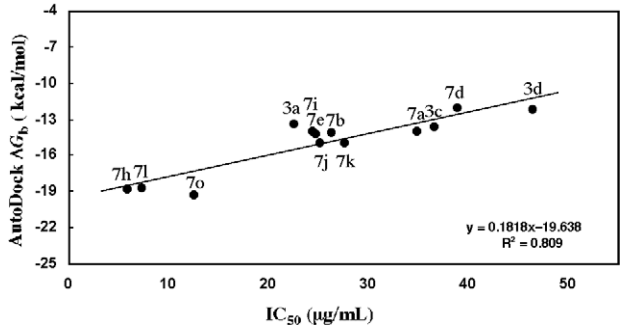


Figure 6. Correlation between the binding free energy (ΔG_b) and IC_{50} of 2-(substituted amino)-10-alkyl-2-deoxo-5-deazaflavins (**3a**,**c**,**d** and **7a**,**b**,**d**,**e**,**h**–**l**,**o**) against human T-cell acute lymphoblastoid leukemia cell line (CCRF-HSB-2).

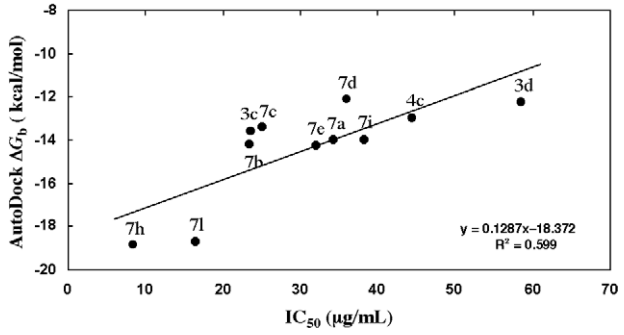


Figure 7. Correlation between the binding free energy (ΔG_b) and IC_{50} of 2-amino- and 2-(substituted amino)-10-alkyl-2-deoxo-5-deazaflavins (**3c**,**d**,**4c**, and **7a**–**e**,**h**,**i**,**l**) against human oral epidermoid carcinoma cell line (KB).

by AutoDock was fairly good for some compounds. Considering the growth inhibition against CCRF-HSB-2 cells, it was noticed that the correlation between IC_{50} of **3a,c,d** and **7a,b,d,e,h**–**l,o** and their AutoDock binding free energies revealed an excellent correlation coefficient (R^2) of 0.809 as represented in Figure 6. Whereas, the growth inhibition against KB cells revealed a reasonable correlation with AutoDock binding free energies for compounds **3c,d**, **4c**, and **7a**–**e,h,i,l** of correlation coefficient (R^2) value of 0.599 as shown in Figure 7.

3. Conclusions

In this study, various novel 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2a**–**j**) were synthesized from 6-(*N*-monoalkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones (**1a**–**j**) by the reaction using Vilsmeier reagent. The similar 2-(substituted amino)-10-alkyl-2-deoxo-5-deazaflavins (**3a**–**e**, **6**, and **7a**–**q**) were synthesized by the facile replacement of C_2-methylthio moiety by amines of different type. 10-Alkyl-2-amino-2-deoxo-5-deazaflavins (**4a**–**c**) were also prepared in better yield than the published method for compounds **4a,b**. Additionally, 5-deazaflavins {pyrimido[4,5-*b*]quinolin-2,4-diones} (**5a**–**c**) were prepared by acidic hydrolysis of 2-deoxo-2-methylthio-5-deazaflavins (**2**) in good yields. In the growth inhibitory activities of **2**–**7** against T-cell acute lymphoblastoid leukemia cell line (CCRF-HSB-2) and human oral epidermoid carcinoma cell line (KB) in vitro, many derivatives showed potential antitumor activities. Among them, compounds **2a,h**, **3b**, and **4b** exhibited the most significant antiproliferative potencies with IC_{50} of 1.64–1.96 and 1.00–3.96 μg/mL against CCRF-HSB-2 and KB cells, respectively. Some other derivatives, namely **2b,f,i,j**, **6**, and **7h,m,p** showed IC_{50} of 4.84–7.40 and 5.42–7.90 μg/mL against CCRF-HSB-2 and KB cells, respectively. These results revealed a promising antitumor activity of many derivatives, which are considered as new leads.

The AutoDock investigation of the synthesized 2-deoxo-5-deazaflavins (**2**–**4**, **6**, and **7**) and the computationally designed 2-deoxo-5-deazaflavins connected with amino acids (**8a,b**) was carried out for lead optimization. Thus, they were docked within c-kit protein tyrosine kinase.

The overall correlation between the growth inhibitory activities (IC_{50}, μg/mL) of the synthesized 5-deazaflavins against tumor cells and the binding affinities predicted by AutoDock was fairly good for some compounds, namely, **3a**,**c,d** and **7a,b**,**d**,**e**,**h**–**l**,**o** against CCRF-HSB-2 cell line, with the correlation coefficient (R^2) of 0.809. While the correlation between IC_{50} of compounds **3c,d, 4c**, and **7a**–**e**,**h**,**i**,**l** against KB cell line was also good with the correlation coefficient (R^2) of 0.599. Further, in order to enhance the binding affinity of these derivatives, 5-deazaflavins connected with amino acids were designed computationally. They exhibited preferential binding affinities into PTK with more hydrogen bonds and lower binding free energies. These computationally designed hybrid compounds may be promising candidates for further antitumor investigation and their syntheses are in progress.

4. Experimental

4.1. Chemistry

Mps were obtained on a Yanagimoto micro melting point apparatus and are 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 NMR spectra were obtained using a Varian VXR 300 MHz spectrophotometer and chemical shift values were expressed in δ values (ppm) relative to tetramethylsilane (TMS) as internal standard. Coupling constants are given in Hz. All NH and OH protons were exchangeable with D_2O. UV spectra were measured in absolute EtOH using Beckman DU-68S UV spectrophotometer and the wavelength value with sh refers to wavelength at which shoulder or inflexion occurs in the absorption. All reagents were of commercial quality and were used without further purification. Organic solvents were dried in the presence of an appropriate drying agent and were stored over suitable molecular sieves. Reaction progress was monitored by analytical thin layer chromatography (TLC) on pre-coated glass plates (silica gel $60F_{254}$-plate-Merck) and the products were visualized by UV light.

4.1.1. General procedure for the preparation of 6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones (1a–j). A mixture of 6-chloro-2-methylthiopyrimidin-4(3*H*)-one[14,16] (3.0 g, 0.017 mol) and an appropriate *N*-alkylaniline (0.051–0.085 mol) in *n*-butanol (20 mL) was refluxed with stirring for 12–72 h. After cooling, the precipitated solid was filtered off to get the first crop. The filtrate was concentrated in vacuo and the residue was crushed with diethyl ether to precipitate powdery crystals, which were filtered off, washed with water, and dried to afford the second crop. The collected solids were crystallized from appropriate solvents with charcoal to afford the corresponding products as colorless needles in 55–83% yields.

4.1.2. 6-(*N*-Methylanilino)-2-methylthiopyrimidin-4(3*H*)-one (1a). Yield, 3.11 g (74%); mp 268–270 °C (from EtOH); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 241 (4.28), 263sh (4.15), 292sh (3.89); IR (ν_{max}/cm^{-1}): 3220 (NH), 1640 (CO); ^{1}H NMR ($CDCl_3$): δ 2.46 (3H, s, 2-SMe), 3.45 (3H, s, 6-NMe), 5.06 (1H, s, 5-H), 7.11–7.30 (3H, m, Ph-*o*,*p*H), 7.36–7.43 (2H, m, Ph-*m*H), 13.24 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{12}H_{13}N_3OS$: C, 58.28; H, 5.30; N, 16.99. Found C, 58.23; H, 5.43; N, 16.95.

4.1.3. 6-(*N*-Ethylanilino)-2-methylthiopyrimidin-4(3*H*)-one (1b). Yield, (2.89 g, 65%); mp 235–236 °C (from EtOH); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 241 (4.56), 261sh (4.40), 293sh (4.10) ; IR (ν_{max}/cm^{-1}): 3200 NH), 1640 (CO); ^{1}H NMR ($CDCl_3$): δ 1.20 (3H, t, J = 6.9 Hz, 6-NCH$_2$-C*H*$_3$), 2.48 (3H, s, 2-SMe), 3.94 (2H, q, J = 6.9 Hz, 6-NC*H*$_2$-CH$_3$), 4.90 (1H, s, 5-H), 7.15–7.22 (2H, m, Ph-*o*H), 7.28–7.34 (2H, m, Ph–*p*H), 7.37–7.45 (2H, m, Ph–*m*H), 13.35 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{13}H_{15}N_3OS$: C, 59.74; H, 5.79; N, 16.08. Found: C, 59.58; H, 5.81; N, 16.10.

