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Antitumor studies. Part 1: Design, synthesis, antitumor activity, and AutoDock study of 2-deoxo-2-phenyl-5-deazaflavins and 2-deoxo-2-phenylflavin-5-oxides as a new class of antitumor agents

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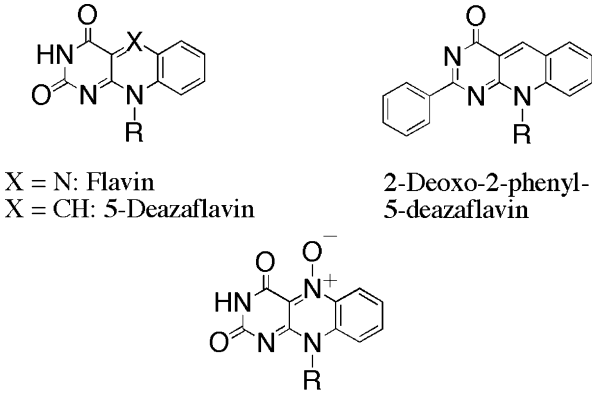
Abstract—Novel 2-deoxo-2-phenyl-5-deazaflavins and 2-deoxo-2-phenylflavin-5-oxides were prepared as a new class of antitumor agents and showed significant antitumor activities against NCI-H 460, HCT 116, A 431, CCRF-HSB-2, andKB cell lines. In vivo investigation, 2-deoxo-10-methyl-2-phenyl-5-deazaflavin exhibited the effective antitumor activity against A 431 human adenocarcinoma cells transplanted subcutaneously into nude mouse. Furthermore, AutoDock study has been done by binding of the flavin analogs into PTK pp60$^{c\text{-}src}$, where a good correlation between their IC_{50} and AutoDock binding free energy was exhibited. In particular, 2-deoxo-2-phenylflavin-5-oxides exhibited the highest potential binding affinity within the binding pocket of PTK.

1. Introduction

During the last three decades there has been considerable interest in synthesis, functional elucidation, and biological evaluation of 5-deazaflavins {5-deazaisoalloxazines, pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-diones}. Actually, 5-deazaflavins have attracted great interest because of the first synthesis as potential flavin antagonists of flavin models[1] and of the discovery that they serve as co-factors for several flavin catalyzed reactions[2] (Scheme 1). They also have the potent broad-spectrum activity against coccidiosis.[3] Recently, we have preliminarily reported the selective protein kinase C (PKC) inhibitory activities of 5-deazaflavins and 2-deoxo-2-phenyl-5-deazaflavins {2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-ones}, and their effective growth inhibition against cancer cell lines such as A 431 cells and HT 1080 cells.[4] Antitumor activities of nitro-5-deazaflavin-



Scheme 1.

Keywords: Antitumor activity; Flavin analog; AutoDock; Protein tyrosine kinase.
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pyrrolecarboxamide hybrid molecules[5] and 5-amino-5-deazaflavin derivatives revealed potential inhibitory activity against L 1210 or KB cells.[6] In addition, cytotoxicities of the nitro-5-deazaflavins were evaluated in vitro for hypoxic and oxic Chinese hamster cells (V79). The 6- and 8-nitro derivatives were generally more toxic against hypoxic cells than oxic cells and showing marked hypoxic selectivity.[7]

Computer-aided drug design tools can generate many useful and powerful models that explain structure–activity relationship (SAR) observations in a quantitative manner. Using a model with a given structure, medicinal chemists can compute the activity of a molecule.[8] Very recently, the impressive technological advances in areas such as structural characterization of biomacromolecules, computer sciences, and molecular biology have made rational drug design feasible.[9] The explosion of genomic, proteomic, and structural information has provided hundreds of new targets and opportunities for future discovery process of lead drug.[10] Many new protein structures of pharmaceutical interest have been elucidated, and a substantial number of those proteins contain an embedded inhibitor. Meanwhile, several drugs that were designed by intensive use of computational methods are currently under investigation through clinical trials.[11] AutoDock is one of the most widely used docking programs in computational binding studies. It is said to offer a reasonable result in comparison with other methods that specialize either in fast database searches or in computationally intensive calculations.[12]

The main goal of this study is the development of potential inhibitors synthesized newly, for example, 5-deazaflavins, flavins, flavin-5-oxides, and their analogs as antitumor agents, based on molecular modeling with the investigation of SAR between new inhibitors and protein tyrosine kinase (PTK). Thereupon, 2-deoxo-2-phenylflavin-5-oxide derivatives were designed and synthesized for the aim to get stronger binding affinity and more hydrogen bonds to the binding pocket of the PTK due to the polar part of the *N*-oxide moiety. In the present paper, we describe the preparation of 2-deoxo-2-phenyl-5-deazaflavins and 2-deoxo-2-phenylflavin-5-oxides and their antitumor activities, and also the AutoDock study for new inhibitors docked into the binding pocket of PTK. In this study, compounds having potential antitumor and PTK inhibitory activities, for example, IC_{50} values less than 25 μM, were docked into the binding pocket of PTK using AutoDock 3.05 program.

