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Antitumor studies. Part 4: Design, synthesis, antitumor activity, and molecular docking study of novel 2-substituted 2-deoxoflavin-5-oxides, 2-deoxoalloxazine-5-oxides, and their 5-deaza analogs

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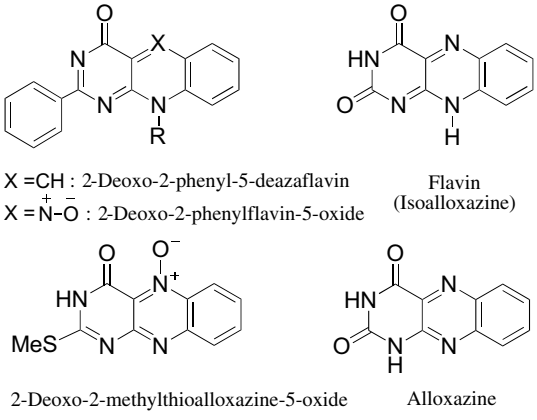
Abstract—Various novel 10-alkyl-2-deoxo-2-methylthioflavin-5-oxides and their 2-alkylamino derivatives were prepared by facile nitrosative cyclization of 6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones followed by nucleophilic replacement of the 2-methylthio moiety by different amines, and acidic hydrolysis of the 2-methylthio moiety afforded the corresponding flavin derivatives. 2-Deoxo-2-methylthio-5-deazaalloxazines and 2-deoxo-2-methylthioalloxazine-5-oxides were also prepared by Vilsmeier reaction and by nitrosation of 6-anilino-2-methylthiopyrimidin-4(3*H*)-ones, respectively. Then, they were subjected to nucleophilic replacement with appropriate amines to produce the corresponding 2-alkylamino derivatives. Regiospecific N_3-alkylation of 2-deoxo-2-methylthioalloxazine-5-oxides was carried out with various alkylating agents in the usual way. The antitumor activities against CCRF-HSB-2 and KB tumor cells have been investigated in vitro, and many compounds showed promising antitumor activities. Furthermore, AutoDock molecular docking into PTK (PDB: 1t46) has been done for lead optimization of the aforementioned compounds as potential PTK inhibitors.

1. Introduction

Cancer remains as a major threat to the public health. In our previous publications,[1–6] a series of synthesized 2-phenylflavin-5-oxide and 2-phenyl-5-deazaflavin analogs (Scheme 1) has been reported for their in vitro significant antitumor activities against different tumor cell lines. Compounds comprising flavin-*N*-oxides have been recently used for treatments of solid tumors, non-solid tumor masses, leukemia, and non-small cell lung cancers involving in situ activator mixed with the flavin-*N*-oxide for a period of time, resulting in damage to the DNA in the cancer cells without substantial damage to the DNA of normal cells.[7] Considering their biological activities, 6-nitro-5-deazaflavin derivatives showed the most potent antitumor activities among the nitro-5-deazaflavin derivatives.[8] The 6- and 8-nitro-5-deazaflavin derivatives generating stable one-electron reduction product(s) showed marked selective cytotoxicities toward hypoxic cells.[9] Furthermore, it has been found that the combination of conjugate molecules of 8-amino-5-deazaflavin with the sialosylalkyl group has been found



Scheme 1. Structures of different flavin and flavin-5-oxide analogs.

Keywords: Antitumor activity; Flavin-5-oxide; Alloxazine-5-oxide; AutoDock.
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to give rise to significant increase in their antitumor activities.[10] Nitro-5-deazaflavin-pyrrolecarboxamide(s) hybrid molecules were evaluated in vitro as novel DNA targeted bioreductive antitumor agents on KB cells.[11]

5-Amino-5-deazaflavins also revealed antiproliferative activity against L1210 and KB tumor cells.[12] The development of a potent riboflavin antagonist is one of the major interests for the fundamental studies for investigation of possible chemotherapeutic activity. Those analogs of riboflavin inhibit flavoenzymes or influence riboflavin metabolism in a variety of biological systems providing a reason to prepare a number of isoalloxazines for evaluation of their therapeutic activity against cancer.[13] Therefore, 8-chloroalloxazine was one of an early series of alloxazines and isoalloxazines prepared for the investigation of the possible antitumor activity of riboflavin inhibitors.[14] And isoalloxazines with a β-hydroxyethyl group at the 9 position as well as some of their esters are also designed as riboflavin antagonists, sometimes as antitumor agents.[15] Moreover, 6,7-dimethyl-9-formylmethyl-isoalloxazine and its thiosemicarbazone analogs were found to be reversible riboflavin antagonists and they showed antitumor activity against the Murphy–Sturm lymphosarcoma in rats.[13] The 10-aryl-5-deazaflavin derivatives were preliminarily reported to act as inhibitors of E3 activity of HMD2 in tumors that retain wild-type P53.[16] Some heterocyclic compounds containing a quinoline ring such as 5-deazaflavins are of importance owing to their miscellaneous biological activities with higher activity toward tumor cells.[17]

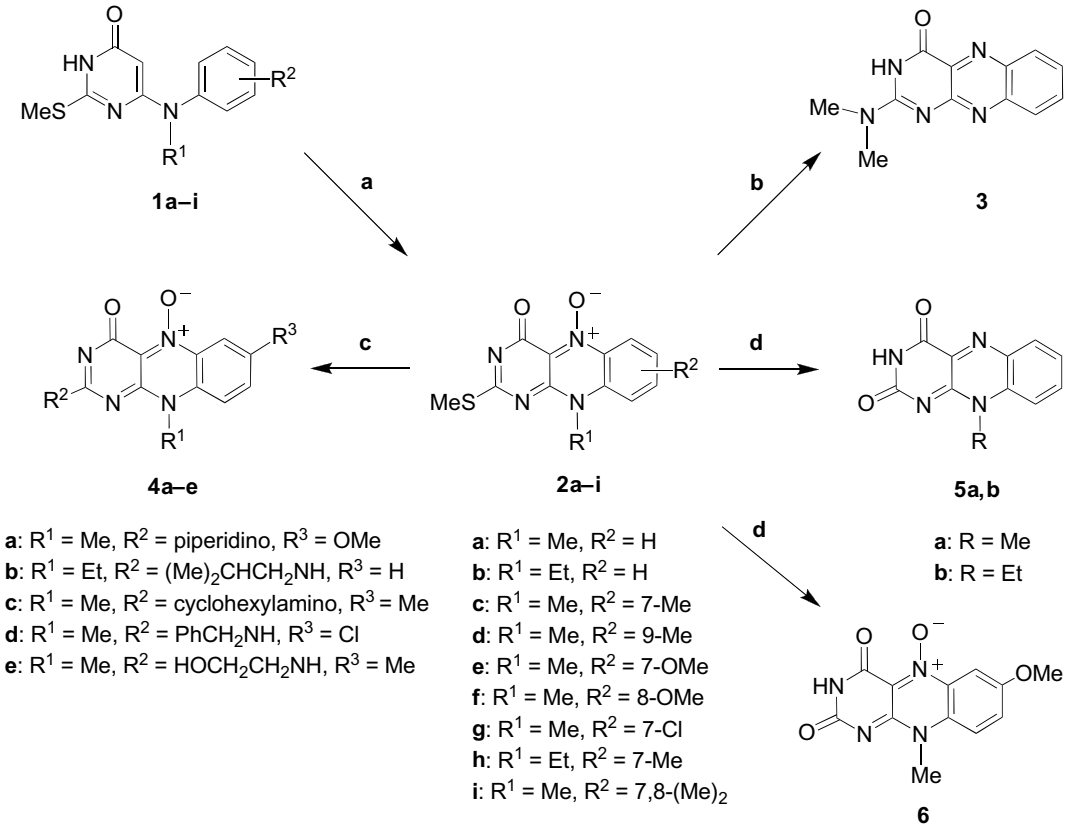
Despite the aforementioned potential antitumor activities of various flavin analogs and the numerous publications on chemistry of the related flavin compounds, information of the 2-methylthio- and 2-alkylamino-flavin analogs is none yet except our previous report.[18] These circumstances led us to seek a convenient synthetic route to the 2-methylthio- and 2-alkylamino-flavin analogs to search their potential antitumor activities and to develop potent antitumor agents showing a higher selectivity toward tumor cells.

Thus, in the present paper, we describe a facile synthesis of 10-alkyl-2-deoxo-2-methylthioflavin-5-oxides, which were used as versatile intermediates for the synthesis of various 2-alkylamino-2-deoxoflavin-5-oxides by nucleophilic substitution involving different primary and secondary alkylamines. Furthermore, they were derived to 10-alkylflavins and 10-alkylflavin-5-oxide as a novel synthetic route. 2-Deoxo-2-methylthio-5-deazaalloxazines and 2-methylthioalloxazine-5-oxides were also prepared from 6-anilino-2-methylthiopyrimidin-4(3*H*)-one. And the N_3-alkyl analogs of 2-deoxo-2-methylthioalloxazin-5-oxides were prepared by alkylation using various alkylating agents. Moreover, 2-deoxo-2-methylthio-5-deazaalloxazines and 2-deoxo-2-methylthioalloxazine-5-oxides were subjected to nucleophilic replacement with some amine derivatives to prepare 2-alkylamino-2-deoxo-5-deazaalloxazines and 2-alkylamino-2-deoxoalloxazines, respectively. These compounds were investigated for their in vitro antitumor activities. In fact, many of the synthesized compounds showed promising antitumor activities against CCRF-HSB-2 and KB tumor cells. The molecular docking study using AutoDock was carried out to get the designed compounds possessing higher binding affinities into PTK. As the result, the correlation between the growth inhibitory activities (IC_{50}, μg/mL) of the synthesized flavin analogs against tumor cells and the AutoDock binding free energies was investigated.

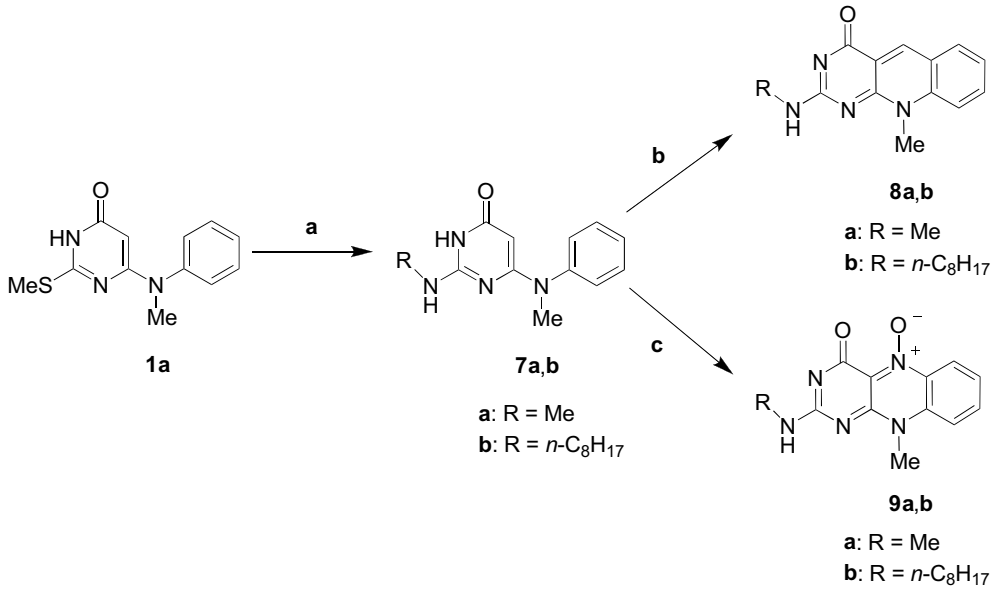
2. Results and discussion

2.1. Chemistry

The requisite starting material, 6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones (**1a**–**j**), was synthesized by treatment of 6-chloro-2-methylthiopyrimidin-4(3*H*)-one[19,20] with appropriate *N*-alkyl anilines in *n*-butanol under reflux for 12–72 h in 55–83% yields.[18] The intended 10-alkyl-2-deoxo-2-methylthioflavin-5-oxides (**2a**–**i**) were prepared by nitrosative cyclization of 6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones (**1a**–**j**) with excess $NaNO_2$ in glacial acetic acid at 10–15 °C for 1–2 h to afford the red compounds in 65–89% yields (Scheme 2). The preparation of such alkylamino derivatives by reaction of alkylthio derivatives with appropriate 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 vessel except when amines of high boiling point are used.[21] Nucleophilic replacement of the methylthio moiety of 10-methyl-2-deoxo-2-methylthioflavin-5-oxides (**2a**) by dimethylamine in steel sealed tube at 160–165 °C (15 kg/cm^2 pressure) for 4 h exhibited the replacement accompanied with the N_{10}-demethylation and N_5-deoxygenation to give 2-deoxo-2-dimethylaminoalloxazine (**3**), where the singlet signal of the N_{10}-methyl at δ 4.1 and 33.5 of ^{1}H NMR and ^{13}C NMR, respectively, was lost. Therefore, to avoid the N_{10}-demethylation, this reaction should be carried out at lower temperature for short time. Interestingly, facile rapid nucleophilic substitution of 10-alkyl-2-deoxo-2-methylthioflavin-5-oxides (**2**) by different amines was carried out by heating the mixture in *n*-butanol under reflux to afford the yellow crystalline solid of 10-alkyl-2-alkylamino-2-deoxoflavin-5-oxides (**4a**–**e**) in 62–89% yields. The pyrimidine moieties of **2a**–**i** undergo nucleophilic substitution on the carbon adjacent to the ring nitrogen which is well authenticated and readily explicable in view of the π-electron-deficient nature.[15] Therefore, various aminations were applied for the replacement of 2-methylthio group. The nucleophilic substitution of 10-alkyl-2-deoxo-2-methylthioflavin-5-oxide analogs (**2**) involves short time reaction (15 min) in comparison with the substitution reaction in case of 2-deoxo-2-methylthio-5-deazaflavin analogs, which needs reaction time of 2–5 h.[18] In contrast, acidic hydrolysis of **2** by heating in 5 N hydrochloric acid under reflux for 7–12 h gave the corresponding 10-alkylflavins (**5a** and **b**) or 7-methoxy-10-methylflavin-5-oxide (**6**) as yellow needles in 68–97% yields as shown in Scheme 2.



Scheme 2. General methods for the preparation of 2-methylthio- and 2-alkylamino-10-alkyl-2-deoxoflavin-5-oxides (**2a**–**i** and **4a**–**e**), 2-deoxo-2-dimethylaminoalloxazine (**3**), flavins (**5a** and **b**), and 7-methoxy-10-methylflavin-5-oxide (**6**). Reagents and conditions: (a) $NaNO_2$, AcOH, 10–15 °C, 1–2 h; (b) 50% aq $NH(Me)_2$, steel sealed tube, 160–165 °C, 4 h; (c) appropriate amines, *n*-BuOH, reflux 15 min; (d) 5 N HCl, reflux, 7–12 h.