4.1.4. 6-(*N*-Methyl-4-methylanilino)-2-methylthiopyrimidin-4(3*H*)-one (1c). Yield, (3.51 g, 79%); mp 301–303 °C (from EtOH); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 242 (4.56), 259sh (4.47), 292sh (4.16); IR (ν_{max}/cm^{-1}): 3200 (NH), 1625 (CO); ^{1}H NMR ($CDCl_3$): δ 2.36 (3H, s, *p*Me), 2.48 (3H, s, 2-SMe), 3.43 (3H, s, 6-N-Me), 5.02 (1H, s, 5-H), 7.09 (2H, d, $J_{2',3'} = J_{5',6'} = 8.1$ Hz, Ar-*o*H), 7.19 (2H, d, $J_{2',3'} = J_{5',6'} = 8.1$ Hz, Ar-*m*H), 12.94 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{13}H_{15}N_3OS$: C, 59.74; H, 5.79; N, 16.08 . Found: C, 59.66; H, 5.70; N, 15.86.

4.1.5. 6-(*N*-Methyl-2-methylanilino)-2-methylthiopyrimidin-4(3*H*)-one (1d). Yield, (2.35 g, 53%); mp 246–248 °C (from MeOH); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 240 (4.59), 267sh (4.33), 290sh (4.09); IR (ν_{max}/cm^{-1}): 3200 (NH), 1620 (CO); ^{1}H NMR ($CDCl_3$): δ 2.15 (3H, s, *o*Me), 2.57 (3H, br s, 2-SMe), 3.39 (3H, s, 6-NMe), 4.62 (1H, br s, 5-H), 7.08–7.13 (1H, m, Ar-*o*H), 7.21–7.25 (3H, m, Ar-*m*,*p*H), 13.07 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{13}H_{15}N_3OS$: C, 59.74; H, 5.74; N, 16.08. Found: C, 59.72; H, 5.97; N, 15.85.

4.1.6. 6-(*N*-Methyl-4-methoxyanilino)-2-methylthiopyrimidin-4(3*H*)-one (1e). Yield, (3.58 g, 76%); mp 292–293 °C (from EtOH); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 246 (4.44), 275sh (4.20), 289sh (4.07); IR (ν_{max}/cm^{-1}): 3200 (NH), 1630 (CO); ^{1}H NMR ($CDCl_3$): δ 2.48 (3H, s, 2-SMe), 3.42 (3H, s, 6-NMe), 3.82 (3H, s, *p*OMe), 4.98 (1 H, s, 5-H), 6.91 (2 H, d, $J_{2',3'} = J_{5',6'} = 9.0$ Hz, Ar-*o*H), 7.12 (2H, d, $J_{2',3'} = J_{5',6'} = 9.0$ Hz, Ar-*m*H), 12.78 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{13}H_{15}N_3O_2S$: C, 56.30; H, 5.45; N, 15.15. Found: C, 56.13; H, 5.44; N, 15.30.

4.1.7. 6-(*N*-Methyl-3-methoxyanilino)-2-methylthiopyrimidin-4(3*H*)-one (1f). Yield, (3.54 g, 75%); mp 196–198 °C (from EtOH); UV (EtOH): λ_{max}/nm (log ε/

$dm^3\ mol^{-1}\ cm^{-1}$): 240 (4.56), 261sh (4.42), 287sh (4.20); IR (ν_{max}/cm^{-1}): 3200 (NH), 1635 (CO); ^1H NMR ($CDCl_3$): δ 2.48 (3H, s, 2-SMe), 3.44 (3H, s, 6-NMe), 3.80 (3H, s, *m*OMe), 5.08 (1H, s, 5-H), 6.73–6.77 (1H, m, Ar-*p*H), 6.78–6.85 (2H, m, Ar-*o*H), 7.30 (1H, t, $J_{4',5'} = J_{5',6'} = 8.4$ Hz, Ar-*m*H), 13.02 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{13}H_{15}N_3O_2S$: C, 56.30; H, 5.45; N, 15.15. Found: C, 56.39; H, 5.48; N, 14.90.

4.1.8. 6-(*N*-Methyl-4-chloroanilino)-2-methylthiopyrimidin-4(3*H*)-one (1g). Yield, (3.98 g, 83%); mp >300 °C (from MeOH); UV (EtOH): λ_{max}/nm (log ε/$dm^3\ mol^{-1}\ cm^{-1}$): 239 (4.51), 262sh (4.43), 291sh (4.21); IR (ν_{max}/cm^{-1}): 3200 (NH), 1620 (CO); ^1H NMR ($CDCl_3$): δ 2.44 (3H, s, 2-SMe), 3.41 (3H, s, 6-NMe), 5.07 (1H, s, 5-H), 6.91 (2H, d, $J_{2',3'} = J_{5',6'} = 8.7$ Hz, Ar-*o*H), 7.12 (2H, d, $J_{2',3'} = J_{5',6'} = 8.7$ Hz, Ar-*m*H), 11.89 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{12}H_{12}ClN_3OS$: C, 51.15; H, 4.29; N, 14.91. Found: C, 50.85; H, 4.28; N, 14.88.

4.1.9. 6-(*N*-Ethyl-4-methylanilino)-2-methylthiopyrimidin-4(3*H*)-one (1h). Yield, (2.86 g, 61%); mp 237–239 °C (from EtOH); UV (EtOH): λ_{max}/nm (log ε/$dm^3\ mol^{-1}\ cm^{-1}$): 242 (4.63), 260sh (4.48), 292sh (4.16); IR (ν_{max}/cm^{-1}): 3200 (NH), 1635 (CO); ^1H NMR ($CDCl_3$): δ 1.19 (3H, t, $J = 7.2$ Hz, 6-NCH_2-CH_3), 2.37 (3H, s, *p*Me), 2.49 (3H, s, 2-SMe), 3.91 (2H, q, $J = 7.2$ Hz, 6-NCH_2-CH_3), 4.86 (1H, s, 5-H), 7.04 (2H, d, $J_{2',3'} = J_{5',6'} = 8.4$ Hz, Ar-*o*H), 7.19 (2H, d, $J_{2',3'} = J_{5',6'} = 8.4$ Hz, Ar-*m*H), 13.13 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{14}H_{17}N_3OS$: C, 61.06; H, 6.22; N, 15.26. Found: C, 61.12; H, 6.24; N, 15.50.

4.1.10. 6-(*N*-Methyl-3,4-dimethylanilino)-2-methylthiopyrimidin-4(3*H*)-one (1i). Yield, (3.65 g, 78%); mp 281–283 °C (from EtOH); UV (EtOH): λ_{max}/nm (logε/$dm^3\ mol^{-1}\ cm^{-1}$): 243 (4.55), 260sh (4.44), 292sh (4.13); IR (ν_{max}/cm^{-1}): 3200 (NH), 1625 (CO); ^1H NMR ($CDCl_3$): δ 2.25 (3H, s, *p*Me), 2.26 (3H, s, *m*Me), 2.49 (3H, s, 2-SMe), 3.42 (3H, s, 6-NMe), 5.00 (1H, s, 5-H), 6.92 (1H, dd, $J_{5',6'} = 7.8$ Hz, $J_{2',6'} = 1.5$ Hz , N-Ar-6′H), 6.98 (1H, d, $J_{2',6'} = 1.5$ Hz, Ar-2′H), 7.14 (1H, d, $J_{5',6'} = 7.8$ Hz, Ar-5′H), 12.74 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{14}H_{17}N_3OS$: C, 61.06; H, 6.22; N, 15.26. Found: C, 61.13; H, 6.17; N, 14.95.

4.1.11. 6-(*N*-Methyl-2,5-dimethoxyanilino)-2-methylthiopyrimidin-4(3*H*)-one (1j). Yield, (3.76 g, 72%); mp 227–229 °C (from EtOH); UV (EtOH): λ_{max}/nm (log ε/$dm^3\ mol^{-1}\ cm^{-1}$): 240 (4.50), 266sh (4.23), 292sh (4.16); IR (ν_{max}/cm^{-1}): 3200 (NH), 1630 (CO); ^1H NMR ($CDCl_3$): δ 2.50 (3H, br s, 2-SMe), 3.36 (3H, s, 6-NMe), 3.75 (3H, s, 2′-OMe), 3.76 (3H, s, 5′-OMe), 4.87 (1H, s, 5-H), 6.73 (1H, d, $J_{4',6'} = 3.0$ Hz, Ar-6′-H), 6.83 (1H, dd, $J_{4',6'} = 9.0$ Hz, $J_{4',6'} = 3.0$ Hz, Ar-4′H), 6.90 (1H, d, $J_{4',6'} = 9.0$ Hz, Ar-3′H), 12.93 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{14}H_{17}N_3O_3S$: C, 54.71; H, 5.57; N, 13.67. Found: C, 54.81; H, 5.62; N, 13.39.

4.1.12. General procedure for the preparation of 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins {10-alkyl-2-methylthiopyrimido[4,5-*b*]quinolin-4(10*H*)-ones} (2a–j). A mixture of 6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones (**1**, 0.01 mol) and phosphoryl chloride (7.7 g, 0.05 mol) in anhydrous dimethylformamide (10 mL) was heated under stirring at 90 °C for 0.5–2 h. Then, the reaction mixture was poured onto ice and neutralized with aqueous ammonia (pH 7). The yellow crystals separated were filtered off, washed with water, dried, and recrystallized from an appropriate solvent to afford the corresponding product as yellow needles in 71–99% yields.