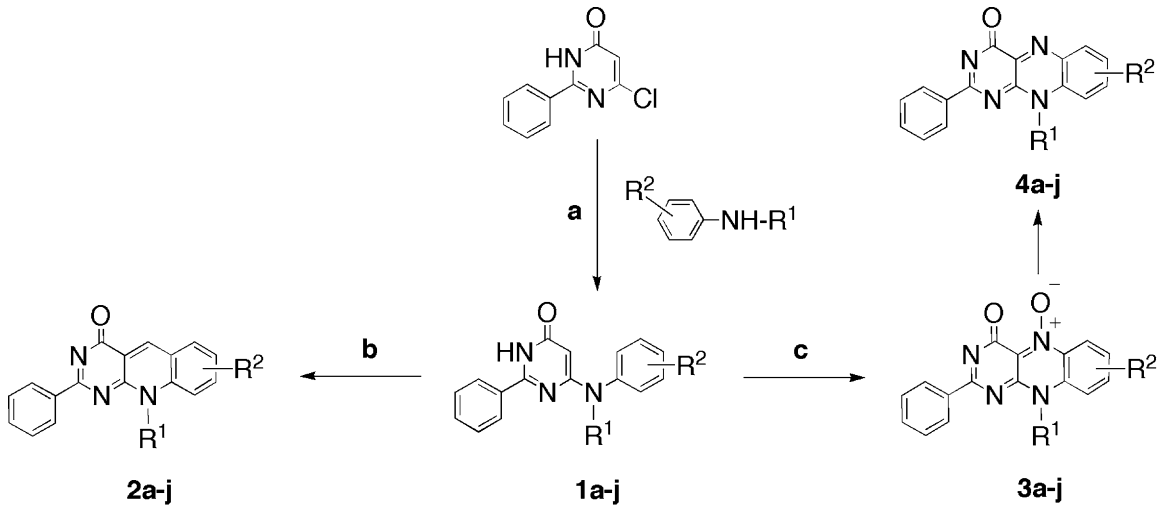
2. Results and discussion

2.1. Chemistry

The required 6-*N*-monoalkylanilino-2-phenylpyrimidin-4(3*H*)-ones (**1a**–**j**), which were used as precursors for synthesis of 2-deoxo-2-phenyl-5-deazaflavins (**2a**–**g**) and 2-deoxo-2-phenylflavin-5-oxides (**3a**–**i**), were synthesized by fusion of 6-chloro-2-phenylpyrimidin-4(3*H*)-one with appropriate *N*-alkyl anilines at 180–200 °C under nitrogen atmosphere for 0.5–5 h according to the known procedure[13] as shown in Scheme 2. The commercially unavailable N-monomethylated anilines were synthesized from appropriate aniline derivatives in 2 steps, by reaction of anilines with ethyl formate under reflux for 15 h to get the *N*-arylformamides followed by reduction using $LiAlH_4$ in THF to afford the corresponding N-monomethylated anilines.[14]

The intended 10-alkylated 2-deoxo-2-phenyl-5-deazaflavins (**2a**–**g**) were prepared by reaction of **1a**, **b**, **d**–**h** with Vilsmeier reagent (*N*,*N*-dimethylformamide–phosphoryl chloride) at 90 °C for 1–4 h, and the 10-unsubstituted derivatives (**2h**–**j**) were prepared from 6-anilino-2-phenylpyrimidin-4(3*H*)-ones[15] in the same conditions of Vilsmeier reaction. This synthetic method is a useful preparation for 5-deazaflavins from 6-(*N*-monoalkylanilino) uracils.[16] The 10-methyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (**2a**) was also prepared by methylation of 2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (**2h**) with excess methyl iodide in the presence of anhydrous potassium carbonate in DMF at room temperature in 80% yield. On the other hand, 2-deoxo-2-phenylflavin-5-oxides (**3a**–**i**) were prepared by nitrosative cyclization of 6-*N*-monoalkylanilino-2-phenylpyrimidin-4(3*H*)-ones (**1a**–**h**, **j**) with excess $NaNO_2$ in AcOH at 10–15 °C for 2–5 h. However, such cyclization of **1i** was difficult due to the peri steric hindrance between the ethyl at the *N*-ethyl and the ortho methyl of aniline moiety.

The structures of the products **1a**–**j** were confirmed in particular by the presence of an equivalent proton resonance at the 5-position as a singlet signal at δ_H 4.8–5.3 in ^1H NMR spectra and by the presence of the C_5 carbon signal at δ_C 86–87 in ^{13}C NMR spectra (Table 1). The cyclized 2-deoxo-2-phenyl-5-deazaflavins (**2a**–**j**) showed a characteristic singlet signal in the lower field at δ_H 9.2–9.4 due to the proton at the 5-position of **2a**–**j** and a signal at δ_C 137–141 due to the carbon at the 5-position. It is implying that the 5-position of **2a**–**j** is the most electron-deficient methine and very reactive to nucleophiles. Whereas, the 2-deoxo-2-phenylflavin-5-oxides (**3a**–**i**) showed no such signals at the 5-position. On the other hand, the UV absorption spectra of the anilinopyrimidones (**1a**–**j**) showed one absorption maximum at 253–257 nm together with an absorption maximum as shoulder at 272–274 nm. Whereas, the 10-alkylated deazaflavins (**2a**–**g**) showed four absorption maxima at 283–297, 336–355, 414–431, and 433–456 nm together with an absorption maximum as shoulder at 262–288 nm, and the 10-unsubstituted deazaflavins (**2h**–**i**) also showed three absorption maxima at 279–290, 364–370, and 374–385 nm, which were observed with hypsochromic shift in the region of the longest wavelength in comparison with those of **2a**–**g** together with an absorption maximum as shoulder at 254–256 nm. In contrast with the above UV absorption spectra, the 2-deoxo-2-phenylflavin-5-oxides (**3a**–**i**) exhibited the absorptions in the region of longer wavelength, that is, four absorption maxima at 303–312, 362–373, 470–496, and 500–528 nm together with an absorption maximum as shoulder at 292–302 nm. All compounds of



2a: 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 = Et, R^2 = 7-Me
h: R^1 = R^2 = H
i: R^1 = H, R^2 = 9-Me
j: R^1 = H, R^2 = 8-OMe

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

3, 4a: R^1 = Me, R^2 = H
b: R^1 = Et, R^2 = H
c: R^1 = Pr^i R^2 = H
d: R^1 = Me, R^2 = 7-Me
e: R^1 = Me, R^2 = 9-Me
f: R^1 = Me, R^2 = 7-OMe
g: R^1 = Me, R^2 = 8-OMe
h: R^1 = Et, R^2 = 7-Me
i: R^1 = Me, R^2 = 6,7,8-Tri-OMe
j: R^1 = R^2 = H

Scheme 2. General method for the preparation of 6-(*N*-alkylanilino)-2-phenylpyrimidin-4(3*H*)-ones(**1a**–**j**), 2-deoxo-2-phenyl-5-deazaflavins (**2a**–**j**), and 2-deoxo-2-phenylflavin-5-oxides (**3a**–**i**). Reagents and conditions: (a) fusion at 180–200 °C, 0.5–5 h, N_2; (b) Vilsmmeier reagent (DMF–$POCl_3$), 90 °C, 1–4 h; (c) $NaNO_2$, AcOH, 10–15 °C, 2–5 h. The compounds **3j** and **4a**–**j** were computationally designed compounds.