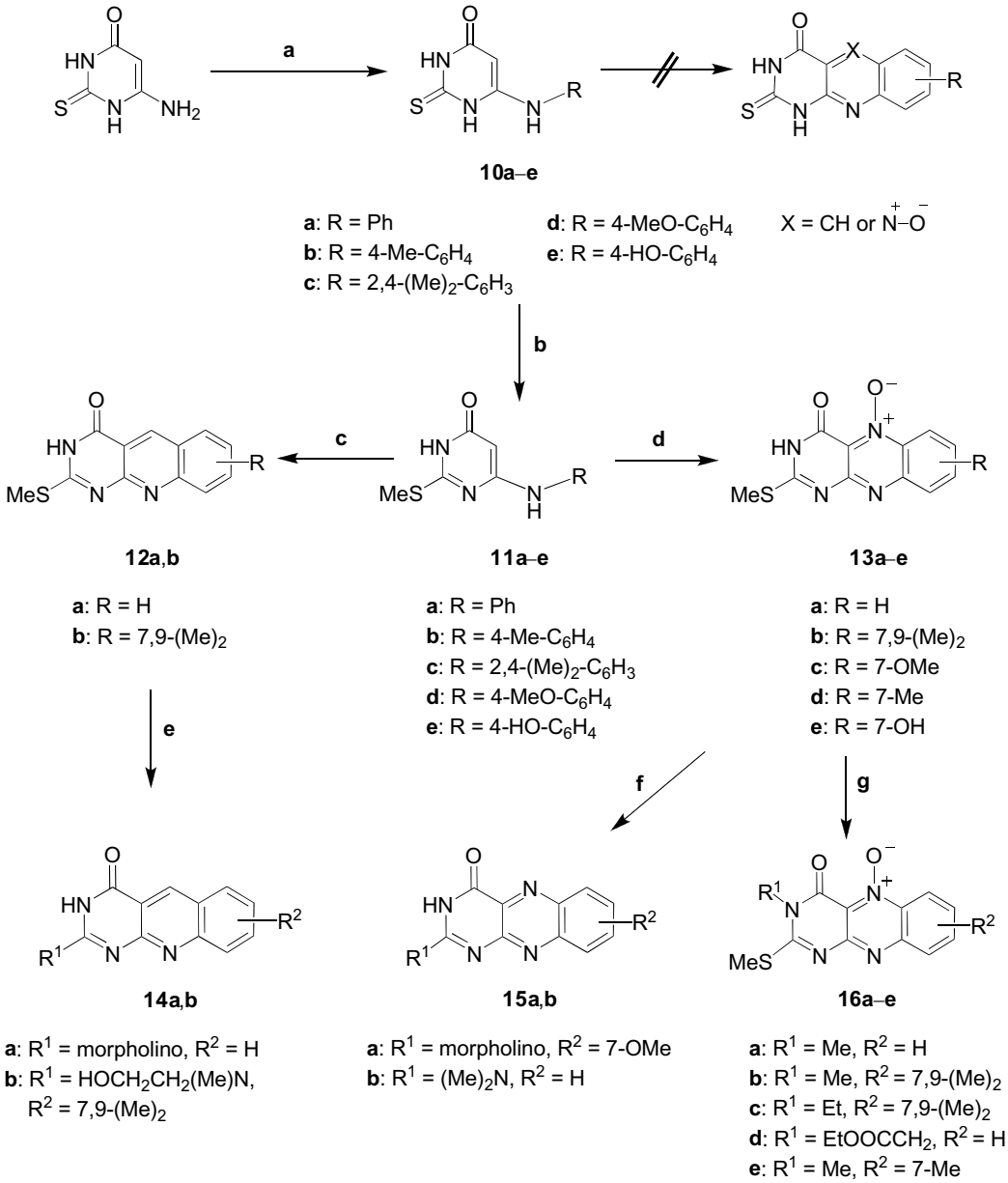


Scheme 3. General methods for the preparation of 2-alkylamino-2-deoxo-10-methyl-5-deazaflavins (**8a** and **b**), and 2-alkylamino-2-deoxo-10-methylflavin-5-oxides (**9a** and **b**). Reagents and conditions: (a) 40% aq NH_2Me, steel sealed tube, 160 °C, 15 h for **7a**; *n*-octylamine, 160 °C, 10 h for **7b**; (b) Vilsmeier reagent (DMF–$POCl_3$), 90 °C, 3 h; (c) $NaNO_2$, AcOH, 10–15 °C, 2–3 h.

Another route for synthesis of 10-alkyl-2-alkylamino-2-deoxo-5-deazaflavins (**8a** and **b**) was established by the nucleophilic substitution of 2-methylthio group of 6-(*N*-methylanilino)-2-methylthiopyrimidin-4(3*H*)-one (**1a**) by an appropriate amine before the cyclization of **1a** as shown in Scheme 3. This nucleophilic substitution

was done either by fusion of **1a** with *n*-octylamine of high boiling point or by heating in steel sealed tube at 150–160 °C with volatile methylamine. Then, the compounds **7a**, **b** were used as precursors for the preparation of 2-alkylamino-2-deoxo-5-deazaflavins (**8a** and **b**) by heating with Vilsmeier reagent at 90 °C for 3 h, and for the preparation of 2-alkylamino-2-deoxoflavin-5-oxides (**9a** and **b**) by nitrosative cyclization involving excess $NaNO_2$ in glacial acetic acid for 2–3 h.

Based on our previous structure based drug design (SBDD) study and docking investigation of different flavin analogs, and aiming to discovery of antitumor agents,[22] it was concluded from the results considering SAR that the higher binding affinities were obtained with the structure features on the flavin or 5-deazaflavin skeleton; NH_2 or Ph group at the C_2-position, H (or alloxazine conformation) or Ph group at the N_{10}-position. Herein, we tried to synthesize the designed analogs based on AutoDock possessing higher binding affinities, namely, 2-deoxo-2-methylthio-5-deazaalloxazines (**12**) and 2-deoxo-2-methylthioalloxazine-5-oxides (**13**). These derivatives can be synthesized from the key intermediate, 6-anilino-2-methylthiopyrimidin-4(3*H*)-ones (**11**), by facile methods of interactions (Scheme 4). The requisite key compounds**11a**–**e** cannot be prepared directly from 6-chloro-2-methylthiopyrimidin-4(3*H*)-ones by adapting the same procedure for the preparation of



Scheme 4. General methods for the preparation of 2-deoxo-5-deazaalloxazines (**12a**, **b** and **14a**, **b**), 2-deoxo-alloxazine-5-oxides (**13a**–**e** and **16a**–**f**), and 2-deoxoalloxazines (**15a** and **b**). Reagents and conditions: (a) $ArNH_2/ArNH_3^+Cl^-$, 170 °C, 12 h; (b) MeI, 2 N KOH, 0–5 °C, 30 min; (c) Vilsmeier reagent (DMF–$POCl_3$), 90 °C, 1 h; (d) $NaNO_2$, AcOH, 10–15 °C, 1–2 h; (e) appropriate amines, DMF or *n*–BuOH, reflux 12–24 h; (f) morpholine, DMF, reflux, 6 h for **15a**; 50% aq $NH(Me)_2$, steel sealed tube, 135 °C, 8 h for **15b**; (g) R^1I or R^1Br, DMF, K_2CO_3, rt, 3–10 h.

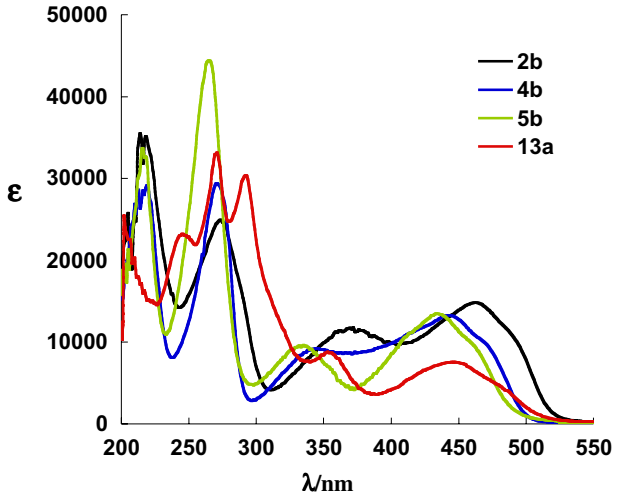


Figure 1. UV–vis spectra of 2-deoxo-10-ethyl-2-methylthioflavin-5-oxide (**2b**), 2-deoxo-10-ethyl-2-isobutylaminoflavin-5-oxide (**4b**), 10-ethylflavin (**5b**), and 2-deoxo-2-methylthioalloxazine-5-oxide (**13a**).

6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones (**1a**–**j**).[18] Both of the 6-chloro and 2-methylthio groups were replaced by the anilines. Therefore, the compounds **11a**–**e** were prepared in a two-step reaction. The first reaction by amine exchange route, namely, the reaction of 6-amino-2-thiouracil with anilines in the presence of their corresponding anilinium chloride salts at high temperature to afford the corresponding 6-anilino-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidins (**10a**–**e**) in 67–83% yields. Compounds **10a**–**e** were prepared by modification of known procedure[23,24] to improve the yields and to get more pure products. Hence, compound **10a** was obtained in higher yield of 83% (literatures[23,24]: 78% and 68% yields). In our proposed procedure, the anilinium chloride derivatives were used in more quantities of 1.5–2.0 equivalents and the crude product was further purified by dissolving it in alkaline solution, then reprecipitation by 10% HCl to get more pure compounds. The second-step reaction involves S-methylation by MeI in alkaline solution in ice bath to afford the 2-methylthio analogs (**11a**–**e**) in excellent yields of 77–98%. Synthesis of 2-thio-5-deazaalloxazines and 2-thioalloxazine-5-oxides was not successful directly from the compounds **10a**–**e**. This may be attributed to oxidative dimerization of the 2-thioxo derivatives. Therefore, the 2-thioxo moiety should be protected by methylation before the cyclization step to get the desired compounds **12** and **13**. The 2-deoxo-2-methylthio-5-deazaalloxazines (**12a** and **b**) were prepared from **11** by Vilsmeier reagent at 90 °C for 1 h as yellow needles in 72 and 63% yields, respectively. 2-Deoxo-2-methylthioalloxazine-5-oxides (**13a**–**e**) were also prepared from **11** by nitrosative cyclization using excess $NaNO_2$ in glacial acetic acid at 10–15 °C and then at room temperature for 1–2 h. After adding $NaNO_2$ to the cold reaction mixture, the formed greenish yellow nitroso intermediates have to be dissolved in acetic acid by mild warming in water bath to enhance the prompt cyclization to afford the red products in 66–89% yields (Scheme 4). Moreover, the nucleophilic replacement of 2-methylthio group for these compounds **12** and **13** by alkylamines was carried out by heating the reaction mixture with appropriate amines in DMF or *n*-butanol under reflux for high boiling point amines (morpholine or *N*-hydroxyethyl-*N*-methylamine) for 6–24 h or in sealing vessel for volatile one (dimethylamine) for 8 h to afford the corresponding yellow crystalline solid of the 2-alkylamino analogs (**14a**, **b** and **15a**, **b**) in 67–86% yields. This replacement was accompanied by deoxygenation of 2-deoxo-2-methylthioalloxazin-5-oxides (**13**) to produce the 2-alkylamino-2-deoxoalloxazine analogs (**15a** and **b**). Interestingly, the regiospecific N_3-alkylation of **13a**–**e** was carried out using excess alkyl iodide or bromide in the presence of anhydrous potassium carbonate in DMF at room temperature to afford the 3-alkyl-2-deoxo-2-methylthioalloxazine-5-oxides (**16a**–**e**) in 71–82% yields. This alkylation is 100% regioselective at the N_3-position, giving highly pure one-spot product without purification by column chromatography as previously described in methylation of 2-deoxo-2-phenyl-5-deazaflavins.[6] The discrimination between the positional isomers, 3-methyl-2-deoxo-2-methylthioalloxazine-5-oxide (**16a**) and 10-methyl-2-deoxo-2-methylthioflavin-5-oxide (**2a**), was accurately proved by obvious different melting points and IR spectra where the 4-oxo group of 3-methyl analog (**16a**) was shown at higher frequency of 1700 cm^{-1}, whereas that of 10-methyl analog (**2a**) was shown at lower frequency of 1645 cm^{-1}. In ^{1}H NMR spectra, the N_3-methyl of **16a** as a singlet signal was shown at 3.64 ppm, while the N_{10}-methyl of **2a** was shown at lower field of 4.13 ppm.

UV–vis, IR, ^{1}H NMR spectra, and elemental analyses were used for determination and identification of the newly assigned structures. The 2-deoxo-2-methylthioflavin-5-oxides (**2a**–**i**) were differentiated from the non-cyclized compounds (**1a**–**i**) by the absence of a singlet signal proton at the 5-position of **1a**–**i** in their ^{1}H NMR spectra and loss of one *ortho*-aromatic proton by the oxidative cyclization. The compounds **2** in UV–vis spectra revealed the characteristic bathochromic shift in the regions of the longest two wavelengths compared with all other flavin analogs, as shown in Figure 1 and experimental part. Considering ^{1}H NMR, the spectra of 10-alkyl-2-deoxo-2-alkylaminoflavin-5-oxides (**4a**–**e**) are characterized by disappearance of the strong characteristic singlet signal of 2-methylthio group, which was assigned for compounds **2a**–**i** at 2.57–2.60 ppm. Interestingly, the phenomenon of reversible interconversion of two isomers at room temperature in case of the 2-monoalkylamino (secondary amino) derivatives (**4b**–**e**, **8a**, **b**, and **9a**, **b**) was observed as tautomerism in ^{1}H NMR spectra as reported the similar phenomenon in the previous paper.[18] 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 the carbonyl group. At higher temperature of 85–100 °C, the coalescence of the duplicated spectrum was observed to produce the single spectrum. 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 tertiary amines. On the other hand, 2-deoxo-2-methylthio-5-deazaalloxazines (**12a** and **b**) showed a characteristic singlet signal of the most electron-deficient C_5-methine proton in the lower field at 8.98 and 9.16 ppm, respectively. 2-Deoxo-2-methylthioalloxazine-5-oxides (**13a**–**e**) can be easily differentiated from the 2-deoxo-2-methylthioflavin-5-oxide analogs (**2a**–**i**) by the presence of singlet signals of 3-NH proton at 12.69–12.79 ppm, whereas such signals of **2a**–**i** were lost. In contrast, the N_{10}-alkyl signals of **2a**–**i** at 4.07–4.19 and 4.77 ppm for methyl and methylene moieties were observed, respectively, whereas such signals of **13a**–**e** did not exist. Both of them have a singlet signal identified to their 2-SMe moiety at 2.57–2.60 ppm. The 2-alkylamino-2-deoxo-5-deazaalloxazines (**14a** and **b**) and 2-alkylamino-2-deoxoalloxazines (**15a** and **b**) are characterized by disappearance of the strong characteristic singlet signals of the 2-methylthio groups of the corresponding precursors (**12** and **13**) at 2.44–2.83 ppm.