4.1.13. 10-Methyl-2-methylthiopyrimido[4,5-*b*]quinolin-4(10*H*)-one (2a). Yield, (2.55 g, 99%); mp 281–282 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/$dm^3\ mol^{-1}\ cm^{-1}$): 222 (4.57), 268 (4.24), 286 (4.42), 340 (4.12), 423 (4.17); IR (ν_{max}/cm^{-1}): 1655 (CO); ^1H NMR ($CDCl_3$): δ 2.62 (3H, s, 2-SMe), 4.29 (3H, s, 10-Me), 7.60 (1H, t, $J_{6,7} = J_{7,8} = 8.7$ Hz, 7-H), 7.81 (1H, d, $J_{8,9} = 8.7$ Hz, 9-H), 7.97 (1H, t, $J_{7,8} = J_{8,9} = 8.7$ Hz, 8-H), 7.02 (1H, d, $J_{6,7} = 8.7$ Hz, 6-H), 9.24 (1H, s, 5-H); Anal. calcd for $C_{13}H_{11}N_3OS$: C, 60.68; H, 4.31; N, 16.33. Found: C, 60.30; H, 4.53; N, 15.94.

4.1.14. 10-Ethyl-2-methylthiopyrimido[4,5-*b*]quinolin-4(10*H*)-one (2b). Yield, (2.69 g, 99%); mp 262–265 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/$dm^3\ mol^{-1}\ cm^{-1}$): 222 (4.64), 268sh (4.32), 286 (4.49), 338 (4.19), 424 (4.25); IR (ν_{max}/cm^{-1}): 1655 (CO); ^1H NMR ($CDCl_3$): δ 1.54 (3H, t, $J = 6.9$ Hz, 10-CH_2-CH_3), 2.63 (3H, s, 2-SMe), 4.96 (2H, q, $J = 6.9$ Hz, 10-CH_2-CH_3), 7.59 (1H, dt, 1 H, $J_{6,7} = J_{7,8} = 9.0$ Hz, $J_{7,9} = 1.5$ Hz, 7-H), 7.82 (1H, d, $J_{8,9} = 9.0$ Hz, 9-H), 7.97 (1H, dt, $J_{7,8} = J_{8,9} = 9.0$ Hz, $J_{6,8} = 1.5$ Hz, 8-H), 8.04 (1H, dd, $J_{6,7} = 9.0$ Hz, $J_{6,8} = 1.5$ Hz, 6-H), 9.24 (1H, s, 5-H); Anal. calcd for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49. Found: C, 62.08; H, 5.01; N, 15.12.

4.1.15. 7,10-Dimethyl-2-methylthiopyrimido[4,5-*b*]quinolin-4(10*H*)-one (2c). Yield, (2.66 g, 98%); mp 279–281 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/$dm^3\ mol^{-1}\ cm^{-1}$): 225 (4.62), 270 (4.36), 288 (4.46), 340 (4.19), 432 (4.23); IR (ν_{max}/cm^{-1}): 1640 (CO); ^1H NMR ($CDCl_3$): δ 2.56 (3H, s, 7-Me), 2.62 (3H, s, 2-SMe), 4.28 (3H, s, 10-Me), 7.71 (1H, d, $J_{8,9} = 8.7$ Hz, 9-H), 7.75–7.80 (1H, m, 8-H), 7.81 (1H, d, $J_{6,8} = 2.1$ Hz, 6-H), 9.16(1H, s, 5-H); Anal. calcd for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49. Found: C, 61.66; H, 4.89; N, 15.27.

4.1.16. 9,10-Dimethyl-2-methylthiopyrimido[4,5-*b*]quinolin-4(10*H*)-one (2d). Yield, (1.93 g, 71%); mp 273–274 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/$dm^3\ mol^{-1}\ cm^{-1}$): 227 (4.56), 270 (4.36), 290 (4.52), 351 (4.26), 429 (4.21); IR (ν_{max}/cm^{-1}): 1650 (CO); ^1H NMR ($CDCl_3$): δ 2.63 (3H, s, 2-SMe), 2.91 (3H, s, 9-Me), 4.35 (3H, s, 10-Me), 7.45 (1H, t, $J_{6,7} = J_{7,8} = 7.5$ Hz, 7-H), 7.70–7.76 (1H, m, 8-H), 7.82 (1H, d, $J_{6,7} = 7.5$ Hz, 6-H), 9.14 (1H, s, 5-H); Anal. calcd for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49. Found: C, 61.73; H, 4.96; N, 15.10.

4.1.17. 7-Methoxy-10-methyl-2-methylthiopyrimido[4,5-*b*]quinolin-4(10*H*)-one (2e). Yield, (2.7 g, 94%); mp 275–277 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 229 (4.48), 275 (4.40), 290 (4.43), 339 (4.12), 450 (4.19); IR (ν_{max}/cm^{-1}): 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 2.63 (3H, s, 2-SMe), 3.97 (3H , s, 7-OMe), 4.31 (3H, s, 10-Me), 7.31 (1H, d, $J_{6,8}$ = 3.0 Hz, 6-H), 7.61 (1H, dd, $J_{8,9}$ = 9.3 Hz, $J_{6,8}$ = 3.0 Hz, 8-H), 7.77 (1H, d, $J_{8,9}$ = 9.3 Hz, 9-H), 9.19 (1H, s, 5-H); Anal. calcd for $C_{14}H_{13}N_3O_2S$: C, 58.52; H, 4.56; N, 14.62. Found: C, 58.26; H, 4.67; N, 14.22.

4.1.18. 8-Methoxy-10-methyl-2-methylthiopyrimido[4,5-*b*]quinolin-4(10*H*)-one (2f). Yield, (2.70 g, 94%); mp 285–287 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 231 (4.60), 256 (4.25), 281 (4.44), 359 (4.05), 417 (4.47); IR (ν_{max}/cm^{-1}): 1630 (CO); ^{1}H NMR ($CDCl_3$): δ 2.60 (3H, s, 2-SMe), 4.07 (3H, s, 8-OMe), 4.21 (3H, s, 10-Me), 7.03 (1H, d, $J_{7,9}$ = 2.4 Hz, 9-H), 7.18 (1H, dd, $J_{6,7}$ = 9.0 Hz, $J_{7,9}$ = 2.4 Hz, 7-H), 7.89 (1H, d, $J_{6,7}$ = 9.0 Hz, 6-H), 9.09 (1H, s, 5-H); Anal. calcd for $C_{14}H_{13}N_3O_2S$: C, 58.52; H, 4.56; N, 14.62. Found: C, 58.42; H, 4.63; N, 14.36.

4.1.19. 7-Chloro-10-methyl-2-methylthiopyrimido[4,5-*b*]-quinolin-4(10*H*)-one (2g). Yield, (2.86 g, 98%); mp 287–289 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 227 (4.63), 272 (4.49), 291 (4.46), 332 (4.12), 434 (4.19); IR (ν_{max}/cm^{-1}): 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 2.62 (3H, s, 2-SMe), 4.27 (3H, s, 10-Me), 7.75 (1H, d, $J_{8,9}$ = 9.3 Hz, 9-H), 7.61 (1H, dd, $J_{8,9}$ = 9.3 Hz, $J_{6,8}$ = 2.4 Hz, 8-H), 7.97 (1H, d, $J_{6,8}$ = 2.4 Hz, 6-H), 9.12 (1H, s, 5-H); Anal. calcd for $C_{13}H_{10}ClN_3OS$: C, 53.52; H, 3.45; N, 14.40. Found: C, 53.55; H, 3.66; N, 14.17.

4.1.20. 10-Ethyl-7-methyl-2-methylthiopyrimido[4,5-*b*]-quinolin-4(10*H*)-one (2h). Yield, (2.71 g, 95%); mp 278–280 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 225 (4.65), 269 (4.41), 289 (4.49), 340 (4.23), 432 (4.26); IR (ν_{max}/cm^{-1}): 1655 (CO); ^{1}H NMR ($CDCl_3$): δ 1.52 (3H, t, J = 7.2 Hz, 10-CH_2-CH_3), 2.56 (3H, s, 7-Me), 2.62 (3H, s, 2-SMe), 4.94 (2H, q, J = 7.2 Hz, 10-CH_2-CH_3), 7.69–7.74 (1H, m, 9-H), 7.76–7.81 (3H, m, 6, 8-H), 9.17 (1H, s, 5-H); Anal. calcd for $C_{15}H_{15}N_3OS$: C, 63.13; H, 5.30; N, 14.73. Found: C, 63.37; H, 5.55; N, 14.51.