3a–**i** showed red color owing to the presence of absorption maximum at ca. 515 nm in the longest wavelength.

2.2. In vitro antitumor activities of deazaflavin and flavin analogs against human tumor cell lines

The synthesized compounds **2a**–**j** and **3a**–**i** were tested in vitro for growth inhibitory activities against various cultured tumor cell lines. Five human tumor cell lines including human lung cancer cell line (NCI-H 460), human colon carcinoma cell line (HCT 116), human adenocarcinoma cell line (A 431), human T-cell acute lymphoblastoid leukemia cell line (CCRF-HSB-2), and human oral epidermoid carcinoma cell line (KB) were used, and the antitumor agents of Ara-C, cisplatin, and adriamycin were also used as positive controls in this study. As can be seen from Table 2, many compounds of flavin analogs such as 2-deoxo-2-phenyl-5-deazaflavins (**2**) and 2-deoxo-2-phenylflavin-5-oxides (**3**) have been found to show fairly good antitumor activities. Although the tested flavin analogs showed slightly poorer antitumor activities than cisplatin against NCI-H 460 cell line, their activities for the compounds **2a**, **2c**, **3a**, and **3f** against HCT 116 cell line were higher than cisplatin. Especially, compound **3f** (IC_{50}: 0.72 μM) exhibited 3-fold more potent antitumor activity than cisplatin (IC_{50}: 2.23 μM). Further, against A 431 cell line they showed less antitumor activities in comparison with adriamycin. Though it is also inferior to the antitumor activity of Ara-C (IC_{50}: 0.15 μM), many compounds showed 0.5–2.0 μM potential growth inhibitory activities against CCRF-HSB-2 cell line. In case against KB cell line, many compounds showed 0.5–5.0 μM potential growth inhibitory activities and the compounds **2e** and **2g** (IC_{50}: 0.5 μM) exhibited more potent antitumor activities than that of Ara-C (IC_{50}: 0.70 μM).

2.3. In vivo antitumor activity against subcutaneously transplanted human adenocarcinoma A 431 xenograft

In vivo research has an advantage to discover drugs as the nature and properties of a chemical tool cannot be considered independently. Therefore, the antitumor activities of compounds **2a** and **2b** against subcutaneously transplanted human adenocarcinoma A 431 xenograft in mice were measured, and the tumor volume and body weights of mice were evaluated. Thus the human adenocarcinoma A 431 was subcutaneously transplanted into BALB/c nude mice, and the administration of test compounds was started on the day (day 1) when the mice show tumor growth in the 100–300 mm^3 range. Since the test compounds prepared here showed poor solubility in water, the administrations were done by intratumoral (it), intraperitoneal (ip) or oral (po) meth-

Table 1. UV and and ^{13}C NMR spectroscopic data for the compounds (**1a–j**, **2a–j**, and **3a–i**)