The UV–vis absorption spectra of the 2-deoxo-2-methylthioflavin analogs located at shorter wavelength (nm) in comparison with the 2-deoxo-2-phenylflavin analogs[6] which have more conjugated system. As shown in Figure 1, the 2-deoxo-2-methylthioflavin-5-oxides (**2a**–**i**) exhibited four absorption maxima, three in the UV region at 219–221, 265–289, and 347–370 nm, and one in the visible region at 457–495 nm with bathochromic shift (red shift) in the regions of the longest two wavelengths in comparison with the 2-deoxo-2-methylthio-5-deazaflavin analogs.[18] All compounds of **2a**–**i** showed red color owing to the presence of absorption maximum at 457–495 nm in the longest wavelength. The acid hydrolyzed products, 10-alkylflavins (**5a** and **b**), exhibited hypsochromic shift mainly in the longest two wavelengths in comparison with the UV–vis spectra of **2a**–**i** as can be seen in Figure 1 and experimental part. The 2-deoxoflavin-5-oxides (**4b**–**e**) substituted by the primary amines at the 2-position exhibited four absorption maxima at 217–223, 266–274, 330–350, and 436–453 nm, and an additional shoulder at 472–487 nm as shown in Figure 1 for compound **4b**. While, 2-deoxo-2-methylthioalloxazine-5-oxides (**13a**–**e**) exhibited five absorption maxima at 241–247, 270–275, 285–298, 351–361, and 443–457 nm.

2.2. In vitro antitumor activities of different 2–methylthioflavin-5-oxides, alloxazin-5-oxides, 5-deazaalloxazines, and their 2-alkylamino derivatives against human tumor cell lines

The compounds (**2**, **4**, **6**, **8**, **9**, **12**, **13**, **15**, and **16**) synthesized in this study were tested in vitro for their growth inhibitory activities against two human cultured tumor cell lines, namely, human T-cell acute lymphoblastoid leukemia cell line (CCRF-HSB-2) and human oral epidermoid carcinoma cell line (KB), by using the modified MTT colorimetric assay.[25] The antitumor agent, cytosine arabinoside (Ara-C), was used as a positive control in this study. As can be noticed in Table 1, some compounds of 2-deoxo-2-methylthioflavin-5-oxides (**2**), 2-deoxo-2-alkylaminoflavin-5-oxides (**4**), flavin-5-oxides (**6**), and 2-deoxo-2-alkylamino-5-deazaflavins (**8**) have been found to show significant antitumor activities, but they were of inferior antitumor activities than that of Ara-C against CCRF-HSB-2 cell line. The activities for the compounds (**2a**, **d**, **4e**, **5a**, **b**, **8a**, and **9a**) were the highest among their analogs (IC_{50}: 1.56, 1.46, 1.46, 1.8, 1.57, 2.7, and 1.74 μg/mL, respectively). Further, against KB cell line they exhibited good growth inhibitory activities of one-third to one-ninth against the antitumor potency of Ara-C (IC_{50}: 0.23 μg/mL), where IC_{50} of compounds **2a**, **d**, **4e**, and **5a**, **b** were of 1.44, 0.61, 1.7, 2.1, and 1.89 μg/mL, respectively. This may reveal a fairly good and less toxic antitumor activity of these flavin analogs in comparison with Ara-C. Moreover, compounds **2b**, **g**, **4b**, **c**, and **6** exhibited moderate potential growth inhibitory activities against CCRF-HSB-2 cell line with IC_{50} of 3.72, 6.66, 6.0, 7.1, and 5.1 μg/mL, respectively. Also compounds **2b**, **4b**, and **6** exhibited prospective growth inhibitory activities against KB cell line with IC_{50} being 8.19, 8.1, and 6.2 μg/mL, respectively.

Table 1. Growth inhibitory activities against CCRF-HSB-2 and KB cells for flavin-5-oxides (**2**, **4**, **6**, and **9a**), flavins (**5**), 5-deazaflavins (**8**), 5-deazaalloxazine (**12a**), alloxazine-5-oxides (**13** and **16**), and alloxazines (**15**)

Compound	Inhibitorty activity against tumor cell lines [IC_{50} (μg/mL)]	
	CCRF-HSB-2	KB
2a	1.56	1.44
2b	3.72	8.19
2c	17.1	27.7
2d	1.46	0.61
2e	9.84	11.0
2f	10.0	12.5
2g	6.66	10.8
2h	34.7	46.3
2i	14.3	30.0
4a	6.18	27.6
4b	6.0	8.1
4c	7.1	33.7
4e	1.46	1.7
5a	1.8	2.1
5b	1.57	1.89
6	5.1	6.2
8a	2.7	14.5
8b[a]	8.9	7.9
9a	1.74	6.93
12a	23.2	44.1
13a	6.77	6.76
13b	6.29	11.8
13c	4.11	5.35
13d	5.86	5.8
15a	41.4	71.7
15b	51.2	63.1
16a	8.63	9.31
16b	8.96	14.2
16c	5.9	10.0
16d	34.2	54.8
16e	7.74	13.4
Ara-C	0.047	0.23

[a] Ref. 18.

In comparison with the antitumor activities of the above studied flavin-5-oxide, 2-alkylaminoflavin-5-oxide, and 5-deazaflavin derivatives (**2**, **4**, **5**, and **8**), which revealed the IC_{50} in the range of 1.46–2.7 and 0.61–2.1 μg/mL against CCRF-HSB-2 and KB tumor cell lines, respectively, and the activities of our previously reported potent 2-deoxo-2-phenylflavin analogs,[6] which revealed the IC_{50} in the range of 0.15–0.68 μg/mL for CCRF-HSB-2 cells and 0.16–0.72 μg/mL for KB cell. It can be explained that the phenyl group at the C_2-position provides better affinity to the PTK enzyme on account of the force of electrostatic attraction between the planar phenyl and the target site pocket of the PTK. Hence, the phenyl derivatives exhibit a good fitting into the active site and better antitumor activity. The results considering SAR revealed that the highest antitumor activities (ca. 1.5 μg/mL) were obtained with the structure features on the flavin-5-oxide, 5-deazaflavin, and alloxazine skeletons; SMe or HNMe group at the C_2-position, H (or alloxazine conformation) or Me group at the N_{10}-position, and unsubstituted quinoxaline nucleus or substituted by 7-Me, or 9-Me group. The SAR revealing moderate antitumor activity (ca. 5.5 μg/mL) was obtained with the structure features: HN-*n*-Bu or oxo at the C_2-position, Me or Et group at the N_{10}-position, and unsubstituted quinoxaline nucleus or substituted by 7-OMe group. Noteworthy, the N_3-alkyl substituted alloxazin-5-oxides exhibited lower antitumor activities than their unsubstituted analogs as shown in Table 1. And the *N*-5-oxide analogs revealed higher potencies than 5-deazaflavins. These results confirm our previously reported SAR study.[22]

2.3. Molecular docking study

Due to their involvement in various forms of cancers, PTKs have become prominent targets for therapeutic intervention. The selective PTK inhibitors into the receptor and non-receptor PTK represent a promising class of antitumor agents. These agents are shown to inhibit multiple features of cancer cells, including proliferation, survival, invasion, and angiogenesis. Depending on the above-mentioned idea, herein we investigated the AutoDock binding affinities into PTK (PDB code, 1t46) for the synthesized flavin analogs (**2**–**6**, **8**, **9**, and **12**–**16**). For the purpose of optimization of the aforementioned lead compounds for the promising antitumor activities, the advanced docking program AutoDock 3.05[26] was used to evaluate the binding free energies of these inhibitors into the target PTK macromolecule.

2.3.1. Validation of the docking performance and accuracy. The validation of the docking accuracy was done by docking of the native co-crystallized STI-571 ligand (Imatinib or Gleevec), 4-(4-methylpiperazin-1-ylmethyl)-*n*-[4-methyl-3-(4-pyridin-3-ylpyrimidin-2-ylamino)phenyl]-benzamide, into its binding site of PTK. The obtained success rates of AutoDock, as mentioned in detail in our previous paper,[18] was highly excellent in comparison with the biological method,[27] where the docked ligand was exactly superimposed on the native co-crystallized one with RMSD being 0.25 Å and binding free energies (ΔG_b) of −18.43 kcal/mol. The hydrogen bonds exhibited between the docked ligand and amino acids were quite similar to those between the native ligand and amino acids.

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 bonds, and RMSD values. The compounds, which revealed the highest binding affinities (in other words, lowest binding

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] (Å)
			Atom of compound	Amino acid	Distance (Å)	Angle (°)	
3	−13.73	8.61E−11	N_3–H	CO of Asp 810	2.06	136.7	3.55
4b	−13.39	1.54E−10	C_4-Oxo	HN of Asp 810	2.03	133.3	1.66
			N_3–N	HN of Asp 810	1.52	136.0	
4c	−14.47	1.35E−11	C_4-Oxo	HN of Cys 673	1.82	124.8	7.32
			C_5-Oxide	HN of Cys 673	1.82	142.0	
4d	−14.47	2.47E−11	C_2–NH	CO of Asp 810	2.39	144.8	4.63
4e	−13.76	8.14E−11	$C_2NCH_2CH_2O$	HM of Asp 810	2.19	132.7	4.33
			C_2–NCH_2CH_2OH	OH of Glu 640	1.94	146.3	
8a	−13.01	2.93E−10	C_2–NH	OH of Glu 640	2.25	132.5	3.96
			C_2–NH	HN of Asp 810	2.23	131.0	
8b	−13.41	1.48E−10	C_2–NH	CO of Cys 673	2.21	137.0	9.07
9a	−12.75	4.53E−10	C_2–NH	OH of Thr 670	1.94	131.0	6.40
9b	−12.97	3.09E−10	C_2–NH	OH of The 670	2.44	173.1	6.11
13c	−8.93	2.87E−07	N_3–H	CO of Asp 810	2.05	137.7	3.92
13d	−8.95	2.77E−07	N_3–H	CO of Asp 810	2.19	145.2	3.98
16b	−9.36	1.39E−07	C_4-Oxo	HN of Cys 673	2.06	132.7	5.48
STI[d]	−18.43	3.08E−14	N_3–N	HN of Cys 673	1.66	161.0	
			NH (H79)	OH of Thr 670	1.86	147.2	0.25
			O29	HN of Asp 810	2.01	133.3	

[a] Binding free energy.
[b] Inhibition constant.
[c] Root mean square deviation.
[d] The native co-crystallized bound ligand (STI-571) of PTK (PDB code: 1t46).

free energies) within PTK and the hydrogen bond interactions into the target macromolecule, are represented in Table 2. These compounds include 2-dimethylamino-2-deoxoalloxazine (**3**), 10-alkyl-2-alkylamino-2-deoxoflavin-5-oxides (**4b–e**), 2-alkylamino-10-methyl-5-deazaflavins (**8a** and **b**), 2-alkylamino-2-deoxo-10-methyl-flavin-5-oxides (**9a** and **b**), 2-methylthioalloxazine-5-oxides (**13c** and **d**), and 3,7,9-trimethyl-2-methylthioalloxazine-5-oxide (**16b**). Compounds 2-(2-hydroxyethylamino)-7,10-dimethyl-2-deoxoflavin-5-oxide (**4e**) and 10-methyl-2-methylamino-2-deoxoflavin-5-oxide (**9a**) as shown in Figures 2 and 3, respectively, were deeply embedded into the catalytic and activation loops of the ATP-binding cleft of the c-Kit receptor tyrosine kinase domain with ΔG_b: −13.76 for **4e** and −12.75 kcal/mol for **9a**. Compound **4e** showed two hydrogen bonds with Asp 810 and Glu 640, whereas **9a** showed one hydrogen bond with Thr 679. These docking results of compounds **4e** and **9a** are highly correlated to their antitumor activities cited in Table 1.

Figure 4 illustrates the comparative docking modes of **8a** and **13d** into the c-Kit receptor PTK together with the bound ligand STI. Compounds **8a** (ΔG_b: −13.01 kcal/mol) and **13d** (ΔG_b: −8.95 kcal/mol) were docked into same groove of the binding site along with STI-ligand and exhibited two (with Glu 640 and Asp 810) and one (with Asp 810) hydrogen bonds, respectively.

The docking for 2-deoxo-2-methylthioflavin-5-oxides (**2**), flavins (**5**), and flavin-5-oxide (**6**) exhibited mostly higher binding energies (lower binding affinities) being −9.11 to −7.43 kcal/mol (ΔG_b) due to less proper fitting into the target site by hydrogen bond formation, in comparison with the binding energies of other derivatives (**3**,

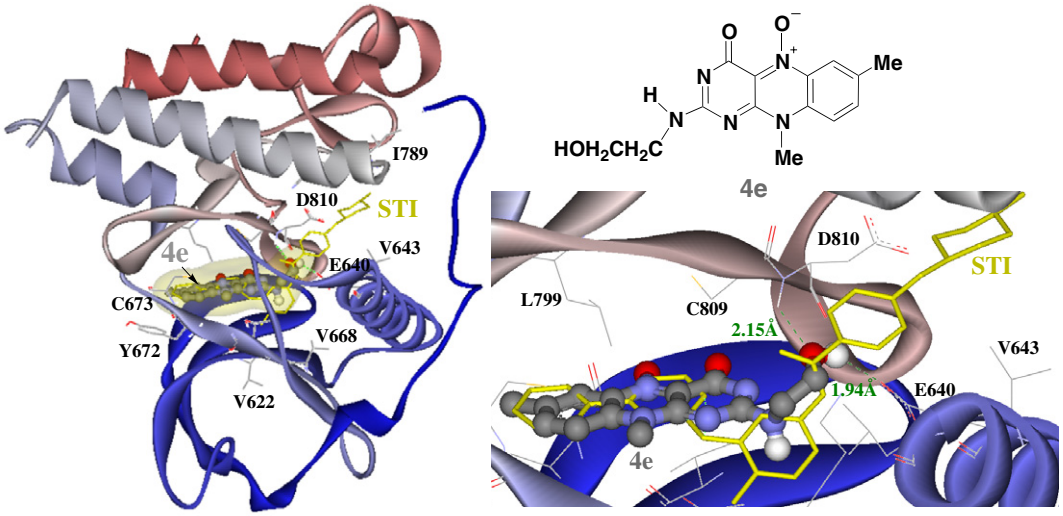


Figure 2. Stereo view of compound 2-(2-hydroxyethylamino)-7,10-dimethyl-2-deoxoflavin-5-oxide **(4e)** (ball and stick, colored by element) deeply embedded into the ATP-binding cleft of the c-Kit receptor PTK domain with the bound ligand STI-571. Hydrogen bonds are shown as green dashed lines.