4.1.21. 7,8,10-Trimethyl-2-methylthiopyrimido[4,5-*b*]-quinolin-4(10*H*)-one (2i). Yield, (2.31 g, 81%); mp 266–268 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 226 (4.65), 270 sh (4.35), 284 (4.48), 351 (4.23), 430 (4.30); IR (ν_{max}/cm^{-1}): 1655 (CO); ^{1}H NMR ($CDCl_3$): δ 2.46 (3H, s, 7-Me), 2.57 (3H, s, 8-Me), 2.62 (3H, s, 2-SMe), 4.26 (3H, s, 10-Me), 7.56 (1H, s, 9-H), 7.70 (1H, s, 6-H), 9.13 (1H, s, 5-H); Anal. calcd for $C_{15}H_{15}N_3OS$: C, 63.13; H, 5.30; N, 14.72. Found: C, 62.83; H, 5.42; N, 14.32.

4.1.22. 6,9-Dimethoxy-10-methyl-2-methylthiopyrimido-[4,5-*b*]quinolin-4(10*H*)-one (2j). Yield, (2.73 g, 86%); mp 251–253 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 213 (4.31), 259 (4.26), 278 sh (4.09), 311 (4.41), 403 (4.33); IR (ν_{max}/cm^{-1}): 1660 (CO); ^{1}H NMR ($CDCl_3$): δ 2.63 (3H, s, 2-SMe), 3.96 (3H, s, 9-OMe), 3.98 (3H, s, 6-OMe), 4.48 (3H, s, 10-Me), 6.78 (1H, d, $J_{7,8}$ = 9.0 Hz, 7-H), 7.31 (1H, d, $J_{8,9}$ = 9.0 Hz, 8-H), 9.63 (1H, s, 5-H); Anal. calcd for $C_{15}H_{15}N_3O_3S$: C, 56.77; H, 4.76; N, 13.24. Found: C, 56.65; H, 4.78; N, 13.01.

4.1.23. General procedure for the preparation of 10-alkyl-2-deoxo-2-dimethylamino-5-deazaflavins {10-alkyl-2-dimethylaminopyrimido[4,5-*b*]quinolin-4(10*H*)-ones} (3a–e). A mixture of 10-alkyl-2-deoxo-2-methylthio-5-deazaflavin (**2**, 5.0 mmol) and 50% aqueous dimethylamine (50 mL) was heated in steel sealed tube at 135 °C (10 kg/cm^2 pressure) for 4 h. After the reaction was complete, the precipitated crystals were collected by filteration, and mother liquid was evaporated in vacuo to get the second crop. The product was washed with water, dried and recrystallized from an appropriate solvent to afford the corresponding product as yellow crystals in 59–96% yields.

4.1.24. 10-Methyl-2-dimethylaminopyrimido[4,5-*b*]quinolin-4(10*H*)-one (3a). Yield, (1.22 g, 96%); mp >300 °C (from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 220 (4.65), 271 (4.68), 279sh (4.57), 320 (4.12), 382sh (4.15), 408 (4.33), 432 (4.28); IR (ν_{max}/cm^{-1}): 1655 (CO); ^{1}H NMR ($CDCl_3$): δ 3.34 (6H, s, 2-N(CH_3)$_2$), 4.12 (3H, s,10-Me), 7.44 (1H, t, $J_{6,7}$ = $J_{7,8}$ = 7.8 Hz, 7-H), 7.65 (1H, d, $J_{8,9}$ = 8.7 Hz, 9-H), 7.81 (1H, t, $J_{7,8}$ = 7.8 Hz, $J_{8,9}$ = 8.7 Hz, 8-H), 7.86 (1H, d, $J_{6,7}$ = 7.8 Hz, 6-H), 8.94 (1H, s, 5-H); Anal. calcd for $C_{14}H_{14}N_4O{\cdot}0.1H_2O$: C, 65.66; H, 5.59; N, 21.88. Found: C, 65.55; H, 5.80; N, 21.50.

4.1.25. 10-Ethyl-2-dimethylaminopyrimido[4,5-*b*]quinolin-4(10*H*)-one (3b). Yield, (0.79 g, 59%); mp >300 °C (from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 220 (4.55), 271 (4.53), 278sh (4.48), 322 (3.96), 383sh (4.04), 407 (4.22), 431 (4.16); IR (ν_{max}/cm^{-1}): 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 1.49 (3H, t, J = 7.2 Hz, 10-CH_2-CH_3), 3.34 (3H, s, 2-NMe), 3.48 (3H, s, 2-NMe), 4.79 (2H, q, J = 7.2 Hz, 10-CH_2-CH_3), 7.43 (1H, dt, $J_{6,7}$ = $J_{7,8}$ = 7.8 Hz, $J_{7,9}$ = 1.8 Hz, 7-H), 7.65 (1H, d, $J_{8,9}$ = 8.7 Hz, 9-H), 7.81 (1H, dt, $J_{7,8}$ = 7.8 Hz, $J_{8,9}$ = 8.7 Hz, $J_{6,8}$ = 1.8 Hz, 8-H), 7.90 (1 H, dd, $J_{6,7}$ = 7.8 Hz, $J_{6,8}$ = 1.8 Hz, 6-H), 8.95 (1H, s, 5-H); Anal. calcd for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 66.86; H, 6.12; N, 20.70.

4.1.26. 7-Methoxy-10-methyl-2-dimethylaminopyrimido[4,5-*b*]quinolin-4(10*H*)-one (3c). Yield, (1.24 g, 87%); mp 291–294 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 225 (4.64), 279 (4.69), 326 (4.18), 400sh (4.09), 428 (4.37), 454 (4.36); IR (ν_{max}/cm^{-1}): 1660 (CO); ^{1}H NMR ($CDCl_3$): δ 3.33 (6H, s, 2-N(CH_3)$_2$), 3.92 (3H, s, 7-OMe), 4.12 (3H, s,10-Me), 7.20 (1H, d, $J_{6,8}$ = 3.0 Hz, 6-H), 7.44 (1H, dd, $J_{8,9}$ = 9.3 Hz, $J_{6,8}$ = 3.0 Hz, 8-H), 7.60 (1 H, d, $J_{8,9}$ = 9.3 Hz, 9-H), 8.88 (1 H, s, 5-H); Anal. calcd for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.03; H, 5.65; N, 19.52.

4.1.27. 7-Chloro-10-methyl-2-dimethylaminopyrimido-[4,5-*b*]quinolin-4(10*H*)-one (3d). Yield, (0.94 g, 65%); mp 291–293 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 225 (4.64), 275 (4.70), 286sh (4.52), 318 (4.06), 390sh (4.10), 415 (4.25), 424 (4.19); IR (ν_{max}/cm^{-1}): 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 3.33 (3H, s, 2-NMe), 3.34 (3H, s, 2-NMe), 4.09 (3H, s,10-Me), 7.59 (1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 7.74 (1H, dd, $J_{8,9}$ = 9.0 Hz, $J_{6,8}$ = 2.4 Hz, 8-H), 7.80 (1H, d, $J_{6,8}$ = 2.4 Hz, 6-H), 8.81 (1H, s, 5-H); Anal. calcd for $C_{14}H_{13}ClN_4O{\cdot}0.2H_2O$: C, 57.52; H, 4.62; N, 19.17. Found: C, 57.40; H, 4.60; N, 19.07.

4.1.28. 7,10-Dimethyl-2-dimethylaminopyrimido[4,5-*b*]-quinolin-4(10*H*)-one (3e). Yield, (1.15 g, 86%); mp >300 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 222 (4.68), 273 (4.68), 281sh (4.59), 323 (4.13), 389sh (4.15), 413 (4.35), 438 (4.30); IR (ν_{max}/cm^{-1}): 1655 (CO); ^{1}H NMR ($CDCl_3$): δ 2.50 (3H, s, 7-Me), 3.33 (6H, s, 2-NMe_2), 4.10 (3H, s,10-Me), 7.55 (1H, d, $J_{8,9}$ = 9.6 Hz, 9-H), 7.61–7.65 (2H, m, 6 and 8-H), 8.88 (1H, s, 5-H); Anal. calcd for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 66.92; H, 6.09; N, 20.88.

4.1.29. General procedure for the preparation of 10-alkyl-2-amino-2-deoxo-5-deazaflavins {10-alkyl-2-aminopyrimido[4,5-*b*]quinolin-4(10*H*)-ones} (4a–c). A mixture of 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2**, 4.0 mmol) and ammonium acetate (10 g, 0.13 mol) was heated under stirring at 160–165 °C for 0.5–3 h. The reaction mixture was cooled, diluted with water (15 mL), neutralized with aqueous ammonia (pH 7), and cooled in refrigerator overnight. The resulting yellow crystals were collected by filtration, dried, and crystallized from an appropriate solvent to give the corresponding products as yellow needles in 57–77% yields.