Compound	λ_{max}/nm (logε/dm^{3}mol^{-1} cm^{-1})	δ_C (75 MHz)[a]
1a	256 (4.62), *273* (4.53)	38.84 (6-NCH$_3$), 86.66 (C$_5$), 127.19 (C$''_4$), 127.43 (C$''_2$ and C$''_6$), 128.09 (C$''_3$ and C$''_5$), 129.14 (C$'_3$ and C$'_5$), 130.04 (C$'_2$ and C$'_6$), 132.06 (C$'_4$), 132.83 (C$'_1$), 145.05 (C$''_1$), 156.20 (C$_6$), 163.50 (C$_2$), 165.99 (C=O)
1b	256 (4.68), *274* (4.54)	14.09 (6-NCH$_2$–*C*H$_3$), 45.79 (6-N*C*H$_2$–CH$_3$) 86.52 (C$_5$), 127.61 (C$''_4$), 128.03 (C$''_2$ and C$''_6$), 128.68 (C$''_3$ and C$''_5$), 129.14 (C$'_3$ and C$'_5$), 130.21 (C$'_2$ and C$'_6$), 131.99 (C$'_4$), 132.96 (C$'_1$), 143.47 (C$''_1$), 156.17 (C$_6$), 163.24 (C$_2$), 165.85 (C=O)
1c	253 (4.73), *274* (4.42)	21.93 (6-NCH(*C*H$_3$)$_2$), 47.64 (6-N*C*H(CH$_3$)$_2$), 87.02 (C$_5$), 127.99 (C$''_4$), 128.32 (C$''_2$ and C$''_6$), 129.16 (C$''_3$ and C$''_5$), 130.0 (C$'_3$ and C$'_5$), 131.12 (C$'_2$ and C$'_6$), 131.95 (C$'_4$), 133.06 (C$'_1$), 139.46 (C$''_1$), 155.91 (C$_6$), 163.75 (C$_2$), 165.65 (C=O)
1d	257 (4.68), *274* (4.54)	21.45 (4″-CH$_3$), 38.87 (6-NCH$_3$), 86.58 (C$_5$), 127.19 (C$''_2$ and C$''_6$), 128.09 (C$''_3$ and C$''_5$), 129.13 (C$''_4$), 130.66 (C$'_3$ and C$'_5$), 132.00 (C$'_2$ and C$'_6$), 132.91 (C$'_4$), 137.04 (C$'_1$), 142.44 (C$''_1$), 156.15 (C$_6$), 163.38 (C$_2$), 165.88 (C=O)
1e	254 (4.73), *273* (4.45)	17.85 (2″-CH$_3$), 37.59 (6-NCH$_3$), 85.81 (C$_5$), 127.36 (C$''_2$), 128.09 (C$'_3$ and C$'_5$), 128.39 (C$''_4$), 129.16 (C$'_4$, C$''_3$ and C$''_6$) , 129.16 (C$''_5$), 132.05 (C$'_2$), 132.92 (C$'_6$), 136.50 (C$'_1$), 142.44 (C$''_1$), 156.18 (C$_6$), 163.25 (C$_2$), 165.93 (C=O)
1f	257 (4.67), *274* (4.49)	39.00 (6-NCH$_3$), 55.90 (4″-OCH$_3$), 86.36 (C$_5$), 115.30 (C$''_2$ and C$''_6$) , 128.05 (C$''_3$ and C$''_5$) , 128.65 (C$'_3$ and C$'_5$), 129.15 (C$'_2$ and C$'_6$), 132.02 (C$'_4$), 132.89 (C$'_1$), 137.87 (C$''_1$), 156.12 (C$''_4$), 158.63 (C$_6$), 163.88 (C$_2$), 165.82 (C=O)
1g	257 (4.52), *272* (4.42)	38.79 (6-NCH$_3$), 55.76 (4″-OCH$_3$), 86.84 (C$_5$), 112.98 (C$''_2$), 113.10 (C$''_4$), 119.62 (C$''_6$), 128.04 (C$'_3$ and C$'_5$), 129.17 (C$'_2$ and C$'_6$), 130.73 (C$''_5$), 132.07 (C$'_4$), 132.82 (C$'_1$), 146.14 (C$''_1$), 156.16 (C$_6$), 161.0 (C$''_3$), 163.46 (C$_2$), 165.83 (C=O)
1h	257 (4.66), *274* (4.49)	14.10 (6-NCH$_2$–*C*H$_3$), 21.48 (4″-CH$_3$), 45.77 (6-N*C*H$_2$–CH$_3$), 86.36 (C$_5$), 127.99 (C$''_2$ and C$''_6$), 128.43 (C$''_3$ and C$''_5$), 129.14 (C$''_4$), 130.83 (C$'_3$ and C$'_5$), 131.95 (C$'_2$ and C$'_6$), 133.01 (C$'_4$), 137.47 (C$'_1$), 140.77 (C$''_1$), 156.07 (C$_6$), 163.38 (C$_2$), 165.70 (C=O)
1i	254 (4.73), *274* (4.43)	14.09 (6-NCH$_2$–*C*H$_3$), 18.0 (2′-CH$_3$), 45.08 (6-N*C*H$_2$–CH$_3$), 85.99 (C$_5$), 127.82 (C$''_2$), 128.02 (C$'_3$ and C$'_5$), 128.31 (C$''_4$), 129.18 (C$''_3$ and C$''_6$), 129.49 (C$''_5$), 131.97 (C$'_2$ and C$'_6$), 133.12 (C$'_4$), 137.01 (C$'_1$), 141.81 (C$''_1$), 156.19 (C$_6$), 163.01 (C$_2$), 165.77 (C=O)