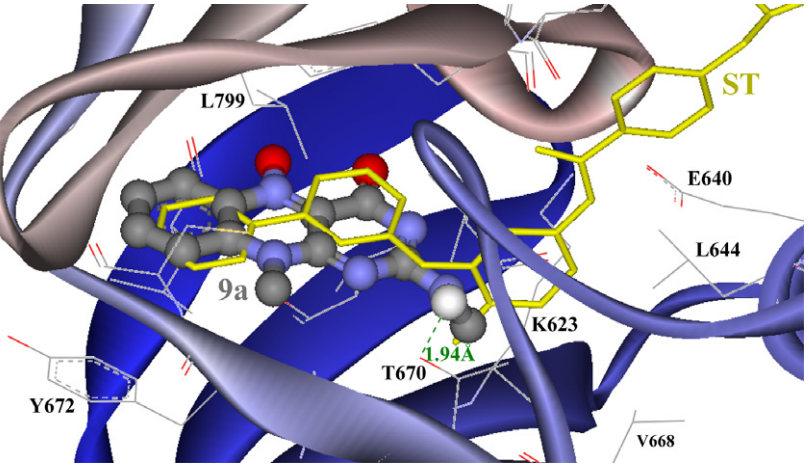


Figure 3. Docking of compound 2-methylamino-2-deoxo-10-methylflavin-5-oxide (**9a**) (ball and stick, colored by element) into the ATP-binding cleft of the c-Kit receptor PTK, exhibiting one hydrogen bond with amino acid Thr 670 (in green dashed lines) and RMSD being 6.40 Å from the bound ligand STI-571.

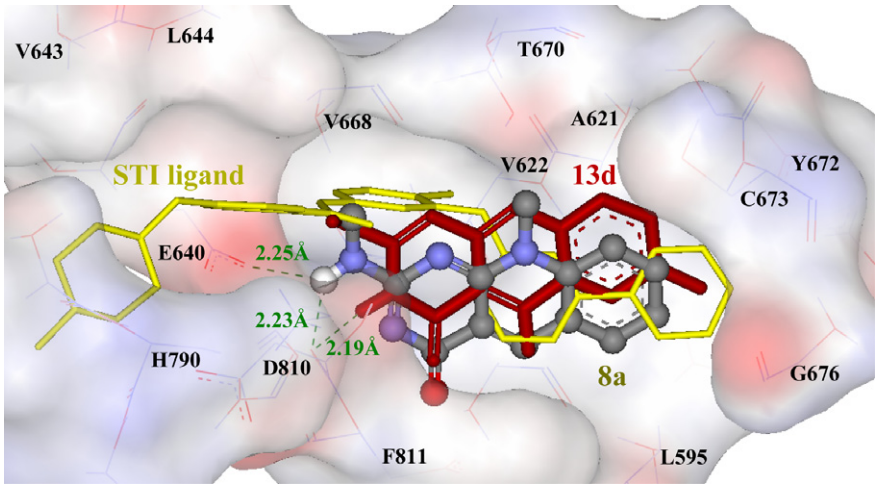


Figure 4. AutoDock binding affinities of compound 2-deoxo-2-methylamino-5-deazaflavin (**8a**; colored by element, ball and stick) and 2-deoxo-7-methyl-2-methylthioalloxazine-5-oxide (**13d**; red, stick) into PTK (1t46). They exhibit two and one hydrogen bonds, respectively, shown as green dashed lines. The binding pocket of the PTK is shown in transparent solid surface with labeled amino acids and the STI ligand is shown as yellow stick.

4, **8**, and **9**) (ΔG_b: −14.83 to −12.75 kcal/mol). However, some compounds showed better antitumor activities in spite of the lower binding affinities as cited in Table 1. It appeared reasonable to conclude the reason for the better antitumor activities as described below. That is, the planar pyrimidoquinoxaline ring of compounds **2**, **5**, and **6** was involved in hydrophobic electrostatic surface interaction, where it was sandwiched within distance of ca. 5.0 Å between the phenyl moieties of Phe 811 and Tyr 672 and the terminal hydrocarbon chain of Leu 595. Also their 2-methylthio group interacts with Leu 644, Val 654, Val 668, and Cys 809 by hydrophobic attraction within distance of ca. 3.62–5.16 Å. This interaction keeps these derivatives (**2**, **5**, and **6**) in the binding site, within RMSD values of 4.09–6.53 Å.

The overall correlation between the growth inhibitory activities of the synthesized flavin analogs against tumor cells and the AutoDock binding affinities was better for some compounds. The growth inhibitory activities of

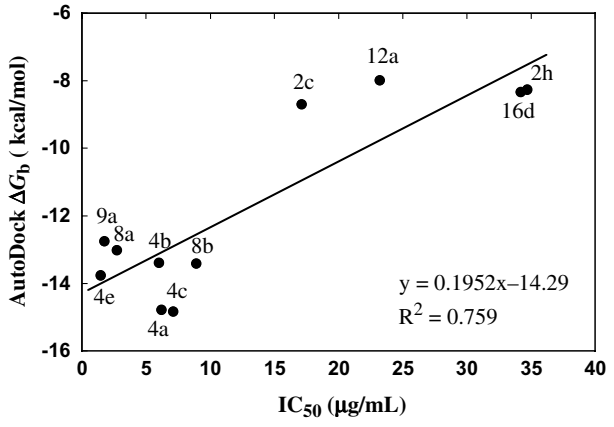


Figure 5. Correlation between the binding free energy (ΔG_b) and IC_{50} (μg/mL) of compounds **2c**, **h**, **4a–c**, **e**, **8a**, **b**, **9a**, **12a**, and **16d** against CCRF-HSB-2 tumor cells.

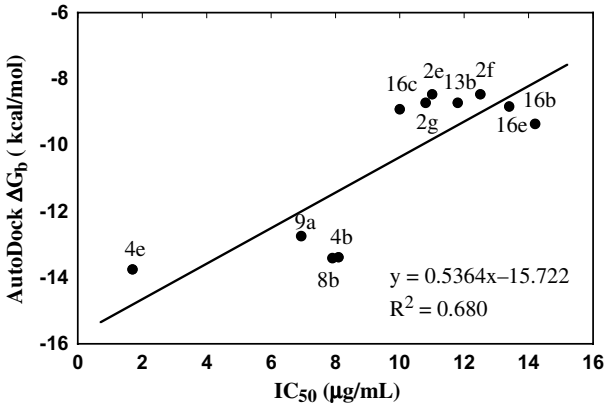


Figure 6. Correlation between the binding free energy (ΔG_b) and IC_{50} (μg/mL) of compounds **2e**, **f**, **g**, **4b**, **e**, **8b**, **9a**, **13b**, and **16b**, **c**, **e** against KB tumor cells.

compounds **2c**, **h**, **4a–c**, **e**, **8a**, **b**, **9a**, **12a**, and **16d** against CCRF-HSB-2 tumor cells were correlated to their AutoDock binding free energies with a good correlation coefficient (R^2) of 0.759 as shown in Figure 5.

Whereas, the growth inhibition against KB tumor cells revealed a reasonable correlation with AutoDock binding free nergies for compounds **2e**, **f**, **g**, **4b**, **e**, **8b**, **9a**, **13b**, and **16b**, **c**, **e** of correlation coefficient (R^2) of 0.68 as shown in Figure 6.

3. Conclusions

In this study, various novel 10-alkyl-2-deoxo-2-methylthioflavin-5-oxides (**2a–i**) were synthesized from 6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-ones (**1a–i**) by nitrosative cyclization. The 2-alkylamino derivatives (**4a–e**) were synthesized by the facile replacement of the C_2-methylthio moiety of **2** by different amines. Flavins (**5a** and **b**) and flavin-5-oxide (**6**) were prepared by

acidic hydrolysis of **2** in excellent yields. Nucleophilic substitution of 2-methylthio group of the non-cyclized 6-(*N*-methylanilino)-2-methylthiopyrimidin-4(3*H*)-one (**1a**) by an appropriate amine was carried out, followed by Vilsmeier reaction to afford the 2-alkylamino-2-deoxo-5-deazaflavins (**8a** and **b**) or by nitrosative cyclization to afford the 2-alkylaminoflavin-5-oxides (**9a** and **b**). Compounds 2-deoxo-2-methylthio-5-deazaalloxazines (**12**) and 2-deoxo-2-methylthioalloxazine-5-oxides (**13**) were facilely synthesized from 6-anilino-2-methylthiopyrimidin-4(3*H*)-ones (**11**) by Vilsmeier reaction and nitrosative cyclization, respectively. The nucleophilic replacement of 2-methylthio group of compounds (**12** and **13**) was carried out by heating with alkylamines to afford their 2-alkylamino analogs (**14** and **15**). Additionally, 3-alkyl-2-methylthioalloxazine-5-oxides (**16a–e**) were prepared by the regiospecific N_3-alkylation of 2-deoxo-2-methylthioalloxazine-5-oxides (**13**) using excess alkyl iodide or bromide.

In vitro growth inhibitory activities of compounds (**2**, **4**, **6**, **8**, **9**, **12**, **13**, **15**, and **16**) against T-cell acute lymphoblastoid leukemia cell line (CCRF-HSB-2) and human oral epidermoid carcinoma cell line (KB) revealed potential antitumor activities of many derivatives. Among them, compounds **2a**, **2d**, **4e**, **5a**, **5b**, **8a**, and **9a** exhibited the most significant antiproliferative potencies with good IC_{50} of 1.46–2.70 and 0.61–2.1 μg/mL against CCRF-HSB-2 and KB cells, respectively. Some other derivatives, namely **2b**, **4b**, **6**, **13a**, **13c**, and **13d**, showed moderate IC_{50} of 3.72–6.77 and 5.35–8.19 μg/mL against CCRF-HSB-2 and KB cells, respectively. These promising antitumor activities provide these derivatives as new lead entities in cancer therapy. Moreover, the structure–activity relationships (SAR) revealed that the flavin-5-oxide, 5-deazaflavin, and alloxazine skeletons with SMe or HNMe group at the C_2-position, alloxazine conformation or Me group at the N_{10}-position, and unsubstituted quinoxaline nucleus or substituted by 7-Me or 9-Me group provide higher antitumor activities.

The AutoDock investigation of the synthesized derivatives (**2–6**, **8**, **9**, and **12–16**) was carried out for lead optimization and they docked into c-kit protein–tyrosine kinase. The correlation between the binding free energies (ΔG_b, kcal/mol) predicted by AutoDock and the growth inhibitory activities (IC_{50}, μg/mL) against CCRF-HSB-2 tumor cells for compounds **2c**, **h**, **4a–c**, **e**, **8a**, **b**, **9a**, **12a**, and **16d** was good with a correlation coefficient (R^2) of 0.759. Also the correlation between and the AutoDock binding free energies and IC_{50} against KB tumor cells for compounds **2e**, **f**, **g**, **4b**, **e**, **8b**, **9a**, **13b**, and **16b**, **c**, **e** was good with a correlation coefficient (R^2) of 0.68.

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 spectro-photometer 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 absorption values followed by sh refer to wavelengths 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 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 10-alkyl-2-deoxo-2-methylthioflavin-5-oxides {10-alkyl-2-methylthio-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxides} (2a–i). To a stirring solution of 6-(*N*-alkylanilino)-2-methylthiopyrimidin-4(3*H*)-one (**1**, 0.01 mol) in acetic acid (5–15 mL) at 10–15 °C was added sodium nitrite (0.02–0.04 mol) by portions, and the mixture was stirred for 1–2 h at room temperature. The solid deposited was collected by suction filtration and washed with water. The filtrate was evaporated in vacuo and diluted with excess water to afford the second crop. The collected solids were dried and crystallized from an appropriate solvent to afford the corresponding products as red needles in 65–89% yields.

4.1.1.1. 10-Methyl-2-methylthio-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (2a). Yield, (2.0 g, 73%); mp 215–217 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 219 (4.53), 269 (4.39), 368 (4.00), 464 (4.09); IR (ν_{max}/cm^{-1}): 1650 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.59 (3H, s, 2-SMe), 4.13 (3H, s, 10-Me), 7.60 (1H, dt, $J_{6,7} = J_{7,8} = 8.7$ Hz, $J_{7,9} = 1.5$ Hz, 7-H), 7.75 (1H, dd, $J_{7,9} = 1.5$ Hz, $J_{8,9} = 8.7$ Hz, 9-H), 7.94 (1H, dt, $J_{7,8} = J_{8,9} = 8.7$ Hz, $J_{6,8} = 1.5$ Hz, 8-H), 8.57 (1H, dd, $J_{6,7} = 8.7$ Hz, $J_{6,8} = 1.5$ Hz, 6-H). Anal. Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.54; H, 3.67; N, 20.43. Found: C, 52.61; H, 3.91; N, 20.59.

4.1.1.2. 10-Ethyl-2-methylthio-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (2b). Yield, (2.57 g, 89%); mp 234–236 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 220 (4.53), 278 (4.38), 371 (4.06), 468 (4.16); IR (ν_{max}/cm^{-1}): 1655 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.53 (3H, t, $J = 7.2$ Hz, 10-CH_2-CH_3), 2.59 (3H, s, 2-SMe), 4.77 (2H, q, $J = 7.2$ Hz, 10-CH_2–CH_3), 7.60 (1H, dt, $J_{6,7} = 8.7$ Hz, $J_{7,8} = 8.4$ Hz, $J_{7,9} = 1.5$ Hz, 7-H), 7.74 (1H, br d, $J_{8,9} = 8.4$ Hz, 9-H), 7.94 (1H, dt, $J_{7,8} = J_{8,9} = 8.4$ Hz, $J_{6,8} = 1.5$ Hz, 8-H), 8.58 (1H, dd, $J_{6,7} = 8.7$ Hz, $J_{6,8} = 1.5$ Hz, 6-H). Anal. Calcd for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.19; N, 19.43. Found: C, 54.31; H, 4.38; N, 19.16.

4.1.1.3. 7,10-Dimethyl-2-methylthio-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (2c). Yield, (2.02 g, 70%); mp 221–223 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}): 221 (4.48), 279 (4.38), 369 (4.00), 472 (4.11); IR (ν_{max}/cm^{-1}): 1650 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.57 (3H, s, 7-Me), 2.59 (3H, s, 2-SMe), 4.11 (3H, s, 10-Me), 7.63 (1H, d, $J_{8,9}$ = 8.4 Hz, 9-H), 7.75 (1H, dd, $J_{8,9}$ = 8.4 Hz, $J_{6,8}$ = 2.1 Hz, 8-H), 8.35 (1H, br s, 6-H). Anal. Calcd for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.19; N, 19.43. Found: C, 54.15; H, 4.26; N, 19.12.