4.1.30. 2-Amino-10-methylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4a). Yield, (0.64 g, 57%); mp 284–288 °C (decomp. from DMF, lit.,[26] 288 °C); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 218 (4.40), 267 (4.44), 319 (3.95), 380sh (3.67), 401 (3.83), 425 (3.72); IR (ν_{max}/cm^{-1}): 3340 and 3470 (NH), 1640 (CO); ^{1}H NMR ($CDCl_3$): δ 4.18 (3H, s,10-NMe), 5.38 (1H, br s, 2-NH, exchangeable with D_2O), 5.74 (1H, br s, 2-NH,exchangeable with D_2O), 7.43 (1H, dt, $J_{6,7}$ = $J_{7,8}$ = 7.8 Hz, $J_{7,9}$ = 1.5 Hz, 7-H), 7.71 (1H, d, $J_{8,9}$ = 8.7 Hz, 9-H), 7.88 (1H, dt, $J_{7,8}$ = 7.8 Hz, $J_{8,9}$ = 8.7 Hz, $J_{6,8}$ = 1.5 Hz, 8-H), 7.90 (1 H, dd, $J_{6,7}$ = 7.8 Hz, $J_{6,8}$ = 1.5 Hz, 6-H), 9.06 (1H, s, 5-H); Anal. calcd for $C_{12}H_{10}N_4O{\cdot}0.3H_2O$: C, 62.22; H, 4.61; N, 24.19. Found: C, 62.35; H, 4.65; N, 23.78.

4.1.31. 2-Amino-10-ethylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4b). Yield, (0.93 g, 77%); mp 264–266 °C (from EtOH, lit.,[26] 266 °C); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 220 (4.56), 268 (4.63), 319 (4.09), 378sh (3.83), 400 (4.02), 424 (3.91); IR (ν_{max}/cm^{-1}): 3320 and 3410 (NH), 1630 (CO); ^{1}H NMR [$(CD_3)_2SO$]: δ 1.36 (3H, t, J = 6.9 Hz, 10-CH_2-CH_3), 4.81 (2H, q, J = 6.9 Hz, 10-CH_2-CH_3), 7.38 (2H, br s, 2-NH_2,exchangeable with D_2O), 7.54 (1 H, dt, $J_{6,7}$ = $J_{7,8}$ = 7.8 Hz, $J_{7,9}$ = 1.2 Hz, 7-H), 7.88–8.06 (2H, m, 8 and 9-H), 8.20 (1H, dd, $J_{6,7}$ = 7.8 Hz, $J_{6,8}$ = 1.2 Hz, 6-H), 8.92 (1 H, s, 5-H); Anal. calcd for $C_{13}H_{12}N_4O{\cdot}H_2O$: C, 60.45; H, 5.46; N, 21.69. Found: C, 60.28; H, 5.49; N, 21.29.

4.1.32. 2-Amino-7,10-dimethylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (4c). Yield, (1.02 g, 85%); mp >300 °C (from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 221 (4.57), 268 (4.66), 320 (4.08), 381sh (3.96), 408 (4.11), 428 (4.02); IR (ν_{max}/cm^{-1}): 3360 and 3330 (NH), 1635 (CO); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.47 (3H, s, 7-Me), 4.09 (3H, s, 10-Me), 7.15 (2H, br s, 2-NH_2, exchangeable with D_2O), 7.75 (1H, dd, $J_{8,9}$ = 9.0 Hz, $J_{6,8}$ = 2.1 Hz, 8-H), 7.82 (1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 7.94 (1 H, br s, 6-H), 8.79 (1H, s, 5-H).; Anal. calcd for $C_{13}H_{12}N_4O{\cdot}0.3H_2O$: C, 63.56; H, 5.17; N, 22.81. Found: C, 63.96; H, 5.34; N, 22.44.

4.1.33. General procedure for the preparation of 10-alkyl-5-deazaflavins {10-alkylpyrimido[4,5-*b*]quinoline-2,4(3*H*, 10*H*)-diones} (5). To 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2**, 0.01 mol) was added 5N hydrochloric acid (250 mL). Then, the mixture was refluxed for 1–2 h and the resulting clear yellow solution was concentrated in vacuo. The yellow residue was crushed with water and neutralized with aqueous ammonia (pH 7). The precipitated powdery crystals were filtered off, washed well with water, dried, and recrystallized from glacial acetic acid to afford the corresponding products as yellow needles in 78–91% yields.

4.1.34. 10-Methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (5a). Yield, (2.07 g, 91%); mp >300 °C (from HOAc, lit.,[27] 359 °C); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 218 (4.57), 261 (4.55), 318 (3.97), 398 (4.04), 420 (3.88); IR (ν_{max}/cm^{-1}): 3140 (NH), 1700 and 1660 (CO); ^{1}H NMR [$(CD_3)_2SO$]: δ 4.04 (3H, s, 10-Me), 7.48–7.61 (1H, br m, 7-H), 7.86–8.03 (2H, br m, 8 and 9-H), 8.18 (1H, br d, $J_{6,7}$ = 7.2 Hz, 6-H), 9.0 (1 H, s, 5-H), 11.06 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{12}H_9N_3O_2{\cdot}0.2H_2O$: C, 62.44; H, 4.10; N, 18.20. Found: C, 62.16; H, 4.24; N, 17.92.

4.1.35. 10-Ethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (5b). Yield, (2.20 g, 91%); mp >300 °C (from HOAc, lit.,[27] 353 °C); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 219 (4.43), 262 (4.65), 319 (4.05), 396 (3.17), 417 (4.06); IR (ν_{max}/cm^{-1}): 3140 (NH), 1700 and 1660 (CO); ^{1}H NMR [$(CD_3)_2SO$]: δ 1.34 (3H, t, J = 7.2 Hz, 10-CH_2-CH_3), 4.74 (2 H, q, J = 7.2 Hz, 10-CH_2-CH_3), 7.54 (1H, dt, $J_{6,7}$ = $J_{7,8}$ = 8.1 Hz, $J_{7,9}$ = 2.4 Hz, 7-H), 7.92–8.01 (2H, m, 8 and 9-H), 8.19 (1H, br d, $J_{6,7}$ = 8.1 Hz, 6-H), 9.0 (1H, s, 5-H), 11.08 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.88; H, 4.85; N, 17.05.

4.1.36. 7-Methoxy-10-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione (5c). Yield, (2.01 g, 91%); mp >300 °C (from HOAc); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 225 (4.69), 262 (4.77), 324 (4.39), 426 (4.41), 447 (4.40); IR (ν_{max}/cm^{-1}): 3140 (NH),

1700 and 1655 (CO); ^{1}H NMR [$(CD_3)_2SO$]: δ 3.91 (3H, s, 7-OMe), 4.05 (3H, s, 10-Me), 7.59 (1H, dd, $J_{6,8}$ = 2.7 Hz, $J_{8,9}$ = 8.7 Hz, 8-H), 7.70 (1H, d, $J_{6,8}$ = 2.7 Hz, 6-H), 7.87 (1H, d, $J_{8,9}$ = 8.7 Hz, 9-H), 8.90 (1H, s, 5-H), 10.65 (1H, br s, 3-NH, exchangeable with D_2O); Anal. calcd for $C_{13}H_{11}N_3O_3{\cdot}0.1H_2O$: C, 60.27; H, 4.36; N, 16.22. Found: C, 60.25; H, 4.68; N, 15.83.

4.1.37. Preparation of 2-*n*-butylamino-10-methylpyrimido[4,5-*b*]quinolin-4-(10*H*)-one (6). *n*-Butylammonium acetate was prepared in situ by slowly adding glacial acetic acid (5.7 mL, 0.1 mol) to *n*-butylamine (7.31 g, 0.1 mol) in an ice bath. 2-Deoxo-10-methyl-2-methylthio-5-deazaflavin (**2**, 0.008 mol) was added to the *n*-butylammonium acetate and the mixture was fused under stirring at 150–165 °C for 1–3 h. After the reaction was complete, water (100 mL) was added to the mixture to give a turbid solution. The residue concentrated in vacuo was dissolved in hot water and brought to pH 8 with sodium bicarbonate to give a yellow crystalline solid, which was dried, and recrystallized from DMF to afford yellow needles.

Yield, (1.28 g, 91%); mp 252–254 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 219 (4.59), 269 (4.63), 273sh (4.60), 321 (4.08), 403 (4.13), 422sh (4.07); IR (ν_{max}/cm^{-1}): 3180 (NH), 1655 (CO); ^{1}H NMR [$(CD_3)_2SO$]: at 100 °C: δ 0.93 (3 H, t, J = 7.2 Hz, 2-N(CH$_2$)$_3$–C*H*$_3$), 1.39 (2H, sextet, J = 7.2 Hz, 2-N(CH$_2$)$_2$–C*H*$_2$–CH$_3$), 1.59 (2H, quintet, J = 7.2 Hz, 2-NCH$_2$–C*H*$_2$–CH$_2$–CH$_3$), 3.30–3.55 (2H, m, 2-NC*H*$_2$–(CH$_2$)$_2$–CH$_3$), 4.07 (3H, s, 10-Me), 7.14 (1H, br s, 2-NH exchangeable with D_2O), 7.45–7.55 (1H, m, 9-H), 7.78–7.87 (2H, m, 7 and 8-H), 8.06 (1H, br d, J = 7.8 Hz, 6-H), 8.77 (1H, s, 5-H); Anal. calcd for $C_{16}H_{18}N_4O{\cdot}0.4H_2O$: C, 66.37; H, 6.54; N, 19.35. Found: C, 66.04; H, 6.52; N, 19.17.