1j	257 (4.65), *274* (4.51)	38.92 (6-NCH$_3$), 56.55 (3″- and 5″-OCH$_3$), 61.35 (4″-OCH$_3$), 86.72 (C$_5$), 104.81 (C$''_2$ and C$''_6$), 128.06 (C$'_2$ and (C$'_6$), 129.20 (C$'_3$ and C$'_5$), 132.17 (C$'_4$), 132.81 (C$'_1$), 137.40 (C$''_4$), 140.72 (C$''_1$), 154.37 (C$''_3$ and C$''_5$), 156.28 (C$_6$), 163.73 (C$_2$), 165.89 (C=O)
2a	*265* (4.42), 284 (4.51), 340 (3.95), 425 (3.88), 453 (3.88)	33.28 (N$_{10}$–CH$_3$), 116.63 (C$_7$), 118.28 (C$_9$), 123.99 (C$_6$), 125.71(C$_8$), 128.42 (C$'_3$ and C$'_5$), 130.08 (C$'_2$ and C$'_6$), 132.32 (C$'_4$), 132.44 (C$_{5a}$), 136.12 (C$'_1$), 137.86 (C$_5$), 141.05 (C$_{4a}$), 145.30 (C$_{9a}$), 157.21 (C$_{10a}$), 171.41 (C$_2$), 172.01 (C=O)
2b	*266* (4.44), 286 (4.55), 338 (4.11), 425 (3.98), 453 (3.99)	13.48 (N$_{10}$–CH$_2$–*C*H$_3$), 41.61 (N$_{10}$–*C*H$_2$–CH$_3$), 116.37 (C$_7$), 118.32 (C$_9$), 124.45 (C$_6$), 125.55 (C$_8$), 128.49 (C$'_3$ and C$'_5$) , 130.24 (C$'_2$ and C$'_6$) , 132.48 (C$'_4$), 132.62 (C$_{5a}$), 136.08 (C$'_1$), 138.22 (C$_5$), 140.11 (C$_{4a}$), 145.29 (C$_{9a}$), 156.93 (C$_{10a}$), 171.76 (C$_2$), 172.13 (C=O)
2c	*288* (4.60), 297 (4.65), 340 (3.28), 431 (4.22), 456 (4.26)	21.19 (7-CH$_3$), 33.26 (N$_{10}$–CH$_3$), 116.45 (C$_7$), 118.19 (C$_9$), 124.15 (C$_6$), 128.43 (C$'_3$ and C$'_5$), 130.10 (C$'_2$ and C$'_6$), 131.22 (C$_8$), 132.30 (C$'_4$), 136.07 (C$_{5a}$), 138.16 (C$'_1$), 138.16 (C$_5$), 139.38 (C$_{4a}$), 144.78 (C$_{9a}$), 151.52 (C$_{10a}$), 171.20 (C$_2$), 172.08 (C=O)
2d	*288* (4.45), 297 (4.46), 344 (4.09), 431 (3.94), 455 (3.93)	25.18 (9-CH$_3$), 40.12 (N$_{10}$–CH$_3$), 117.64 (C$_9$), 125.71 (C$_7$), 125.82 (C$_6$), 127.55 (C$_8$), 128.41 (C$'_3$ and C$'_5$), 130.09 (C$'_2$ and C$'_6$), 130.89 (C$'_4$), 132.41 (C$_{5a}$), 138.04 (C$'_1$), 139.32 (C$_5$), 140.52 (C$_{4a}$), 146.10 (C$_{9a}$), 159.26 (C$_{10a}$), 171.24 (C$_2$), 172.11 (C=O)
2e	*268* (4.46), 292 (4.54), 342 (4.10), 428 (4.02), 454 (4.02)	30.05 (N$_{10}$–CH$_3$), 56.31 (7-OCH$_3$), 99.74 (C$_9$), 116.28 (C$_6$), 117.99 (C$_8$), 120.59 (C$_{5a}$), 128.45 (C$'_3$ and C$'_5$), 130.23 (C$'_2$ and C$'_6$), 132.25 (C$'_4$), 134.58 (C$_7$), 136.51 (C$'_1$), 140.99 (C$_5$), 142.38 (C$_{4a}$), 144.03 (C$_{9a}$), 154.42 (C$_{10a}$), 171.96 (C$_2$), 172.97 (C=O)
2f	*262* (4.42), 283 (4.60), 355 (4.02), 414 (4.24), 433 (4.37)	33.96 (N$_{10}$–CH$_3$), 57.49 (8-OCH$_3$), 99.46 (C$_7$), 115.51 (C$_9$), 117.78 (C$_6$), 119.86 (C$_{5a}$), 129.14 (C$'_3$ and C$'_5$), 129.86 (C$'_2$ and C$'_6$), 132.49 (C$'_4$), 134.65 (C$'_1$), 139.11 (C$_5$), 144.16 (C$_{4a}$), 144.78 (C$_{9a}$), 157.74 (C$_{10a}$), 166.63 (C$_8$), 171.33 (C$_2$), 172.12 (C=O)