4.1.1.4. 9,10-Dimethyl-2-methylthio-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (2d). Yield, (2.05 g, 71%); mp 200–202 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}): 219 (4.55), 268 (4.47), 377 (4.10), 466 (4.04); IR (ν_{max}/cm^{-1}): 1650 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.58 (3H, s, 2-SMe), 2.88 (3H, s, 9-Me), 4.19 (3H, s, 10-Me), 7.47 (1H, t, $J_{6,7}$ = $J_{7,8}$ = 8.1 Hz, 7-H), 7.69 (1H, d, $J_{7,8}$ = 8.1 Hz, 8-H), 8.42 (1H, d, $J_{6,7}$ = 8.1 Hz, 6-H). Anal. Calcd for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.19; N, 19.43. Found: C, 54.47; H, 4.49; N, 19.39.

4.1.1.5. 7-Methoxy-10-methyl-2-methylthio-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (2e). Yield, (2.37 g, 78%); mp >300 °C (from DMF); UV (EtOH): λ_{max}/nm ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}): 220 (4.66), 289 (4.61), 365 (4.14), and 495 (4.28); IR (ν_{max}/cm^{-1}): 1640 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.59 (3H, s, 2-SMe), 3.98 (3H, s, 7-OMe), 4.14 (3H, s, 10-Me), 7.56 (1H, dd, $J_{8,9}$ = 9.3 Hz, $J_{6,8}$ = 2.7 Hz, 8-H), 7.69 (1H, d, $J_{8,9}$ = 9.3 Hz, 9-H), 7.96 (1H, d, $J_{6,8}$ = 2.7 Hz, 6-H). Anal. Calcd for $C_{13}H_{12}N_4O_3S$: C, 51.31; H, 3.97; N, 18.41. Found: C, 51.19; H, 4.10; N, 18.32.

4.1.1.6. 8-Methoxy-10-methyl-2-methylthio-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (2f). Yield, (2.04 g, 67%); mp 214–217 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}): 228 (4.50), 265 (4.34), 379 (4.26), 457 (4.23); IR (ν_{max}/cm^{-1}): 1630 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.57 (3H, s, 2-SMe), 4.06 (3H, s, 8-OMe), 4.07 (3H, s, 10-Me), 6.96 (1H, d, $J_{7,9}$ = 2.4 Hz, 9-H), 7.14 (1H, dd, $J_{6,7}$ = 9.6 Hz, $J_{7,9}$ = 2.4 Hz, 7-H), 8.45 (1H, d, $J_{6,7}$ = 9.6 Hz, 6-H). Anal. Calcd for $C_{13}H_{12}N_4O_3S$: C, 51.31; H, 3.97; N, 18.41. Found: C, 51.28; H, 4.40; N, 18.40.

4.1.1.7. 7-Chloro-10-methyl-2-methylthio-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (2g). Yield, (2.0 g, 65%); mp 303–305 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}): 220 (4.39), 268 (4.34), 347 (4.72), 469 (3.85); IR (ν_{max}/cm^{-1}): 1660 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.60 (3H, s, 2-SMe), 4.10 (3H, s, 10-Me), 7.68 (1H, d, $J_{8,9}$ = 9.6 Hz, 9-H), 7.87 (1H, dd, $J_{8,9}$ = 9.6 Hz, $J_{6,8}$ = 2.4 Hz, 8-H), 8.54 (1H, d, $J_{6,8}$ = 2.4 Hz, 6-H). Anal. Calcd for $C_{12}H_9ClN_4O_2S$: C, 46.68; H, 2.94; N, 18.15. Found: C, 46.89; H, 3.09; N, 17.88.

4.1.1.8. 10-Ethyl-7-methyl-2-methylthio-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (2h). Yield, (2.6 g, 86%); mp 236–238 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}): 220 (4.45), 273 (4.31), 368 (3.87), 470 (3.93); IR (ν_{max}/cm^{-1}): 1650 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.51 (3H, t, J = 7.2 Hz, 10-CH_2–CH_3), 2.57 (3H, s, 7-Me), 2.59 (3H, s, 2-SMe), 4.76 (2H, q, J = 7.2 Hz, 10-CH_2–CH_3), 7.68 (1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 7.75 (1H, dd, $J_{8,9}$ = 9.0 Hz, $J_{6,8}$ = 1.5 Hz, 8-H), 8.37 (1H, br s, 6-H). Anal. Calcd for $C_{14}H_{14}N_4O_2S$: C, 55.61; H, 4.67; N, 18.53. Found: C, 55.33; H, 4.79; N, 18.16.

4.1.1.9. 7,8,10-Trimethyl-2-methylthio-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (2i). Yield, (2.45 g, 81%); mp >300 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}): 222 (4.64), 280 (4.59), 378 (4.29), 474 (4.35); IR (ν_{max}/cm^{-1}): 1650 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.52 (3H, s, 7-Me), 2.53 (3H, s, 8Me), 2.57 (3H, s, 2-SMe), 4.10 (3H, s, 10-Me), 7.49 (1H, s, 6-H), 8.21 (1H, s, 9-H). Anal. Calcd for $C_{14}H_{14}N_4O_2S$: C, 55.61; H, 4.67; N, 18.53. Found: C, 55.85; H, 4.68; N, 18.38.

4.1.1.10. Preparation of 2-deoxo-2-dimethylaminoalloxazine {2-dimethylamino-4-oxo-3,4-dihydrobenzo[*g*]pteridine} (3). A mixture of 2-deoxo-10-methyl-2-methylthioflavin-5-oxide (**2a**, 1.4 g, 5.0 mmol) and 50% aqueous dimethylamine (50 mL) was heated in steel sealed tube at 160–165 °C (15 kg/cm^2 pressure) for 4 h. After the reaction was complete, the precipitated crystals were collected by filtration, and mother liquor was evaporated in vacuo to get the second crop. The product was washed with water, dried, and recrystallized from EtOH to afford the yellow crystals in 89% yield.

Yield, (1.07 g, 89%); mp >300 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm ($\log \varepsilon$/dm^3 mol^{-1} cm^{-1}): 222 (4.39), 268 (4.32), 288 (4.32), 335 (3.63), 376sh (3.59), 398 (3.71), 429 (3.77); IR (ν_{max}/cm^{-1}): 3190 (NH), 1700 (C=O); ^{1}H NMR ($CDCl_3$): δ 3.42 (6H, s, 2-N–(CH_3)$_2$), 7.66 (1H, dt, $J_{6,7}$ = $J_{7,8}$ = 8.4 Hz, $J_{7,9}$ = 1.5 Hz, 7-H), 7.81 (1H, dt, $J_{7,8}$ = $J_{8,9}$ = 8.4 Hz, $J_{6,8}$ = 1.5 Hz, 8-H), 8.04 (1H, br d, $J_{8,9}$ = 8.4 Hz, 9-H), 8.23 (1H, br d, $J_{6,7}$ = 8.4 Hz, 6-H), 10.13 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{12}H_{11}N_5O{\cdot}0.2H_2O$: C, 58.86; H, 4.69; N, 28.60. Found: C, 58.78; H, 4.87; N, 28.58.

4.1.2. General procedure for the preparation of 10-alkyl-2-alkylamino-2-deoxoflavin-5-oxides {10-alkyl-2-alkylamino-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxides} (4a–e). A mixture of 10-alkyl-2-deoxo-2-methylthioflavin-5-oxides (**2**, 5.0 mmol) and an appropriate alkylamine (0.025–0.05 mol) in *n*-butanol (30 mL) was refluxed with stirring for 15 min. After the clear yellow solution obtained under reflux was cooled under refrigeration overnight, the resulting yellow crystals were collected by filtration to get the first crop. The filtrate was concentrated in vacuo and the residue was treated with ethanol or water to get the second crop free from amines. The collected solids were dried and recrystallized from an appropriate solvent to give the corresponding products as bright yellow-orange needles in 62–89% yields.

4.1.2.1. 7-Methoxy-10-methyl-2-piperidino-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (4a). Yield, (1.06 g,

62%); mp 276–278 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 223 (4.37), 266 (4.28), 284 (4.48), 350 (3.88), 444sh (3.95), 476 (4.17), 506 (4.14); IR (ν_{max}/cm^{-1}): 1645 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.66 (2H, br s, 4′-CH_2), 3.74–3.80 (4H, m, 3′ and 5′-CH_2), 3.92 (3H, s, 7-OMe), 4.04–4.12 (4H, m, 2′ and 6′-CH_2), 4.09 (3H, s, 10-Me), 7.47 (1H, dd, $J_{8,9}$ = 9.3 Hz, $J_{6,8}$ = 2.7 Hz, 8-H), 7.56 (1H, d, $J_{8,9}$ = 9.3 Hz, 9-H), 7.75 (1H, d, $J_{6,8}$ = 2.7 Hz, 6-H). Anal. Calcd for $C_{17}H_{19}N_5O_3$: C, 59.81; H, 5.61; N, 20.52. Found: C, 59.58; H, 5.44; N, 20.75.

4.1.2.2. 10-Ethyl-2-isobutylamino-4-oxo-4,10-dihydrobenzo[*g*]pteridin-5-oxide (4b). Yield, (1.30 g, 83%); mp 264–267 °C (decomp., from EtOH-*n*-hexane); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 219 (4.47), 271 (4.47), 343sh (3.96), 443 (3.12), 472sh (3.98); IR (ν_{max}/cm^{-1}): 3195 (NH), 1620 (C=O); ^{1}H NMR ($CDCl_3$): δ 0.99–1.02 (6H, m, NH–CH_2–CH–(CH_3)$_2$), 1.46–1.56 (3H, m, 10-CH_2–CH_3) 1.92–2.08 (1H, m, NH–CH_2–C*H*–(CH_3)$_2$), 3.44–3.51 (2H, m, NH–CH_2–CH–(CH_3)$_2$), 4.75 (2H, q, J = 6.9 Hz, 10-CH_2–CH_3), 5.75 (1H, br s, 2-NH, exchangeable with D_2O), 7.53–7.65 (2H, m, 7 and 9-H), 7.76–7.88 (1H, m, 8-H), 7.32–7.40 (1H, m, 6-H). Anal. Calcd for $C_{16}H_{19}N_5O_2$: C, 61.33; H, 6.11; N, 22.35. Found: C, 61.51; H, 6.44; N, 22.43.

4.1.2.3. 2-Cyclohexylamino-7,10-dimethyl-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (4c). Yield, (1.05 g, 62%); mp 288–291 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 220 (4.56), 271 (4.57), 341 (3.94), 436 (4.10), 483sh (3.83); IR (ν_{max}/cm^{-1}): 3270 (NH), 1665 (C=O); ^{1}H NMR [(CD_3)$_2$SO], 100 °C: δ 1.10–1.68 (6H, m, 3′, 4′, and 5′-CH_2), 1.68–1.98 (4H, m, 2′ and 6′-CH_2), 2.49 (3H, s, 7-Me), 3.95 (3H, s, 10-Me), 4.02–4.06 (1H, br m, 1′-CH), 7.37 (1H, br s, 2-NH, exchangeable with D_2O), 7.67–7.78 (1H, m, 9-H), 7.87 (1H, br s, 6-H), 7.95 (1H, br d, $J_{8,9}$ = 9.0 Hz, 8-H). Anal. Calcd for $C_{18}H_{21}N_5O_2$: C, 63.70; H, 6.24; N, 20.64. Found: C, 63.99; H, 6.34; N, 20.25.

4.1.2.4. 2-Benzylamino-7-chloro-10-methyl-4-oxo-4,10-dihydroenzo[*g*]pteridine-5-oxide (4d). Yield, (1.34 g, 73%); mp >300 °C (from EtOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 221 (4.85), 274 (4.90), 330 (4.24), 453 (4.38), 487sh (4.24); IR (ν_{max}/cm^{-1}): 3190 (NH), 1655 (C=O); ^{1}H NMR [(CD_3)$_2$SO], at 100 °C: δ 4.00 (3H, s, 10-Me), 4.60–4.80 (2H, m, 2-NH–CH_2), 7.20–7.52 (5H, m, 2-CH_2–C_6H_5), 7.82–8.02 (2H, m, 2-NH and 9-H), 8.10–8.28 (2H, m, 6 and 8-H). Anal. Calcd for $C_{18}H_{14}ClN_5O_2{\cdot}0.25H_2O$: C, 58.07; H, 3.93; N, 18.81. Found: C, 57.89; H, 4.28; N, 18.42.

4.1.2.5. 2-(2-Hydroxyethylamino)-7,10-dimethyl-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (4e). Yield, (1.34 g, 89%); mp 266–268 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 217 (4.62), 273 (4.64), 344 (4.12), 453 (4.24), 483sh (4.11); IR (ν_{max}/cm^{-1}): 3290 (OH), 3200 (NH), 1660 (C=O); ^{1}H NMR [(CD_3)$_2$SO], at 100 °C: δ 2.49 (3H, s, 7-Me), 3.38–3.46 (2H, m, 2-NHCH_2CH$_2$OH), 3.48–3.60 (2H, m, 2-NHCH$_2$CH_2OH), 3.97 (3H, s, 10-Me), 4.86 (1H, br s, 2-NHCH$_2$CH$_2$O*H*, exchangeable with D_2O), 7.42 (1H, br s, 2-N*H*, exchangeable with D_2O), 7.70–7.81 (1H, m, 9-H), 7.85–7.97 (2H, m, 6 and 8-H). Anal. Calcd for $C_{14}H_{15}N_5O_3$: C, 55.81; H, 5.02; N, 23.24. Found: C, 56.04; H, 4.95; N, 23.34.

4.1.3. General procedure for the preparation 10-alkylflavins {10-alkylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-diones} (5a, b) and 7-methoxy-10-methylflavin-5-oxide {7-methoxy-10-methyl-2,4-dioxo-2,3,4,10-tetrahydro-benzo[*g*]pteridine-5-oxide} (6). To 10-alkyl-2-deoxo-2-methylthioflavin-5-oxides (**2**, 0.01 mol) was added 5 N hydrochloric acid (250 mL). Then, the mixture was refluxed for 7–12 h, and the resulting clear yellow solution was concentrated in vacuo. The yellow residue was treated with water and neutralized with aqueous ammonia (pH 7). The precipitate powdery crystals were filtered off, washed well with water, dried, and recrystallized from acetic acid to afford the corresponding products as yellow needles in 68–97% yields.