4.1.38. General procedure for the preparation of 2-(substituted amino)-10-alkyl-2-deoxo-5-deazaflavins {2-(substituted amino)-10-alkylpyrimido[4,5-*b*]quinolin-4(10*H*)-ones} (7a–q). A mixture of 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2**, 5.0 mmol) and an appropriate primary or secondary amine (25–35 mmol) in *n*-butanol (30 mL) was refluxed with stirring for 2–5 h. After the clear yellow solution was kept overnight in refrigerator, the precipitated yellow crystalline solid was collected by filtration to get the first crop. The filtrate was concentrated in vacuo and the residue was treated with appropriate solvent, such as water or ethanol for the piperidine, morpholine, *N*-(2-hydroxyethyl)-*N*-methylamine, and N-methylpiperazine derivatives and petroleum ether for the *n*-octylamine derivative, to get the second crop of the crude product free from amines. The collected solids were dried and recrystallized from an appropriate solvent to give the corresponding products as bright yellow needles in 70–99% yields.

4.1.39. 2-[*N*-(2-Hydroxyethyl)-*N*-methylamino]-10-methylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (7a). Yield, (1.11 g, 78%); mp 251–253 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 221 (4.51), 272 (4.49), 278sh (4.45), 321 (3.92), 383sh (4.00), 408 (4.18), 431 (4.12); IR (ν_{max}/cm^{-1}): 3260 (OH), 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 2.01 (1H, br s, OH, exchangeable with D_2O), 3.39 (3H, s, 2-NMe), 3.96 (4H, br s, 2-N-*CH*$_2$-*CH*$_2$), 4.12 (3H, s, 10-Me), 7.44 (1H, t, $J_{6,7}$ = $J_{7,8}$ = 8.1 Hz, 7-H), 7.65 (1H, d, $J_{8,9}$ = 8.1 Hz, 9-H), 7.82 (1H, t, $J_{7,8}$ = $J_{8,9}$ = 8.1 Hz, 8-H), 7.89 (1H, d, $J_{6,7}$ = 8.1 Hz, 6-H), 8.95 (1H, s, 5-H); Anal. calcd for $C_{15}H_{16}N_4O_2{\cdot}0.4\,H_2O$: C, 61.80; H, 5.81; N, 19.22. Found: C, 61.93; H, 5.71; N, 18.91.

4.1.40. 10-Ethyl-2-[N-(2-hydroxyethyl)-*N*-methylamino]-pyrimido[4,5-*b*]quinolin-4-(10*H*)-one (7b). Yield, (1.19 g , 80%); mp 250–252 °C (from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 221 (4.72), 271 (4.68), 279sh (4.63), 319 (4.12), 384sh (4.22), 408 (4.39), 432 (4.32); IR (ν_{max}/cm^{-1}): 3260 (OH), 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 1.51 (3H, t, J = 6.9 Hz, 10-CH$_2$-C*H*$_3$), 1.81 (1H, br s, OH, exchangeable with D_2O), 3.40 (3H, s, 2-NMe), 3.93–3.99 (4H, m, 2-NC*H*$_2$-C*H*$_2$-), 4.80 (2H, q, J = 6.9 Hz, 10-C*H*$_2$-CH$_3$), 7.47 (1H, t, $J_{6,7}$ = $J_{7,8}$ = 7.8 Hz, 7-H), 7.68 (1H, d, $J_{8,9}$ = 7.8 Hz, 9-H), 7.85 (1H, dt, $J_{7,8}$ = $J_{8,9}$ = 7.8 Hz, $J_{6,8}$ = 1.5 Hz, 8-H), 7.93 (1H, d, $J_{6,7}$ = 7.8 Hz, 6-H), 8.97 (1H, s, 5-H); Anal. calcd for $C_{16}H_{18}N_4O_2$: C, 64.41; H, 6.08; N, 18.78. Found: C, 64.13; H, 6.06; N, 18.50.

4.1.41. 10-Ethyl-2-[N-(2-hydroxyethyl)-N-methylamino]-7-methylpyrimido[4,5-*b*]quinolin-4-(10*H*)-one (7c). Yield, (1.16 g, 74%); mp 272–274 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 224 (4.64), 274 (4.62), 282sh (4.53), 325 (4.09), 387sh (4.09), 414 (4.30), 438 (4.26); IR (ν_{max}/cm^{-1}): 3210 (OH), 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 1.31 (3H, t, J = 6.9 Hz, 10-CH$_2$–*CH*$_3$), 1.81 (1H, br s, OH, exchangeable with D_2O), 2.51 (3H, s, 7-Me), 3.39 (3H, s, 2-NMe), 3.96 (4H, br s, 2-NC*H*$_2$–C*H*$_2$-), 4.77 (2H, q, J = 6.9 Hz, 10-C*H*$_2$–CH$_3$), 7.54–7.61 (2H, m, 6 and 8-H), 7.66 (1H, d, $J_{8,9}$ = 8.1 Hz, 9-H), 8.91 (1H, s, 5-H); Anal. calcd for $C_{17}H_{20}N_4O_2$: C, 65.37; H, 6.45; N, 17.94. Found: C, 65.21; H, 6.46; N, 17.84.

4.1.42. 2-[N-(2-Hydroxyethyl)-*N*-methylamino]-8-methoxy-10-methylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (7d). Yield, (1.19 g, 76%); mp 288–291 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 230 (4.69), 269 (4.53), 274sh (4.50), 354sh (4.04), 380sh (4.18), 401 (4.46), 423 (4.50); IR (ν_{max}/cm^{-1}): 3200 (OH), 1645 (CO); ^{1}H NMR ($CDCl_3$): δ 3.31 (3H, s, 2-NMe), 3.58–3.74 (2H, m, 2-N-C*H*$_2$-CH$_2$), 3.75–3.84 (2H, m, 2-N-CH$_2$-C*H*$_2$), 4.00 (3H, s, 8-OMe), 4.09 (3H, s,10-Me), 4.64 (1H, br s, OH, exchangeable with D_2O), 7.15 (1H, br d, $J_{6,7}$ = 8.4 Hz, 7-H), 7.24 (1 H, br s, 9-H), 8.06 (1H, d, $J_{6,7}$ = 8.4 Hz, 6-H), 8.78 (1H, s, 5-H); Anal. calcd for $C_{16}H_{18}N_4O_3.0.1H_2O$: C, 60.79; H, 5.80; N, 17.72. Found: C, 60.72; H, 5.77; N, 17.65.

4.1.43. 10-Methyl-2-piperidinopyrimido[4,5-*b*]quinolin-4(10*H*)-one (7e). Yield, (1.19 g, 81%); mp 275–277 °C (from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 221 (4.62), 273 (4.62), 278sh (4.60), 320 (4.03), 384sh (4.15), 410 (4.31), 434 (4.24); IR (ν_{max}/cm^{-1}): 1660 (CO); ^{1}H NMR ($CDCl_3$): δ 1.68 (6H, br s, 3′, 4′ and 5′-*CH*$_2$), 3.98–4.07 (4H, m, 2′ and 6′-*CH*$_2$),

4.11 (3H, s, 10-Me), 7.44 (1H, dt, $J_{6,7}$ = 8.7 Hz, $J_{7,8}$ = 7.8 Hz, $J_{7,9}$ = 1.5 Hz, 7-H), 7.64 (1 H, d, $J_{8,9}$ = 8.7 Hz, 9-H), 7.80 (1H, dt, $J_{7,8}$ = 7.8, Hz $J_{8,9}$ = 8.7 Hz, $J_{6,8}$ = 1.5 Hz, 8-H), 7.87 (1H, dd, $J_{6,7}$ = 8.7 Hz, $J_{6,8}$ = 1.5 Hz, 6-H), 8.93 (1H, s, 5-H); Anal. calcd for $C_{17}H_{18}N_4O{\cdot}0.1H_2O$: C, 68.94; H, 6.19; N, 18.92. Found: C, 68.66; H, 6.17; N, 18.89.

4.1.44. 7-Chloro-10-methyl-2-morpholinopyrimido[4,5-*b*]quinolin-4(10*H*)-one (7f). Yield, (1.29 g, 78%), mp >300 °C (from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 225 (4.65), 276 (4.61), 286sh (4.53), 319 (4.02), 392sh (4.07), 416 (4.23), 441 (4.19); IR (ν_{max}/cm^{-1}): 1660 (CO); ^{1}H NMR ($CDCl_3$): δ 3.73–3.82 (4H, m, 2′ and 6′-NCH_2), 4.03-4.15 (4H, m, 3′ and 5′-OCH_2), 4.11 (3H, s, 10-Me), 7.61 (1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 7.77 (1H, dd, $J_{8,9}$ = 9.0 Hz, $J_{6,8}$ = 2.4 Hz, 8-H), 7.85 (1H, d, $J_{6,8}$ = 2.4 Hz, 6-H), 8.87 (1H, s, 5-H); Anal. calcd for $C_{16}H_{15}ClN_4O_2{\cdot}0.4H_2O$: C, 56.86; H, 4.71; N, 16.58. Found: C, 56.86; H, 4.61; N, 16.24.