(*continued on next page*)

Table 1 (*continued*)

Compound	λ_{max}/nm (log ε/dm^{3}mol^{-1} cm^{-1})	δ_C (75 MHz)[a]
2g	*287* (4.72), 297 (4.74), 336 (4.37), 430 (4.35), 455 (4.37)	13.50 (N_{10}–CH_2–*C*H_3), 21.14 (7-CH_3), 41.55 (N_{10}–*C*H_2–CH_3), 116.20 (C_7), 118.35 (C_9), 124.64 (C_6), 128.44 (C'_3 and C'_5), 130.22 (C'_2 and C'_6), 131.47(C_8), 132.28 (C'_4), 135.82 (C_{5a}), 138.03 C'_1), 138.43 (C_5), 138.46 (C_{4a}), 144.68 (C_{9a}), 156.53 (C_{10a}), 171.56 (C_2), 172.11 (C=O)
2h	*256* (4.35), 286 (4.47), 364 (3.91), 382 (4.14)	116.41 (C_7), 127.21 (C_8), 127.96 (C_6), 128.95 (C'_3 and C'_5), 129.01 (C_9), 129.44 (C'_2 and C'_6), 130.27 (C'_4), 132.84 (C_{5a}), 133.58 (C'_1), 138.89 (C_5), 141.90 (C_{4a}), 144.72 (C_{9a}), 155.29 (C_{10a}), 163.95 (C_2), 168.15 (C=O)
2i	*254* (4.71), 290 (4.86), 365 (4.24), 385 (4.09)	18.40 (9-CH_3), 115.97 (C_9), 126.73 (C_7), 126.92 (C_6), 127.92 (C_8), 128.80 (C'_3 and C'_5), 129.18 (C'_2 and C'_6), 132.49 (C'_4), 132.91 (C_{5a}), 133.45 (C'_1), 136.81 (C_5), 138.77 (C_{4a}), 145.35 (C_{9a}), 156.15 (C_{10a}), 163.78 (C_2), 168.21 (C=O)
2j	*255* (4.63), 279 (4.88), 370 (4.52), 374 (4.48)	56.44 (8-OCH_3), 106.95 (C_7), 120.68 (C_9), 122.59 (C_6), 128.77 (C'_3 and C'_5), 129.26 (C'_2 and C'_6), 131.39 (C'_4), 132.56 (C_{5a}), 133.50 (C'_1), 137.93 (C_5), 139.54 (C_{4a}), 143.68 (C_{9a}), 154.22 (C_8), 156.42 (C_{10a}), 163.67 (C_2), 168.47 (C=O)
3a	*292* (4.35), 303 (4.37), 366 (4.28), 472 (4.20), 500 (4.14)	33.40 (N_{10}–CH_3), 118.89 (C_7), 121.43 (C_9), 127.54 (C_6), 129.04 (C'_3 and C'_5), 129.84 (C'_2 and C'_6), 130.21 (C_8), 132.63 (C'_4), 135.88 C'_1), 136.29 (C_{5a}), 137.34 (C_{9a}), 137.97 (C_{4a}), 154.25 (C_{10a}), 164.38 (C_2), 166.68 (C=O)
3b	*293* (4.40), 303 (4.42), 365 (4.35), 472 (4.27), 500 (4.23)	13.17 (N_{10}–CH_2–*C*H_3), 41.32 (N_{10}–*C*H_2–CH_3), 118.37 (C_9), 121.59 (C_7), 127.32 (C_8), 128.94 (C'_3 and C'_5), 129.66 (C'_2 and C'_6), 130.04 (C_6), 132.47 (C'_4), 134.73 C'_1), 136.24 (C_{5a}), 137.97 (C_{9a}), 145.92 (C_{4a}), 153.45 (C_{10a}), 164.30 (C_2), 166.21 (C=O)
3c	*293* (4.41), 304 (4.21), 365 (4.08), 470 (4.02), 500 (3.93)	20.27 (N_{10}–CH(*C*H_3)$_2$), 51.86 (N_{10}–*C*H(CH_3)$_2$), 119.07 (C_7), 121.71 (C_9), 127.06 (C_6), 129.00 (C'_3 and C'_5), 129.59 (C'_2 and C'_6), 130.82 (C_8), 132.46 (C'_4), 134.93 (C'_1), 135.59 (C_{5a}), 137.76 (C_{9a}), 139.53 (C_{4a}), 153.80 (C_{10a}), 164.74 (C_2), 166.55 (C=O)
3d	*293* (4.46), 303 (4.47), 366 (4.34), 478 (4.27), 507 (4.26)	21.06 (7-CH_3), 33.21 (N_{10}–CH_3), 118.55 (C_7), 120.15 (C_9), 128.78 (C'_3 and C'_5), 129.54 (C'_2 and C'_6), 129.94 (C_6), 131.94 (C_8), 132.29 (C'_4), 137.01 (C'_1), 137.66 (C_{5a}), 137.88 (C_{9a}), 138.03 (C_{4a}), 153.55 (C_{10a}), 164.53 (C_2), 166.31 (C=O)
3e	*294* (4.33), 307 (4.38), 368 (4.23), 477 (4.11), 508 (4.06)	24.13 (9-CH_3), 40.12 (N_{10}–CH_3), 119.53 (C_9), 127.29 (C_7), 128.06 (C_6), 129.04 (C'_3 and C'_5), 129.76 (C'_2 and C'_6), 130.11 (C_8), 132.66 (C'_4), 133.06 C'_1), 137.88 (C_{5a}), 139.93 (C_{9a}), 140.41 (C_{4a}), 156.25 (C_{10a}), 164.33 (C_2), 166.76 (C=O).
3f	*302* (4.47), 312 (4.48), 362 (4.20), 496 (4.21), 528 (4.22)	33.92 (N_{10}–CH_3), 56.63 (7-OCH_3), 113.37 (C_6), 115.21 (C_8), 118.01 (C_9), 128.81 (C'_3 and C'_5), 129.90 (C'_2 and C'_6), 132.57 (C'_4), 134.04 C'_1), 137.26 (C_{5a}), 141.15 (C_{9a}), 147.07 (C_{4a}), 152.14 (C_7), 153.81 (C_{10a}), 165.01 (C_2), 167.94 (C=O)
3g	*293* (4.39), 305 (4.32), 363 (4.02), 493 (4.32), 522 (4.27)	33.65 (N_{10}–CH_3), 58.78 (8-OCH_3), 100.07 (C_7), 116.33 (C_9), 118.38 (C_6), 128.85 (C'_3 and C'_5), 129.89 (C'_2 and C'_6), 132.43 (C'_4), 134.64 C'_1), 138.55 (C_{5a}), 143.38 (C_{9a}), 146.04 (C_{4a}), 152.24 (C_8), 157.71 (C_{10a}), 164.04 (C_2), 166.37 (C=O)
3h	*294* (4.48), 306 (4.49), 366 (4.38), 479 (4.31), 510 (4.29)	13.18 (N_{10}–CH_2–*C*H_3), 21.06 (7-CH_3), 41.61 (N_{10}–*C*H_2–CH_3), 118.19 (C_7), 120.43 (C_9), 122.93 (C_6), 128.82 (C'_3 and C'_5), 129.53 (C'_2 and C'_6), 132.27 (C_8), 132.83 (C'_4), 137.11 (C'_1), 137.78 (C_{5a}), 137.95 (C_{9a}), 147.53 (C_{4a}), 156.49 (C_{10a}), 164.70 (C_2), 166.42 (C=O)
3i	*301* (4.43), 309 (4.41), 373 (4.00), 472 (4.21), 500 (4.18)	33.92 (N_{10}–CH_3), 58.01 (6- and 8-OCH_3), 61.80 (7-OCH_3), 93.57 (C_9), 100.02 (C_7), 128.71 (C'_3 and C'_5), 129.74 (C'_2 and C'_6), 131.89 (C'_4), 132.28 (C'_1), 135.98 (C_{5a}), 137.12 (C_{9a}), 138.11 (C_{4a}), 149.42 (C_{10a}), 151.65 (C_6), 151.84 (C_8), 165.21 (C_2), 168.27 (C=O)

[a] Compounds **la**–**j**, **2a**–**e**, and **2g** were measured in $CDC1_3$, while compounds **2f**, **2h**–**2j**, and **3a**–**i** were measured in $(CD_3)_2SO$.

ods. Table 3 shows the antitumor activity of 10-methyl derivative (**2a**) and 10-ethyl derivative (**2b**) of 2-deoxo-2-phenyl-5-deazaflavin against A 431 human adenocarcinoma cells. After the compound (**2a**, 25 mg/kg, it) was given into the tumor cells by intratumoral administration, the tumor growth was inhibited significantly as can be seen in the *T*/*C* %, namely, the tumor volume was reduced to 57.2% after 17 days of the administration. But the similar intratumoral administration of compound (**2b**) did not inhibit the tumor growth. The antitumor activity of compound (**2a**, 100 mg/kg, ip) was further investigated by intraperitoneal administration. After the compound (**2a**) was given into the tumor cells by ip, the tumor volume was reduced to 53.4% after 10 days of the administration, whereas staurosporine (1 mg/kg, ip) similarly reduced to 84.9% the tumor vol-

Table 2. Growth inhibitory activities against various tumor cell lines of 2-deoxo-2-phenyl-5-deazaflavins (**2a**–**j**) and 2-deoxo-2-phenylflavin-5-oxides (**3a**–**i**)