4.1.3.1. 10-Methylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (5a). Yield, (2.05 g, 90%); mp >300 °C (from HOAc; lit.[28] mp >360 °C); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 214 (4.31), 265 (4.39), 332 (3.74), 436 (3.81); IR (ν_{max}/cm^{-1}): 3170 (NH), 1720, 1660 (C=O); ^{1}H NMR [(CD_3)$_2$SO]: δ 3.98 (3H, s, 10-Me), 7.62–7.69 (1H, m, 7-H), 7.93–7.98 (2H, m, 8 and 9-H), 8.13 (1H, br d, $J_{6,7}$ = 7.8 Hz, 6-H), 11.10 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{11}H_8N_4O_2{\cdot}0.6H_2O$: C, 55.28; H, 3.88; N, 23.44. Found: C, 55.47; H, 3.70; N, 23.02.

4.1.3.2. 10-Ethylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (5b). Yield, (2.35 g, 97%); mp >300 °C (from HOAc; lit.[28] mp 347 °C); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 216 (4.53), 265 (4.65), 335 (3.98), 434 (4.13); IR (ν_{max}/cm^{-1}): 3180 (NH), 1720, 1680 (C=O); ^{1} H NMR ($CDCl_3$): δ 1.33 (3H, t, J = 7.2 Hz, 10-CH_2–CH_3), 4.65 (2H, q, J = 7.2 Hz, 10-CH_2–CH_3), 7.65 (1H, d t, $J_{6,7}$ = $J_{7,8}$ = 7.8 Hz, $J_{7,9}$ = 1.2 Hz, 7-H), 7.92–8.03 (2H, m, 8 and 9-H), 8.14 (1H, dd, $J_{6,7}$ = 7.8 Hz, $J_{6,8}$ = 1.2 Hz, 6-H), 11.39 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.10; H, 4.40; N, 23.06.

4.1.3.3. 7-Methoxy-10-methyl-2,4-dioxo-2,3,4,10-tetrahydrobenzo[*g*]pteridine-5-oxide (6). Yield, (1.86 g, 68%); mp >300 °C (from HOAc); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 216 (4.57), 272 (4.63), 339 (4.04), 474 (4.18); IR (ν_{max}/cm^{-1}): 3160 (NH), 1710, 1665 (C=O); ^{1}H NMR [(CD_3)$_2$SO]: δ 3.93 (3H, s, 7-OMe), 3.99 (3H, s, 10-Me), 7.59 (1H, d, $J_{6,8}$ = 3.0 Hz, 6-H), 7.63–7.65 (1H, m, 8-H), 7.91(1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 11.03 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{12}H_{10}N_4O_4$: C, 52.56; H, 3.68; N, 20.43. Found: C, 52.87; H, 3.94; N, 20.13.

4.1.4. Preparation of 2-methylamino-6-(*N*-methylanilino)pyrimidin-4(3*H*)-one (7a). A mixture of 6-(*N*-methylanilino)-2-methylthiopyrimidin-4(3*H*)-one (**1a**, 0.5 g, 2.0 mmol) and 40% aqueous methylamine (50 mL) in

steel sealed tube was heated at 160 °C (10 kg/cm^2 pressure) for 15 h. After the reaction was complete, the resulting clear yellow solution was concentrated in vacuo. The yellow residue was treated with ethyl acetate to get the solid, which was filtered off, washed with water, dried, and crystallized from DMF to afford the pure product as colorless needles.

Yield, (0.40 g, 87%); mp 219–221 °C (from DMF); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 229 (4.49), 258sh (4.15), 284 (4.21); IR (ν_{max}/cm^{-1}): 3310, 3176 (NH), 1625 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.88 (3H, s, 2-NMe), 3.44 (3H, s, 6-NMe), 4.67 (1H, s, 5-H), 5.52 (1H, br s, 2-NH, exchangeable with D_2O), 7.01–7.44 (5H, m, Ph–H), 12.19 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33. Found: C, 62.23; H, 6.07; N, 24.33.

4.1.4.1. Preparation of 6-(*N*-methylanilino)-2-*n*-octylaminopyrimidin-4(3*H*)-one (7b). A mixture of 6-(*N*-methylanilino)-2-methylthiopyrimidin-4(3*H*)-one (**1a**, 1.24 g, 5.0 mmol) and *n*-octylamine (2.58 g, 0.02 mol) was heated at 160 °C for 10 h. After cooling, the resulting mixture was triturated with water and acidified with acetic acid to remove the excess *n*-octylamine as salt. The product was extracted from the mixture with dichloromethane (3× 50 mL) and the extract was washed with saturated brine (2× 20 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent was removed under reduced pressure to afford the product **7b** as orange oil. The product which was dried under vacuum was used for the next step without further purification.

Yield, (1.41 g, 86%); oily compound; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 230 (4.56), 259sh (4.28), 282 (4.29); IR (ν_{max}/cm^{-1}): 3278, 3156 (NH), 1640 (C=O); ^{1}H NMR ($CDCl_3$): δ 0.84–0.92 (3H, m, 2-N–$(CH_2)_7$–CH_3), 1.22–1.33 (10H, m, 2-N–$(CH_2)_2$–$(CH_2)_5$–CH_3), 1.38–1.59 (2H, m, 2-N–CH_2–CH_2–$(CH_2)_5$–CH_3), 3.27 (2H, q, J = 7.2 Hz, 2-N–CH_2–$(CH_2)_6$–CH_3), 3.41 (3H, s, 6-NMe), 4.66 (1H, s, 5-H), 5.82 (1H, br s, 2-NH, exchangeable with D_2O), 7.19–7.28 (3H, m, Ph-*o*, *p*H), 7.34–7.42 (2H, m, Ph-*m*H), 11.57 (1H, br s, 3-NH, exchangeable with D_2O).

4.1.4.2. General procedure for the preparation of 2-alkylamino-2-deoxo-10-methyl-5-deazaflavins {2-alkylamino-10-methylpyrimido[4,5-*b*]quinolin-4(10*H*)-ones} (8a,b). A mixture of 2-alkylamino-6-(*N*-methylanilino)-pyrimidin-4(3*H*)-ones (**7**, 5.0 mmol) and phosphoryl chloride (3.83 g, 25 mmol) in anhydrous DMF (5 mL) was heated under stirring at 90 °C for 3 h. Then, the reaction mixture was poured onto ice and treated with aqueous ammonia (pH 8). The separated yellow crystals were filtered off, washed with water, dried and recrystallized from an appropriate solvent to afford the products as yellow needles in 60–82% yields.

4.1.4.3. 10-Methyl-2-methylaminopyrimido[4,5-*b*]quinolin-4-(10*H*)-one (8a). Yield, (0.99 g, 82%); mp 259–251 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 221 (5.07), 269 (4.20), 275sh (4.16), 321 (3.67), 410 (3.66); IR (ν_{max}/cm^{-1}): 3290 (NH), 1705 (C=O); ^{1}H NMR ($CDCl_3$): δ 3.34 (3H, s, 2-NMe),4.10 (3H, s, 10-Me), 7.46–7.58 (1H, m, 7-H), 7.67 (1H, br s, 2-NH, exchangeable with D_2O), 7.82–7.98 (2H, br m, 8 and 9-H), 8.11 (1H, br d, $J_{6,7}$ = 7.8 Hz, 6-H), 8.82 (1H, s, 5-H). Anal. Calcd for $C_{13}H_{12}N_4O{\cdot}0.7H_2O$: C, 61.75; H, 5.34; N, 22.16. Found: C, 62.04; H, 5.74; N, 22.0.

4.1.4.4. 10-Methyl-2-*n*-octylaminopyrimido[4,5-*b*]quinolin-4-(10*H*)-one (8b).[18] Yield, (1.02 g, 60%); mp 187–189 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 220 (4.45), 269 (4.54), 275sh (4.47), 320 (3.98), 406 (4.05); IR (ν_{max}/cm^{-1}): 3220 (NH), 1720 (C=O); ^{1}H NMR [$(CD_3)_2SO$], at 100 °C: δ 0.86 (3H, t, J = 7.2 Hz, 2-N$(CH_2)_7$–CH_3), 1.20–1.42 (10H, m, 2-N$(CH_2)_2$–$(CH_2)_5$–CH_3), 1.60 (2H, quint, J = 7.2 Hz, 2-NCH_2–CH_2–$(CH_2)_5$–CH_3), 3.35–3.51 (2H, m, 2-NCH_2–$(CH_2)_6$–CH_3), 4.07 (3H, s, 10-Me), 7.17 (1H, br s, 2-NH, exchangeable with D_2O), 7.42–7.53 (1H, m, 7-H), 7.75–7.93 (2H, m, 8 and 9-H), 8.07 (1H, d, J = 8.1 Hz, 6-H), 8.77 (1H, s, 5-H). Anal. Calcd for $C_{20}H_{26}N_4O{\cdot}0.1H_2O$: C, 70.60; H, 7.76; N, 16.47. Found: C, 70.30; H, 7.59; N, 16.14.

4.1.5. General procedure for the preparation of 2-alkylamino-2-deoxo-10-methylflavin-5-oxides {2-alkylamino-10-methyl-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxides} (9a,b). To a stirring solution of 2-alkylamino-6-(*N*-methylanilino)pyrimidin-4(3*H*)-ones (**7**, 5.0 mmol) in acetic acid (5–15 mL) at 10–15 °C was added sodium nitrite (0.01–0.02 mol) by portions, and the mixture was stirred at room temperature for 2–3 h. The solid deposited was collected by suction filtration and washed well with water. The filtrate was concentrated in vacuo and diluted with excess water to afford the second crop. The collected solids were washed with diluted ammonia solution, dried, and crystallized from an appropriate solvent to afford the corresponding products as orange needles in 76–88% yields.

4.1.5.1. 10-Methyl-2-methylamino-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (9a). Yield, (1.13 g, 88%); mp >300 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 216 (4.27), 247sh (4.34), 274 (4.48), 348 (3.99), 455 (4.05); IR (ν_{max}/cm^{-1}): 3290 (NH), 1670 (C=O); ^{1}H NMR [$(CD_3)_2SO$], at 85 °C: δ 2.89 (3H, s, 2-NMe), 3.93 (3H, s, 10-Me), 7.31 (1H, br s, 2-NH, exchangeable with D_2O), 7.43–7.62 (1H, m, 7-H), 7.78–8.0 (2H, m, 8 and 9-H), 8.34 (1H, d, J = 8.1 Hz, 6-H). Anal. Calcd for $C_{12}H_{11}N_5O_2{\cdot}0.2H_2O$: C, 55.25; H, 4.41; N, 26.85. Found: C, 55.09; H, 4.54; N, 26.85.

4.1.5.2. 10-Methyl-2-*n*-octylamino-4-oxo-4,10-dihydrobenzo[*g*]pteridine-5-oxide (9b). Yield, (1.35 g, 76%); mp 172–174 °C (from EtOAc); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 216 (4.40), 246sh (4.29), 273 (4.47), 347 (3.95), 444 (4.05); IR (ν_{max}/cm^{-1}): 3270 (NH), 1655 (C=O); ^{1}H NMR [$(CD_3)_2SO$], at 100 °C: δ 0.89 (3H, t, J = 6.6 Hz, 2-N$(CH_2)_7$–CH_3), 1.22–1.45 (10H, m, 2-N$(CH_2)_2$–$(CH_2)_5$–CH_3), 1.61 (2H, quint, J = 6.6 Hz, 2-NCH_2–CH_2–$(CH_2)_5$–CH_3), 3.30–3.51 (2H, m, 2-NCH_2–

$(CH_2)_6$–CH_3), 3.96 (3H, s, 10-Me), 7.32 (1H, br s, 2-NH, exchangeable with D_2O), 7.43–7.65 (1H, m, 7-H), 7.75–8.0 (2H, m, 8 and 9-H), 8.36 (1H, d, J = 8.4 Hz, 6-H). Anal. Calcd for $C_{19}H_{25}N_5O_2$: C, 64.20; H, 7.09; N, 19.70. Found: C, 64.14; H, 6.83; N, 19.48.

4.1.6. General procedure for the preparation of 6-(*N*-anilino)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidines (10a–e). A mixture of 6-amino-2-thiouracil (7.10 g, 0.05 mol), an appropriate aniline (0.1 mol) together with anilinium chloride (0.075–0.10 mol) was heated at 170 °C for 4–12 h. The cooled mixture was diluted with 65% ethanol (200 mL) to afford the solid product, which was collected by filtration, dissolved in hot ca. 5% NaOH solution, and reprecipitated by neutralization with 10% HCl to get more pure product. The solid deposited was collected by suction filtration, washed with water, dried, and crystallized from a mixture of DMF and H_2O to afford the corresponding products as colorless needles in 67–83% yields.

4.1.6.1. 6-Anilino-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine (10a). Yield, (9.10 g, 83%); mp 296–298 °C (decomp., from DMF–H_2O); (lit.,[23,24] 287–288 and 281–283 °C); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 205 (4.47), 277 (4.57); IR (ν_{max}/cm^{-1}): 3335, 3220, 3105 (NH), 1670 (C=O); ^{1}H NMR ($CDCl_3$): δ 5.19 (1H, s, 5-H), 7.15–7.23 (3H, m, Ph-*o*, *p*H), 7.34–7.40 (2H, m, Ph-*m*H), 7.78 (1H, s, 6-NH), 11.28 (1H, br s, 1-NH), 11.41 (1H, br s, 3-NH). Anal. Calcd for $C_{10}H_9N_3OS{\cdot}0.1H_2O$: C, 54.33; H, 4.19; N, 19.01. Found: C, 54.27; H, 4.20; N, 18.80.

4.1.6.2. 6-(4-Methylanilino)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine (10b). Yield, (7.82 g, 67%); mp 293–295 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 205 (4.41), 255 (4.24); IR (ν_{max}/cm^{-1}): 3330, 3210, 3120 (NH), 1655 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.30 (3H, s, Me), 4.85 (1H, s, 5-H), 7.11 (2H, br d, J_{AB} = 7.8 Hz, Ar-*o*H), 7.21 (2H, br d, J_{AB} = 7.8 Hz, Ar-*m*H), 8.06 (1H, s, 6-NH), 11.49 (1H, br s, 1-NH), 11.86 (1H, br s, 3-NH). Anal. Calcd for $C_{11}H_{11}N_3OS$: C, 56.63; H, 4.75; N, 18.01. Found: C, 56.32; H, 5.08; N, 17.65.

4.1.6.3. 6-(2,4-Dimethylanilino)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine (10c). Yield, (9.77 g, 79%); mp 293–295 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 204 (4.45), 275 (4.41); IR (ν_{max}/cm^{-1}): 3350, 3190, 3095 (NH), 1650 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.27 (6H, s, 2′ and 4′-CH_3), 5.50 (1H, s, 5-H), 6.69 (1H, s, 6-NH), 7.09 (1H, s, Ar-3′-H), 7.50–7.57 (1H, m, Ar-5′-H), 8.14 (1H, d, $J_{5',6'}$ = 8.1 Hz, Ar-6′-H), 9.04 (1H, s, 1-NH), 11.98 (1H, br s, 3-NH). Anal. Calcd for $C_{12}H_{13}N_3OS$: C, 58.28; H, 5.30; N, 16.99. Found: C, 58.05; H, 5.39; N, 17.14.