4.1.45. 10-Methyl-2-morpholinopyrimido[4,5-*b*]quinolin-4-(10*H*)-one (7g). Yield, (1.47 g, 99%); mp 296–298 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 220 (4.63), 272 (4.60), 279sh (4.55), 319 (4.07), 383sh (4.08), 406 (4.26), 431 (4.20); IR (ν_{max}/cm^{-1}): 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 3.74–3.83 (4H, m, 2′ and 6′-NCH_2), 4.04–4.13 (4H, m, 3′ and 5′-OCH_2), 4.14 (3H, s,10-Me), 7.48 (1H, t, $J_{6,7}$ = $J_{7,8}$ = 7.8 Hz, 7-H), 7.68 (1H, d, $J_{8,9}$ = 8.7 Hz, 9-H), 7.85 (1H, dt, $J_{7,8}$ = 7.8 Hz, $J_{8,9}$ = 8.7 Hz, $J_{6,8}$ = 1.5 Hz, 8-H), 7.91 (1 H, d, $J_{6,7}$ = 7.8 Hz, 6-H), 8.99 (1 H, s, 5-H); Anal. calcd for $C_{16}H_{16}N_4O_2{\cdot}0.4\ H_2O$: C, 63.31; H, 5.58; N, 18.46. Found: C, 63.61; H, 5.84; N, 18.41.

4.1.46. 10-Methyl-2-(4-methylpiperazinyl)pyrimido[4,5-*b*]quinolin-4-(10*H*)-one (7h). Yield, (1.08 g, 70%); mp 235–237 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 220 (4.56), 272 (4.56), 277sh (4.53), 322 (3.99), 382sh (4.06), 407 (4.24), 430 (4.17); IR (ν_{max}/cm^{-1}): 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 2.35 (3H, br s, 4′-Me), 2.42–2.58 (4H, m, 3′ and 5′-CH_2), 4.06–4.11 (4H, m, 2′ and 6′-CH_2), 4.13 (3H, s,10-Me), 7.46 (1H, t, $J_{6,7}$ = $J_{7,8}$ = 7.8 Hz, 7-H), 7.68 (1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 7.83 (1H, dt, $J_{7,8}$ = 7.8 Hz, $J_{8,9}$ = 9.0 Hz, $J_{6,8}$ = 1.5 Hz, 8-H), 7.91 (1H, d, $J_{6,7}$ = 7.8 Hz, 6-H), 8.96 (1H, s, 5-H); Anal. calcd for $C_{17}H_{19}N_5O{\cdot}0.4H_2O$: C, 64.50; H, 6.30; N, 22.12. Found: C, 64.28; H, 6.22; N, 21.92.

4.1.47. 7-Methoxy-10-methyl-2-morpholinopyrimido[4,5-*b*]quinolin-4-(10*H*)-one (7i). Yield, (1.57 g, 96%); mp 296–299 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 226 (4.47), 276 (4.49), 328 (4.03), 400sh (3.92), 427 (4.16), 453 (4.18); IR (ν_{max}/cm^{-1}): 1655 (CO); ^{1}H NMR ($CDCl_3$): δ 3.72–3.82 (4H, m, 2′ and 6′-NCH_2), 3.93 (3H, s, 7-OMe), 4.00–4.12 (4H, m, 3′ and 5′-OCH_2), 4.13 (3H, s, 10-Me), 7.22 (1H, d, $J_{6,8}$ = 2.7 Hz, 6-H), 7.47 (1H, dd, $J_{8,9}$ = 9.3 Hz, $J_{6,8}$ = 2.7 Hz, 8-H), 7.68 (1H, d, $J_{8,9}$ = 9.3 Hz, 9-H), 8.91 (1H, s, 5-H); Anal. calcd for $C_{17}H_{18}N_4O_3{\cdot}H_2O$: C, 59.29; H, 5.85; N, 16.27. Found: C, 59.30; H, 5.76; N, 15.89.

4.1.48. 7,10-Dimethyl-2-piperidinopyrimido[4,5-*b*]quinolin-4-(10*H*)-one (7j). Yield, (1.48 g, 96%); mp 292–293 °C (from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 222 (4.72), 275 (4.71), 283sh (4.64), 322 (4.13), 391sh (4.18), 415 (4.38), 440 (4.33); IR (ν_{max}/cm^{-1}): 1640 (CO); ^{1}H NMR ($CDCl_3$): δ 1.67 (6H, br s, 3′, 4′ and 5′-CH_2), 2.49 (3H, s, 7-Me), 3.97–4.06 (4H, m, 2′ and 6′-CH_2), 4.09 (3H, s, 10-Me), 7.53 (1H, d, $J_{8,9}$ = 9.3 Hz, 9-H), 7.58–7.65 (2H, m, 6 and 8-H), 8.86 (1H, s, 5-H); Anal. calcd for $C_{18}H_{20}N_4O{\cdot}1.1$-$H_2O$: C, 65.87; H, 6.82; N, 17.07. Found: C, 66.16; H, 7.22; N, 17.16.

4.1.49. 10-Ethyl-7-methyl-2-morpholinopyrimido[4,5-*b*]-quinolin-4-(10*H*)-one (7k). Yield, (1.27 g, 78%); mp >300 °C (from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 225 (4.63), 274 (4.62), 283sh (4.55), 326 (4.12), 387sh (4.09), 413 (4.32), 437 (4.29); IR (ν_{max}/cm^{-1}): 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 1.48 (3H, t, J = 7.2 Hz, 10-CH_2-CH_3), 2.51 (3H, s, 7-Me), 3.70–3.85 (4H, m, 2′ and 6′-NCH_2), 3.99–4.16 (4 H, m, 3′ and 5′-OCH_2), 4.76 (2H, q, J = 7.2 Hz, 10-CH_2-CH_3), 7.48 (1H, d, $J_{8,9}$ = 9.3 Hz, 9-H), 7.64–7.68 (2H, m, 6 and 8-H), 8.93 (1H, s, 5-H); Anal. calcd for $C_{18}H_{20}N_4O_2$: C, 66.65; H, 6.21; N, 17.27. Found: C, 66.44; H, 6.37; N, 17.40.

4.1.50. 7-Chloro-10-methyl-2-(4-methylpiperazinyl)-pyrimido[4,5-*b*]quinolin-4-(10*H*)-one (7l). Yield, (1.46 g, 85%); mp >300 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 225 (4.65), 275 (4.65), 284sh (4.59), 319 (4.02), 390sh (4.09), 416 (4.27), 442 (4.21); IR (ν_{max}/cm^{-1}): 1660 (CO); ^{1}H NMR ($CDCl_3$): δ 2.34 (3H, s, 4′-Me), 2.45–2.53 (4H, m, 3′ and 5′-CH_2), 4.05–4.15 (4H, m, 2′ and 6′-CH_2), 4.10 (3H, s, 10-Me), 7.60 (1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 7.75 (1H, dd, $J_{8,9}$ = 9.0 Hz, $J_{6,8}$ = 2.1 Hz, 8-H), 7.81 (1 H, d, $J_{6,8}$ = 2.1 Hz, 6-H), 8.82 (1H, s, 5-H); Anal. calcd for $C_{17}H_{18}ClN_5O{\cdot}0.1H_2O$: C, 59.08; H, 5.31; N, 20.26. Found: C, 58.72; H, 5.43; N, 20.69.

4.1.51. 7-Chloro-10-methyl-2-piperidinopyrimido[4,5-*b*]-quinolin-4-(10*H*)-one (7m). Yield, (1.46 g, 89%); mp >300 °C (from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 226 (4.77), 276 (4.78), 286sh (4.71), 317 (4.11), 393sh (4.24), 420 (4.41), 445 (4.35); IR (ν_{max}/cm^{-1}): 1645 (CO); ^{1}H NMR ($CDCl_3$): δ 1.70 (6H, br s, 3′, 4′ and 5′-CH_2), 3.96–4.06 (4H, m, 2′ and 6′-NCH_2), 4.08 (3H, s, 10-Me), 7.57 (1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 7.72 (1H, d, $J_{8,9}$ = 9.0 Hz, 8-H), 7.79 (1H, s, 6-H), 8.79 (1H, s, 5-H); Anal. calcd for $C_{17}H_{17}ClN_4O{\cdot}1.1H_2O$: C, 58.57; H, 5.55; N, 16.07. Found: C, 58.23; H, 5.46; N, 15.82.

4.1.52. 7,8,10-Trimethyl-2-morpholinopyrimido[4,5-*b*]-quinolin-4-(10*H*)-one (7n). Yield, (1.28 g, 79%); mp 287–289 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 225 (4.75), 276 (4.70), 336 (4.18), 387sh (4.15), 410 (4.38), 434 (4.67); IR (ν_{max}/cm^{-1}): 1650 (CO); ^{1}H NMR ($CDCl_3$): δ 2.41 (3H, s, 7-Me), 2.52 (3H, s, 8-Me), 3.73–3.81 (4H, m, 2′ and 6′-NCH_2), 4.02–4.09 (4H, m, 3′ and 5′-OCH_2), 4.11 (3H, s, 10-Me), 7.44 (1H, s, 9-H), 7.60 (1H, s, 6 H), 8.91

(1H, s, 5-H); Anal. calcd for $C_{18}H_{20}N_4O_2{\cdot}0.4H_2O$: C, 65.20; H, 6.32; N, 16.90. Found: C, 65.05; H, 6.38; N, 17.10.