Compound	Inhibitory activity against tumor cell lines [IC_{50} (μM)]				
	NCI-H 460	HCT 116	A 431	CCRF-HSB-2	KB
2a	2.23	2.11	1.44	11.83	2.33
2b	ND	ND	0.76	1.79	2.62
2c	2.08	1.68	2.45	1.49	1.86
2d	ND	ND	ND	0.70	3.98
2e	3.10	2.91	ND	1.23	0.50
2f	ND	ND	ND	2.14	5.04
2g	ND	ND	ND	0.73	0.51
2h	ND	ND	3.88	3.83	5.12
2i	ND	ND	ND	299.32	>350.00
2j	ND	ND	ND	24.41	62.69
3a	2.72	1.80	1.31	0.97	1.08
3b	ND	ND	ND	0.96	2.38
3c	6.05	6.58	ND	0.98	2.18
3d	4.39	3.25	ND	0.50	1.75
3e	ND	ND	ND	1.29	3.28
3f	1.33	0.72	ND	ND	ND
3g	ND	ND	ND	ND	ND
3h	ND	ND	ND	1.71	2.97
3i	3.72	3.76	ND	ND	ND
Cisplatin	0.82	2.23	ND	ND	ND
Ara-C	ND	ND	ND	0.15	0.70
Adriamycin	ND	ND	0.05	ND	ND

ND, not done.

Table 3. Antitumor activity of 5-deazaflavin analogs (**2a** and **2b**) against human adenocarcinoma A 431 xenograft model[a]

Compound	Dose (mg/kg)	Tumor volume *T*/*C* (%)	Weight change (g)
Intratumoral administration (it after 17 days)			
Control		100	−0.8
2a	25	57.2	+0.1
2b	25	109	−1.6
Staurosporine	1	[b]	−0.6
Intraperitoneal administration (ip after 10 days)			
Control		100	−0.4
2a	100	53.4	−1.7
Staurosporine	1	84.9	−1.0
Oral administration (po after 18 days)			
Control		100	−0.4
2a	75	97.6	−0.9
2a	150	51.8	−0.4
Staurosporine	1	72.9	−2.0

[a] A 431 cells were implanted subcutaneously into nude mice and the administration (it, ip, and po) was started on the day when the mice show tumor growth in the range of 100–300 mm^3. The tumor volume (*T*/*C* %) was measured on the 17 days (it), 10 days (ip), and 18 days (po) after the treatment of chemicals.

[b] Because the necrosis occurred, the reduced tumor volume could not be evaluated.

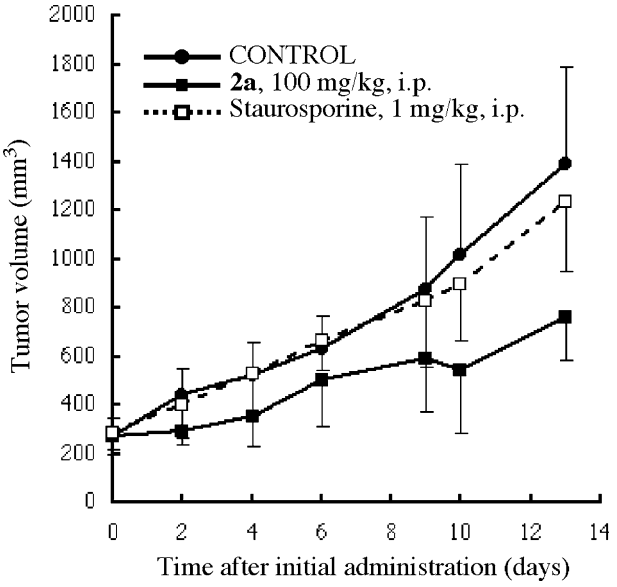


Figure 1. A 431 tumor volume implanted into nude mouse treated with chemicals (ip) after several days. A 431 cells were implanted subcutaneously into nude mice and the administration was started on the day when the mice show tumor growth in the 100–300 mm^3 range.

ume. The staurosporine was used only 1 mg/kg as a positive control because it was too toxic. Figure 1 shows A 431 tumor volume implanted into nude mouse treated with chemicals (ip) after several days. Depending on the gradual deduction of the tumor volume with the progress of time after the initial administration, the more effectual antitumor activity of 2-deoxo-10-methyl-2-phenyl-5-deazaflavin (**2a**, 100 mg/kg, ip) than that of staurosporine was observed. Moreover, the antitumor activity of compound (**2a**, 75 and 100 mg/kg, po) was investigated by oral administration as shown in Table 3. After the compound (**2a**) was given by po, the tumor volume was reduced to 97.6% (dose: 75 mg/kg) and 51.8% (dose: 150 mg/kg) after 18 days of the administration, whereas staurosporine reduced to 72.9% the tumor volume. Figure 2 also shows A 431 tumor volume

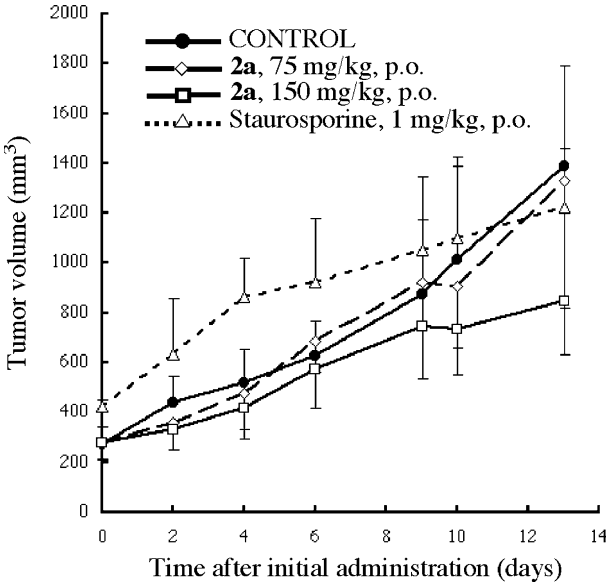


Figure 2. A 431 tumor volume implanted into nude mouse treated with chemicals (po) after several days. A 431 cells were implanted subcutaneously into nude mice and the administration was started on the day when the mice show tumor growth in the 100–300 mm^3 range.

implanted into nude mouse treated with chemicals (po) after several days. Depending on the gradual deduction of the tumor volume with the progress of time after the initial administration, the more effectual antitumor activity of 2-deoxo-10-methyl-2-phenyl-5-deazaflavin (**2a**, 150 mg/kg, po) than that of staurosporine (1 mg/kg, po) was observed, but the dose of 75 mg/kg was insufficient antitumor activity.