4.1.6.4. 6-(4-Methoxyanilino)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine (10d). Yield, (9.60 g, 77%); mp 284–286 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 204 (4.36), 278 (4.35); IR (ν_{max}/cm^{-1}): 3340, 3240, 3100 (NH), 1665 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 3.76 (3H, s, OMe), 4.66 (1H, s, 5-H), 6.98 (2H, dd, $J_{2',3'} = J_{5',6'}$ = 8.7 Hz, $J_{2',6'}$ = 2.1 Hz, Ar-*o*H), 7.17 (2H, dd, $J_{2',3'} = J_{5',6'}$ = 8.7 Hz, $J_{3',5'}$ = 2.1 Hz, Ar-*m*H), 7.92 (1H, s, 6-NH), 11.53 (1H, br s, 1-NH), 11.83 (1H, br s, 3-NH). Anal. Calcd for $C_{11}H_{11}N_3O_2S$: C, 53.00; H, 4.45; N, 16.86. Found: C, 52.87; H, 4.52; N, 16.70.

4.1.6.5. 6-(4-Hydroxyanilino)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine (10e). Yield, (8.35 g, 71%); mp >300 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 208 (4.43), 279 (4.47); IR (ν_{max}/cm^{-1}): 3330 (OH), 3285, 3205, 3095 (NH), 1685 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 4.60 (1H, s, 5-H), 6.79 (2H, dd, $J_{2',3'} = J_{5',6'}$ = 8.7 Hz, $J_{2',6'}$ = 2.1 Hz, Ar-*o*H), 7.05 (2H, dd, $J_{2',3'} = J_{5',6'}$ = 8.7 Hz, $J_{3',5'}$ = 2.1 Hz, Ar-*m*H), 7.81 (1H, s, 6-NH), 9.55 (1H, s, OH), 11.51 (1H, br s, 1-NH), 11.81 (1H, br s, 3-NH). Anal. Calcd for $C_{10}H_9N_3O_2S$: C, 51.05; H, 3.86; N, 17.86. Found: C, 50.79; H, 4.19; N, 17.63.

4.1.7. General procedure for the preparation of 6-anilino-2-methylthio-4-oxo-3,4-dihydropyrimidines (11a–e). To a solution of 6-anilino-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidines (**10**, 0.01 mol) in 2 N KOH (250 mL) at 0–5 °C was added methyl iodide (2.84 g, 0.02 mol) and the solution was shaken vigorously under chilling for 30 min. The solid precipitated was collected by filtration and the filtrate was neutralized with 10% HCl to get the second crop. The collected solid products were dissolved in hot ca. 5% NaOH solution and reprecipitated by neutralization with 10% HCl to get the more pure products. The solid products were collected by suction filtration and washed with water, dried, and crystallized from an appropriate solvent to afford the corresponding silver colored crystals in 77–98% yields.

4.1.7.1. 6-Anilino-2-methylthio-4-oxo-3,4-dihydropyrimidine (11a). Yield, (2.10 g, 90%); mp 294–295 °C (decomp., from MeOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 204 (4.46), 258 (4.41), 297 (4.16); IR (ν_{max}/cm^{-1}): 3250, 3140 (NH), 1640 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.47 (3H, s, 2-SMe), 5.32 (1H, s, 5-H), 6.98 (1H, br t, J = 7.5 Hz, Ph-*p*H), 7.29 (2H, br t, J = 7.5 Hz, Ph-*m*H), 7.42 (2H, br d, J = 7.5 Hz, Ph-*o*H), 9.06 (1H, s, 6-NH), 11.84 (1H, br s, 3-NH). Anal. Calcd for $C_{11}H_{11}N_3OS$: C, 56.63; H, 4.75; N, 18.01. Found: C, 56.32; H, 4.78; N, 17.66.

4.1.7.2. 6-(4-Methylanilino)-2-methylthio-4-oxo-3,4-dihydropyrimidine (11b). Yield, (1.90 g, 77%); mp 252–254 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 204 (4.39), 258 (4.38), 305 (4.09); IR (ν_{max}/cm^{-1}): 3300, 3160 (NH), 1640 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.34 (3H, s, Ar-*p*Me), 2.54 (3H, s, 2-SMe), 5.51 (1H, s, 5-H), 6.47 (1H, s, 6-NH), 7.12 (2H, d, J_{AB} = 8.7 Hz, Ar-*o*H), 7.16 (2H, d, J_{AB} = 8.7 Hz, Ar-*m*H), 12.76 (1H, br s, 3-NH). Anal. Calcd for $C_{12}H_{13}N_3OS$: C, 58.28; H, 5.30; N, 16.99. Found: C, 58.06; H, 5.41; N, 16.71.

4.1.7.3. 6-(2,4-Dimethylanilino)-2-methylthio-4-oxo-3,4-dihydropyrimidine (11c). Yield, (2.56 g, 98%); mp

258–260 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 208 (4.52), 245 (4.50), 286 (4.10); IR (ν_{max}/cm^{-1}): 3390, 3140 (NH), 1640 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.22 (3H, s, 2′-Me), 2.32 (3H, s, 4′-Me), 2.53 (3H, s, 2-SMe), 5.04 (1H, s, 5-H), 6.16 (1H, s, 6-NH), 7.00 (1H, d, $J_{5',6'}$ = 7.8 Hz, Ar-5′-H), 7.06 (1H, s, Ar-3′-H), 7.12 (1H, d, $J_{5',6'}$ = 7.8 Hz, Ar-6′-H), 12.26 (1H, br s, 3-NH). Anal. Calcd for $C_{13}H_{15}N_3OS$: C, 59.74; H, 5.79; N, 16.08. Found: C, 59.44; H, 5.85; N, 15.89.

4.1.7.4. 6-(4-Methoxyanilino)-2-methylthio-4-oxo-3,4-dihydropyrimidine (11d). Yield, (2.24 g, 85%); mp 229–231 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 209 (4.45), 257 (4.48), 300 (4.21); IR (ν_{max}/cm^{-1}): 3310, 3160 (NH), 1640 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.53 (3H, s, 2-SMe), 3.81 (3H, s, 4′-OMe), 5.32 (1H, s, 5-H), 6.33 (1H, s, 6-NH), 6.88 (2H, d, $J_{A,B}$ = 9.0 Hz, Ar-*o*H), 7.15 (2H, d, $J_{A,B}$ = 9.0 Hz, Ar-*m*H), 11.98 (1H, br s, 3-NH). Anal. Calcd for $C_{12}H_{13}N_3O_2S$: C, 54.74; H, 4.98; N, 15.96. Found: C, 54.78; H, 5.02; N, 15.79.

4.1.7.5. 6-(4-Hydroxyanilino)-2-methylthio-4-oxo-3,4-dihydropyrimidine (11e). Yield, (2.34 g, 94%); mp 293–295 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 209 (4.54), 252 (4.49), 296 (4.20); IR (ν_{max}/cm^{-1}): 3400 (OH), 3380, 3210 (NH), 1640 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.45 (3H, s, 2-SMe), 5.06 (1H, s, 5-H), 6.72 (2H, d, $J_{A,B}$ = 8.7 Hz, Ar-*o*H), 7.13 (2H, d, $J_{A,B}$ = 8.7 Hz, Ar-*m*H), 8.75 (1H, s, 6-NH), 9.23 (1H, s, OH), 11.86 (1H, br s, 3-NH). Anal. Calcd for $C_{11}H_{11}N_3O_2S{\cdot}0.1H_2O$: C, 52.62; H, 4.50; N,16.74. Found: C, 52.53; H, 4.68; N, 16.35.

4.1.8. General procedure for the preparation of 2-deoxo-2-methylthio-5-deazaalloxazines {2-methylthiopyrimido[4,5-*b*]quinolin-4(3*H*)-ones} (12a,b). A mixture of 6-anilino-2-methylthio-4-oxo-3,4-dihydropyrimidines (**11**, 0.01 mol) and phosphoryl chloride (7.7 g, 0.05 mol) in anhydrous DMF (10 mL) was heated under stirring at 90 °C for 1 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 products as pale yellow needles in 63–72% yields.

4.1.8.1. 2-Methylthiopyrimido[4,5-*b*]quinolin-4(3*H*)-one (12a). Yield, (1.75 g, 72%), mp 292–294 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 226 (4.49), 239sh (4.35), 273sh (4.54), 283 (4.64), 313sh (3.78), 366 (3.86), 381 (3.75); IR (ν_{max}/cm^{-1}): 3160 (NH), 1680 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.83 (3H, s, 2-SMe), 7.58 (1H, br t, $J_{6,7}$ = $J_{7,8}$ = 7.5 Hz, 7-H), 7.86 (1H, br t, $J_{7,8}$ = $J_{8,9}$ = 7.5 Hz, 8-H), 7.99 (1H, br d, $J_{8,9}$ = 7.5 Hz, 9-H), 8.22 (1H, br d, $J_{6,7}$ = 7.5 Hz, 6-H), 9.16 (1H, s, 5-H), 9.43 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{12}H_9N_3OS{\cdot}0.1H_2O$: C, 58.81; H, 3.78; N, 17.15. Found: C, 58.79; H, 4.08; N, 16.76.

4.1.8.2. 7,9-Dimethyl-2-methylthiopyrimido[4,5-*b*]quinolin-4(3*H*)-ne (12b). Yield, (1.71 g, 63%), mp >300 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 230 (4.33), 244sh (4.38), 278sh (4.55), 287 (4.62), 320sh (3.91), 369 (3.80), 393 (3.66); IR (ν_{max}/cm^{-1}): 3160 (NH), 1660 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.47 (3H, s, 7-Me), 2.66 (3H, s, 9-Me), 2.70 (3H, s, 2-SMe), 7.61 (1H, s, 8-H), 7.77 (1H, s, 6-H), 8.98 (1H, s, 5-H), 12.69 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{14}H_{13}N_3OS{\cdot}0.4H_2O$: C, 60.37; H, 4.99; N, 15.09. Found: C, 60.15; H, 5.04; N, 14.88.

4.1.9. General procedure for the preparation of 2-deoxo-2-methylthioalloxazine-5-oxides {2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxides} (13a–e). To a stirring solution of 6-anilino-2-methylthio-4-oxo-3,4-dihydropyrimidines (**11**, 0.01 mol) in acetic acid (5–15 mL) at 10–15 °C was added sodium nitrite (0.02–0.04 mol) by portions, and the mixture was stirred at room temperature with occasional warming in water bath to enhance the cyclization for 1–2 h. The solid deposited was collected by suction filtration and washed with water. The filtrate was concentrated in vacuo and the residue was diluted with excess water or neutralized with aqueous ammonia (pH 7) to afford the second crop. The solid dried was crystallized from a mixture of DMF and water to afford the corresponding products as yellow or orange needles in 66–89% yields.

4.1.9.1. 2-Methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (13a). Yield, (2.32 g, 89%); mp >300 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 244 (4.30), 270 (4.45), 292 (4.42), 355 (3.87), 446 (3.81); IR (ν_{max}/cm^{-1}): 3200 (NH), 1690 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.44 (3H, s, 2-SMe), 7.55 (1H, d t, $J_{6,7}$ = $J_{7,8}$ = 8.7 Hz, $J_{7,9}$ = 1.5 Hz, 7-H), 7.76–7.87 (2H, m, 6 and 8-H), 8.32 (1H, dd, $J_{6,7}$ = 8.7, $J_{6,8}$ = 0.9 Hz, 6-H), 12.69 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{11}H_8N_4O_2S$: C, 50.76; H, 3.10; N, 21.53. Found: C, 50.58; H, 3.39; N, 21.30.

4.1.9.2. 7,9-Dimethyl-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (13b). Yield, (2.36 g, 82%); mp >300 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 247 (4.42), 275 (4.79), 298 (4.71), 361 (3.92), 453 (3.99); IR (ν_{max}/cm^{-1}): 3180 (NH), 1680 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.49 (3H, s, 7-Me), 2.61 (3H, s, 2-SMe), 2.62 (3H, s, 9-Me), 7.61 (1H, br s, 8-H), 7.98 (1H, br s, 6-H), 12.76 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.19; N, 19.43. Found: C, 54.25; H, 4.53; N, 19.37.

4.1.9.3. 7-Methoxy-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (13c). Yield, (2.38 g, 82%); mp >300 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 242 (4.19), 275sh (4.61), 285 (4.72), 352 (3.83), 457 (3.94); IR (ν_{max}/cm^{-1}): 3175 (NH), 1695 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.59 (3H, s, 2-SMe), 3.97 (3H, s, 7-OMe), 7.60 (1H, dd, $J_{8,9}$ = 9.3 Hz, $J_{6,8}$ = 2.7 Hz, 8-H), 7.67 (1H, d,

$J_{6,8}$ = 2.7 Hz, 6-H), 7.93 (1H, d, $J_{8,9}$ = 9.3 Hz, 9-H), 12.78 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{12}H_{10}N_4O_3S$: C, 49.65; H, 3.47; N, 19.30. Found: C, 49.79; H, 3.76; N, 19.33.

4.1.9.4. 7-Methyl-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (13d). Yield, (1.81 g, 66%); mp >300 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 241 (4.10), 274 (4.56), 295 (4.50), 351 (3.85), 443 (3.85); IR (ν_{max}/cm^{-1}): 3175 (NH), 1695 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.55 (3H, s, 7-Me), 2.59 (3H, s, 2-SMe), 7.78 (1H, d, $J_{8,9}$ = 8.4 Hz, 9-H), 7.90 (1H, br d, $J_{8,9}$ = 8.4 Hz, 8-H), 8.16 (1H, br s, 6-H), 12.79 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.54; H, 3.67; N, 20.43. Found: C, 52.58; H, 4.06; N, 20.19.

4.1.9.5. 7-Hydroxy-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (13e). Yield, (1.81 g, 66%); mp >300 °C (decomp., from DMF–H_2O); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 222 (4.35), 280 (4.28), 422 (3.70); IR (ν_{max}/cm^{-1}): 3260 (OH), 3100 (NH), 1700 (C=O); ^{1}H NMR [$(CD_3)_2SO$]: δ 2.61 (3H, s, 2-SMe), 7.39 (1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 8.24 (1H, dd, $J_{8,9}$ = 9.0 Hz, $J_{6,8}$ = 2.7 Hz, 8-H), 8.73 (1H, d, $J_{6,8}$ = 2.7 Hz, 6-H), 11.67 (1H, s, 7-OH), 13.19 (1H, br s, 3-NH exchangeable with D_2O). Anal. Calcd for $C_{11}H_8N_4O_3S{\cdot}0.6H_2O$: C, 46.02; H, 3.23; N, 19.52. Found: C, 45.61; H, 3.41; N, 19.49.