4.1.53. 7-Methoxy-10-methyl-2-(4-methylpiperazinyl)-pyrimido[4,5-*b*]quinolin-4-(10*H*)-one (7o). Yield, (1.54 g, 85%); mp 237–239 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 226 (4.60), 280 (4.68), 327 (4.18), 400sh (4.05), 427 (4.32), 453 (4.34); IR (ν_{max}/cm^{-1}): 1640 (CO); ^{1}H NMR ($CDCl_3$): δ 2.34 (3 H, br s, 4′-Me), 2.45–2.53 (4H, m, 3′ and 5′-CH_2), 3.92 (3H, s, 7-OMe), 4.04–4.10 (4H, m, 2′ and 6′-CH_2), 4.12 (3H, s, 10-Me), 7.20 (1H, d, $J_{6,8}$ = 2.7 Hz, 6-H), 7.45 (1H, dd, $J_{8,9}$ = 9.6 Hz, $J_{6,8}$ = 2.7 Hz, 8-H), 7.61 (1H, d, $J_{8,9}$ = 9.6 Hz, 9-H), 8.89 (1H, s, 5-H); Anal. calcd for $C_{18}H_{21}N_5O_2{\cdot}0.4H_2O$: C, 62.38; H, 6.34; N, 20.21. Found: C, 62.65; H, 6.23; N, 19.80.

4.1.54. 10-Ethyl-2-(2-hydroxyethylamino)pyrimido[4,5-*b*]-quinolin-4-(10*H*)-one (7p). Yield, (1.14 g, 80%); mp 267–270 °C (decomp. from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 221 (4.42), 268 (4.44), 274sh (4.41), 321 (3.90), 404sh (4.01), 425sh (3.93); IR (ν_{max}/cm^{-1}): 3250 (OH), 3190 (NH), 1640 (CO);^{1}H NMR [$(CD_3)_2SO$] at 90 °C: δ 1.40 (3H, t, J = 6.9 Hz, 10-CH_2-CH_3), 3.39–3.50 (2H, m, 2-NH-CH_2-CH_2-OH), 3.56–3.62 (2H, m, 2-NH-CH_2-CH_2-OH), 3.96 (1H, br s, 2-NH-CH_2-CH_2-O*H*, exchangeable with D_2O), 4.80 (2H, q, J = 6.9 Hz, 10-CH_2-CH_3), 7.19 (1H, br s, 2-N*H*,exchangeable with D_2O), 7.44–7.58 (1H, m, 9-H), 7.84–7.98 (1H, m, 7 and 8-H), 8.15 (1H, br d, $J_{6,7}$ = 8.1 Hz, 6-H), 8.80 (1H, s, 5-H); Anal. calcd for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.19; H, 5.73; N, 19.48.

4.1.55. 10-Methyl-2-*n*-octylaminopyrimido[4,5-*b*]quinolin-4-(10*H*)-one (7q). Yield, (1.46 g, 86%); mp 183–185 °C (decompo, from EtOH); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 220 (4.60), 269 (4.63), 274sh (4.60), 320 (4.07), 404 (4.19), 422sh (4.15); IR (ν_{max}/cm^{-1}): 3180 (NH), 1655 (CO); ^{1}H NMR [$(CD_3)_2SO$] at 90 °C: δ 0.86 (3H, t, J = 7.2 Hz, 2-N$(CH_2)_7$-CH_3), 1.24–1.42 (10H, m, 2-N$(CH_2)_2$ – $(CH_2)_5$-CH_3), 1.65 (2H, m, 2-NCH_2-CH_2-$(CH_2)_5$-CH_3), 3.40–3.61 (2H, m, 2-NCH_2-$(CH_2)_6$-CH_3), 4.21 (3H, s, 10-Me), 7.52–7.74 (1H, m, 7-H), 7.90–8.12 (2H, m, 8 and 9-H), 8.14–8.30 (1H, m, 6-H), 8.85 (1H, br s, 2-N*H*,exchangeable with D_2O), 9.12 (1H, s, 5-H); Anal. calcd for $C_{20}H_{26}N_4O$: C, 70.98; H, 7.74; N, 16.55. Found: C, 70.66; H, 7.59; N, 16.26.

4.2. Growth inhibitory activities of 2-substituted 10-alkyl-2-deoxo-5-deazaflavins (2–4, 6, 7) and 5-deazaflavin (5) against human tumor cell lines

The procedure was carried out using the modified MTT assay[28] to determine the inhibitory effects of test compounds on cell growth in vitro as mentioned in detail in our previous paper.[1] Two human tumor cell lines of human T-cell acute lymphoblastoid leukemia (CCRF-HSB-2) and human oral epidermoid carcinoma (KB) were used in this study.

4.3. Molecular docking study

The advanced docking program AutoDock 3.0.5[24] was used to evaluate the binding free energy of the inhibitors within the macromolecules. The individual components of the program include AutoTors, AutoGrid, and AutoDock. AutoTors defines which bonds in the ligand are rotatable, affecting the degrees of freedom (DOF) of the ligand and hence the complexity of computations. AutoGrid pre-calculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. AutoDock performs the task of the docking. First, the ligand moves randomly in any one of six degrees of freedom, namely 3 translation degrees and 3 rotation degrees, 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.

4.3.1. Preparation of ligands and target protein tyrosine kinase. The compounds involved in this study as ligands include 10-alkyl-2-deoxo-2-methylthio-5-deazaflavins (**2a**–**j**), 2-amino- and 2-(*N*-substituted amino)-10-alkyl-2-deoxo-5-deazaflavins (**3**,**4**,**6**, and **7**), and the computationally designed compounds connected with amino acids (**8a,b**) were studied for their binding activities into PTK. Since ligands are not peptides, Gasteiger-Hückel charge was assigned and then non-polar hydrogens were merged. The rigid roots of each ligands were defined automatically instead of picking manually. The amide bonds were made non-rotatable. The three-dimensional structures of the aforementioned compounds were constructed using Chem3D 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 crystal structures of c-Kit receptor protein-tyrosine kinase in complex with STI-571 (Imatinib or Gleevec) were extracted from the RCSB Protein Data Bank http://www.rcsb.org/pdb/Welcome.do ccessed on August, 20, 2005. All bound waters, ligand were removed from the protein. For the target, the amino acids of the ligand binding site were defined using data in pdbsum http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/ accessed on January 8, 2006. The polar hydrogen model was chosen for the crystal structure. The proteins were assigned with Kollman-united charge. The polar hydrogens and charges were added.

4.3.2. Grid generation and run of molecular docking. The grid maps representing the native ligand in the actual docking target site were calculated with AutoGrid. The grids (one for each atom type in the ligand, plus one for electrostatic interactions) were chosen to be sufficiently large to include not only the active site but also significant portions of the surrounding surface. The three-dimensional grids, 80 Å grid size (x, y, z) with a spacing of 0.375 Å, were created because the location of the native ligand in the crystal structure was known. The cubic grid box was centered in the catalytic active region and encompassed the binding site where the

ligands were embedded. Then automated docking studies were carried out using AutoDock version 3.0.5.[24] Of the three different search algorithms offered by AutoDock, 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 (Autodock Tool Kit) on PC which is associated with Autodock 3.0.5.[24] For all docking parameters, default values were used with 10 independent docking runs for each docking case.

4.3.3. Molecular modeling and analysis of the docked results. There are two kinds of free energies output by Autodock. One is the predicted binding free energy that includes the intermolecular energy and torsional free energy, and the other the docking energy that includes the intermolecular and intramolecular energies. The former is only reported at the end of a docking operation, while the latter is used for selecting better individuals of population during a docking run.[24] We used only the binding free energy of the first type 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. Each of the clusters that exhibited significant negative interaction energies was examined by Accelrys, DS modeling 1.7 [Accelrys Inc., San Diego, CA (2006)] to determine their binding orientations, molecular modeling, evaluation of the hydrogen bonds, and for measuring RMSD, which was measured as distance between the centroids of the docked inhibitor and the native ligand. The mode of interaction of the native ligand within PTK was used as a standard docked model as well as for RMSD calculation. The correct hydrogen bond interaction was considered according to Taylor et al.,[29] who showed that **C**–**H**···**O** in crystals contacts occur within certain distance (3.0–4.0 Å) and angle (**C**–**H**···**O**, 90–180°) ranges. However, the more linear hydrogen bond is likely to be more stronger.[30] Moreover, there is general agreement that for carbonyl acceptors, the H···**O**=**C** angle is distributed around 120°. Therefore, in our modeling results we consider the hydrogen bond angle $\geqslant$120° to be of a reasonable strength.

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References and notes

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