4.1.10. General procedure for the preparation of 2-alkylaminopyrimido[4,5-*b*]quinolin-4-(3*H*)-one (14a,b). A mixture of 2-methylthiopyrimido[4,5-*b*]quinolin-4(3*H*)-ones (**12a**, 5.0 mmol) and appropriate amine (0.035–0.05 mol) in DMF (30 mL) for compound **14a** or in *n*-butanol (20 mL) for compound **14b** was refluxed with stirring for 15–24 h. After the resulting clear yellow solution was kept in refrigerator overnight, the yellow crystals precipitated were collected by filtration to get the first crop. The filtrate was concentrated in vacuo to remove the excess amine and the residue was treated with water to get the second crop free from amine. The collected solids were dried and recrystallized from ethanol to give the pure products as yellow needles in 82–84% yields.

4.1.10.1. 2-Morpholinopyrimido[4,5-*b*]quinolin-4(3*H*)-one (14a). Yield, (1.19 g, 84%); mp 288–291 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 225 (4.53), 263 (4.45), 280 (4.57), 321sh (3.88), 408 (3.97), 434 (3.39); IR (ν_{max}/cm^{-1}): 3260 (NH), 1660 (C=O);^{1}H NMR ($CDCl_3$): δ 3.82–3.90 (4H, m, 2′ and 6′-CH_2), 3.90–4.01 (4H, m, 3′ and 5′-CH_2), 7.47 (1H, t, $J_{6,7}$ = $J_{7,8}$ = 7.2 Hz, 7-H), 7.79 (1H, t, $J_{7,8}$ = $J_{8,9}$ = 7.2 Hz, 8-H), 7.92 (1H, d, $J_{8,9}$ = 7.2 Hz, 9-H), 7.98–8.12 (1H, m, 6-H), 8.96 (1H, s, 5-H), 10.65 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{15}H_{14}N_4O_2{\cdot}0.2H_2O$: C, 63.02; H, 5.08; N, 19.60. Found: C, 62.76; H, 5.19; N, 19.36.

4.1.10.2. 2-[*N*-(2-Hydroxyethyl)-*N*-methylamino]-7,9-dimethylpyrimido[4,5-*b*]quinolin-4(3*H*)-one (14b). Yield, (1.22 g, 82%); mp 273–275 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 246 (4.42), 266 (4.64), 287 (4.36), 322 (3.96), 366 (3.65); IR (ν_{max}/cm^{-1}): 3260 (NH), 1660 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.48 (3H, s, 7-Me), 2.74 (3H, s, 9-Me), 3.35 (3H, s, 2-NMe), 3.73 (2H, br s, 2-N–CH_2–CH_2–OH), 3.88 (2H, br s, 2-N–CH_2–CH_2–OH), 5.50 (1H, br s, 2-N–CH_2–CH_2–O*H*, exchangeable with D_2O), 7.50 (1H, s, 8-H), 7.52 (1H, s, 6-H), 8.82 (1H, s, 5-H), 11.09 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{16}H_{18}N_4O_2{\cdot}0.2$EtOH: C, 64.05; H, 6.29; N, 18.22. Found: C, 63.74; H, 6.29; N, 17.83.

4.1.10.3. Preparation of 7-methoxy-2-morpholino-4-oxo-3,4-dihydrobenzo[*g*]pteridine (15a). A mixture of 7-methoxy-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (**13c**, 2.90 g, 0.01 mol) and morpholine (8.71 g, 0.10 mol) in DMF (30 mL) was refluxed with stirring for 6 h. After the resulting solution was kept in refrigerator overnight, the yellow crystals precipitated were collected by filtration to get the product which was washed with ethyl acetate, dried, and recrystallized from DMF to give the yellow pure product **15a**.

Yield, (2.10 g, 67%); mp >300 °C (decomp., from DMF); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 224 (4.14), 275 (4.47), 332 (3.51), 446 (3.85); IR (ν_{max}/cm^{-1}): 3135 (NH), 1698 (C=O); ^{1}H NMR ($CDCl_3$): δ 3.92 (4H, br m, 2′ and 6′-CH_2), 3.93 (4H, br m, 3′ and 5′-CH_2), 3.98 (3H, s, 7-OMe), 7.45–7.57 (2H, m, 6 and 8-H), 7.96 (1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 10.62 (1H, br s, 3-NH, exchangeable with D_2O). Anal. Calcd for $C_{15}H_{15}N_5O_3$: C, 57.50; H, 4.83; N, 22.35. Found: C, 57.15; H, 5.02; N, 22.44.

4.1.10.4. Preparation of 2-dimethylamino-4-oxo-3,4-dihydrobenzo[*g*]pteridine (15b). A mixture of 2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (**13a**, 2.6 g, 0.01 mol) and 50% aqueous dimethylamine (50 mL) was heated in steel sealed tube at 135 °C (10 kg/cm^2 pressure) for 8 h. After the reaction was complete, the resulting clear yellow solution was concentrated in vacuo and the yellow solid residue was washed with water, dried, and crystallized from ethanol to afford the pure product as yellow crystals.

Yield, (2.08 g, 86%); mp >300 °C (decomp., from H_2O); UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 221 (4.38), 269 (4.31), 287 (4.29), 334 (3.62), 376sh (3.58), 399 (3.68), 430 (3.78); IR (ν_{max}/cm^{-1}): 1700 (C=O); ^{1}H NMR ($CDCl_3$): δ 3.39 (6H, s, 2-N (CH_3)$_2$), 7.64 (1H, dt, $J_{6,7}$ = $J_{7,8}$ = 8.4 Hz, $J_{7,9}$ = 1.5 Hz, 7-H), 7.79 (1H, dt, $J_{7,8}$ = $J_{8,9}$ = 8.4 Hz, $J_{6,8}$ = 1.5 Hz, 8-H), 8.02 (1H, d, $J_{8,9}$ = 8.4 Hz, 9-H), 8.21 (1H, d, $J_{6,7}$ = 8.4 Hz, 6-H). Anal. Calcd for $C_{12}H_{11}N_5O{\cdot}0.1H_2O$: C, 59.30; H, 4.64; N, 28.81. Found: C, 59.23; H, 4.72; N, 28.41.

4.1.11. General procedure for the preparation of 3-alkyl-2-deoxo-2-methylthioalloxazine-5-oxides {3-alkyl-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxides} (16a–e). A mixture of 2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxides (**13**, 5.0 mmol), alkyl iodide or alkyl bromide (0.015–0.025 mol), and anhy-

drous potassium carbonate (2.76 g, 0.02 mol) in DMF (30 mL) was stirred at room temperature for 3–10 h. After the reaction was complete, the insoluble potassium carbonate was filtered off and washed with CH_2Cl_2. The combined solution of the filtrate and washing solution of CH_2Cl_2 was concentrated in vacuo. The residue was dissolved in water and extracted with CH_2Cl_2 (3× 50 mL), dried over anhydrous magnesium sulfate and filtered. Then, the solvent was removed in vacuo to afford the solid, which was collected by filtration, dried, and crystallized from ethanol to afford the corresponding products as yellow crystals in 71–82% yields.

4.1.11.1. 3-Methyl-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (16a). Yield, (1.13 g, 82%); mp 231–233 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 226 (3.72), 272 (4.32), 299 (4.40), 337sh (3.77), 422 (3.64), 445sh (3.48); IR (ν_{max}/cm^{-1}): 1700 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.79 (3H, s, 2-SMe), 3.64 (3H, s, 3-NMe), 7.66 (1H, d t, $J_{6,7}$ = 9.0 Hz, $J_{7,8}$ = 8.7 Hz, $J_{7,9}$ = 1.5 Hz, 7-H), 7.85 (1H, d t, $J_{7,8}$ = $J_{8,9}$ = 8.7 Hz, $J_{6,8}$ = 1.5 Hz, 8-H), 8.13 (1H, br d, $J_{8,9}$ = 8.7, 9-H), 8.56 (1H, br d, $J_{6,7}$ = 9.0, 6-H). Anal. Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.54; H, 3.67; N, 20.43. Found: C, 52.67; H, 3.94; N, 20.74.

4.1.11.2. 3,7,9-Trimethyl-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (16b). Yield, (1.21 g, 80%); mp 264–266 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 225 (3.79), 280 (4.52), 304 (4.55), 344sh (3.72), 431 (3.72), 458sh (3.50); IR (ν_{max}/cm^{-1}): 1698 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.54 (3H, s, 7-Me), 2.79 (3H, s, 2-SMe), 2.81 (3H, s, 9-Me), 3.63 (3H, s, 3-NMe), 7.53 (1H, br s, 8-H), 8.20 (1H, br s, 6-H). Anal. Calcd for $C_{14}H_{14}N_4O_2S$: C, 55.61; H, 4.67; N, 18.53. Found: C, 55.20; H, 4.92; N, 18.20.

4.1.11.3. 3-Ethyl-7,9-dimethyl-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (16c). Yield, (1.12 g, 71%); mp 210–212 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 227 (3.63), 280 (4.49), 304 (4.56), 343sh (3.77), 432 (3.77), 457sh (3.56); IR (ν_{max}/cm^{-1}): 1698 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.41 (3H, t, J = 7.2 Hz, 3-NCH$_2$–CH_3), 2.54 (3H, s, 7-Me), 2.79 (3H, s, 2-SMe), 2.80 (3H, s, 9-Me), 4.22 (2H, q, J = 7.2 Hz, 3-NCH_2–CH$_3$), 7.53 (1H, br s, 8-H), 8.20 (1H, br s, 6-H). Anal. Calcd for $C_{15}H_{16}N_4O_2S$: C, 56.94; H, 5.10; N, 17.71. Found: C, 56.75; H, 5.25; N, 17.38.

4.1.11.4. 3-(Ethoxycarbonylmethyl)-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridin-5-oxide (16d). Yield, (1.28 g, 74%); mp 212–214 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 219 (4.31), 272 (4.51), 299 (4.63), 336sh (3.95), 415 (3.79), 443sh (3.60); IR (ν_{max}/cm^{-1}): 1740, 1705 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.31 (3H, t, J = 7.2 Hz, 3-CH$_2$COOCH$_2$CH_3), 2.81 (3H, s, 2-SMe), 4.28 (2H, q, J = 7.2 Hz, 3-NCH$_2$COOCH_2CH$_3$), 4.91 (2H, s, 3-NCH_2COOCH$_2$CH$_3$), 7.68 (1H, d t, $J_{6,7}$ = $J_{7,8}$ = 8.7 Hz, $J_{7,9}$ = 1.8 Hz, 7-H), 7.88 (1H, d t, $J_{7,8}$ = $J_{8,9}$ = 8.7 Hz, $J_{6,8}$ = 1.8 Hz, 8-H), 8.14 (1H, dd, $J_{8,9}$ = 8.7 Hz, $J_{7,9}$ = 1.8 Hz, 9-H), 8.55 (1H, dd, $J_{6,7}$ = 8.7 Hz, $J_{6,8}$ = 1.8 Hz, 6-H). Anal. Calcd for $C_{15}H_{14}N_4O_4S$: C, 52.02; H, 4.07; N, 16.18. Found: C, 52.24; H, 4.38; N, 15.85.

4.1.11.5. 3,7-Dimethyl-2-methylthio-4-oxo-3,4-dihydrobenzo[*g*]pteridine-5-oxide (16e). Yield, (1.14 g, 79%); mp 211–213 °C (decomp., from EtOH); UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 229 (3.42), 278 (4.10), 299 (4.07), 338sh (3.48), 427 (3.37), 449sh (3.21); IR (ν_{max}/cm^{-1}): 1700 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.60 (3H, s, 7-Me), 2.78 (3H, s, 2-SMe), 3.64 (3H, s, 3-NMe), 7.68 (1H, dd, $J_{8,9}$ = 8.7 Hz, $J_{6,8}$ = 2.1 Hz, 8-H), 8.02 (1H, d, $J_{8,9}$ = 8.7 Hz, 9-H), 8.34 (1H, br s, 6-H). Anal. Calcd for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.19; N, 19.43. Found: C, 54.48; H, 4.34; N, 19.17.

4.2. 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[25] to determine the inhibitory effects of test compounds, namely, 10-alkyl-2-deoxo-2-methylthioflavin-5-oxides (**2a**–**i**), 10-alkyl-2-alkylamino-2-deoxoflavin-5-oxides (**4a**–**e**), 10-alkylflavins (**5a** and **b**), 7-methoxy-10-methylflavin-5-oxide (**6**), 2-alkylamino-2-deoxo-10-methyl-5-deazaflavins (**8a** and **b**), 2-deoxo-10-methyl-2-methylaminoflavin-5-oxide (**9a**), 2-deoxo-2-methylthio-5-deazaalloxazine (**12a**), 2-deoxo-2-methylthioalloxazine-5-oxides (**13a**–**d**), 2-alkylamino-2-deoxoalloxazines (**15a** and **b**), and 3-alkyl-2-deoxo-2-methylthioalloxazine-5-oxides (**16a**–**e**), on cell growth in vitro as mentioned in detail in our previous paper.[6] 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.

4.3. Molecular docking study

The automated docking studies were carried out using AutoDock version 3.0.5.[26] 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 80 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 (AutoDock Tool Kit) on PC which is associated with AutoDock 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.

4.3.1. Preparation of ligands and target protein–tyrosine kinase. The compounds involved in this study as ligands include 10-alkyl-2-deoxo-2-methylthioflavin-5-oxides (**2**), 2-dimethylaminoalloxazine (**3**), 10-alkyl-2-alkylamino-2-deoxoflavin-5-oxides (**4**), 10-alkylflavins (**5**), 7-methoxy-10-methylflavin-5-oxide (**6**), 2-alkylamino-2-deoxo-10-methyl-5-deazaflavins (**8**), 2-alkylamino-2-deoxo-10-methylflavin-5-oxides (**9**), 2-methylthio-2-deoxo-5-deazaalloxazines (**12**), 2-deoxo-2-methylthioalloxazine-5-oxides (**13**), 2-alkylamino-2-deoxo-5-deazaalloxazines (**14**), 2-alkylamino-2-deoxoalloxazines (**15**), and **3**-alkyl-2-deoxo-2-methylthioalloxazine-5-oxides (**16**) which were studied for their binding activities into PTK. 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 c-Kit receptor protein-tyrosine kinase in complex with STI-571 (Imatinib or Gleevec) were retrieved from the RCSB Protein Data Bank http://www.rcsb.org/pdb/Welcome.do (PDB code: 1t46)[27] accessed on April 4, 2006. All bound waters, ligand were removed 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/thornton-srv/databases/pdbsum/ accessed on June 10, 2006.

4.3.2. 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 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 (STI-571) within the crystal structure of c-Kit receptor protein–tyrosine kinase 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 more stronger.[29] Therefore, in our modeling results we consider the hydrogen bond angle within about 120° to be of a reasonable strength.

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