Accordingly, the antitumor activity of compound **2a** in vivo was shown to be more effective than compound **2b** though compound **2a** exhibited weaker activity than that of compound **2b** against A 431 tumor cells in vitro as shown in Table 2. There is no fixed rule that the compound, which has biological activity in vitro, must exhibit the same effect in vivo. For example, the nonselective protein kinase inhibitor, staurosporine, did not exhibit any antitumor activity in vivo though it has much greater antiproliferative activity than UCN-01 (7-hydroxy-staurosporine) in vitro.[17]

2.4. Molecular docking study

The computer simulated automated docking studies were performed using the widely distributed molecular docking software, AutoDock 3.05.[18] The AutoDock study of 2-deoxo-2-phenyl-5-deazaflavins (**2a**–**j**), 2-deoxo-2-phenylflavin-5-oxides (**3a**–**i**), and the computationally designed **3j** and 2-deoxo-2-phenylflavins (**4a**–**j**) was carried out, and they were docked within the protein tyrosine kinase (PTK $pp60^{c\text{-}src}$, PDB code: 1skj). As shown in Table 4, their AutoDock binding free energies (ΔG_b, kcal/mol) and inhibition constants (K_i) were obtained. Among them, all flavin-5-oxides (**3a**–**j**) exhibited the lowest free energy between −7.84 and −5.95 kcal/mol. In other words, they possess the highest potential binding affinity into the binding site of the 3D macromolecule (PTK, 1skj). The 5-deazaflavins (**2a**–**g**) and the computationally designed N-deoxidated derivatives, 2-deoxo-2-phenylflavins (**4a**–**j**), showed less binding affinity than the *N*-oxides (**3a**–**j**). The higher affinity is presumably attributed to the formation of more and/or tighter hydrogen bonds between the C_4-oxo of flavin-*N*-oxides and several amino acids at the binding site owing to the increased electronegativity of the oxo at the 4-posision due to the *N*-oxide moiety. Therefore, the flavin-5-oxides (**3a**–**j**) were docked deeply within the groove of the binding pocket of PTK forming more hydrogen bonds with Arg 12, Arg 32, Glu 35, and Lys 60. Considering the fitting of the 5-deazaflavins (**2a**–**g**) and flavin analogs (**4a**–**i**) into the PTK binding pocket, it was demonstrated that their binding energies were higher in comparison with the flavin-*N*-oxides (**3a**–**j**). Hence, they were partially superimposed on the original docked ur2 ligand (4-[3-carboxymethyl-3-(4-phosphonooxybenzyl)ureido]-4-[(3-cyclohexylpropyl)-methylcarbamoyl]butyric acid), namely, they are docked slightly

Table 4. AutoDock binding free energies (ΔG_b) and inhibition constants (K_i) for 2-deoxo-2-phenyl-5-deazaflavins (**2a**–**j**), 2-deoxo-2-phenylflavin-5-oxides (**3a**–**j**), and 2-deoxo-2-phenylflavins (**4a**–**j**) docked within protein tyrosine kinase

Compound[a]	K_i[b]	ΔG_b (kcal/mol)
2a	3.78E-04	−4.67
2b	0.01	−2.50
2c	0.09	−1.44
2d	0.08	−1.53
2e	0.01	−2.68
2f	0.02	−2.45
2g	0.09	−1.42
2h	1.29E-06	−8.03
2i	0.00	−4.18
2j	3.97E-04	−4.93
3a	4.99E-06	−7.23
3b	4.45E-06	−7.30
3c	2.70E-05	−6.23
3d	8.45E-06	−6.92
3e	3.55E-06	−7.44
3f	2.19E-05	−6.36
3g	5.21E-06	−7.21
3h	4.68E-06	−7.27
3i	4.35E-05	−5.95
3j[c]	1.71E-06	−7.84
4a	2.20E-04	−4.99
4b	0.02	−2.27
4c	—[d]	+0.22
4d	0.01	−2.61
4e	0.04	−1.89
4f	0.02	−2.41
4g	0.03	−2.05
4h	0.03	−2.02
4i	0.01	−1.38
4j	1.95E-06	−7.79

[a] The 2-deoxo-2-phenyflavins (**4a**–**j**), which are the *N*-deoxidated derivatives of 2-deoxo-2-phenylflavin-5-oxides (**3a**–**j**), are computationally designed compounds.
[b] Inhibition constant.
[c] This is a computationally designed compound.
[d] This cannot be calculated because ΔG_b is a positive value.

away from the groove of the binding site. It is noteworthy that the unsubstituted derivatives (**2h**, **3j**, and **4j**) exhibited especially stronger binding affinities to possess the lower binding free energy ca. −8 kcal/mol and also the derivatives (**2a**, **2i**, **2j**, and **4a**) having a methyl group at the 10-position or a methyl or methoxy group on the benzene of the benzopteridine ring showed the binding free energy between −4.99 and −4.18 kcal/mol. It was clarified that the derivatives possessing less binding free energy than −4.2 kcal/mol are allowed to fit well into the groove of the binding site.

The overall good correlation between the growth inhibitory activities (IC_{50},μM) of the flavin analogs against tumor cells and the binding affinities (lower binding free energy) predicted by AutoDock was made clear as indicated in Tables 2 and 4. It was found that the stronger binding affinity, the more potent inhibitory activity against tumor cells. Especially, the correlation between the binding free energy (ΔG_b) and IC_{50} (μM) values for compounds **2a**–**f** and **3a**–**d** against CCRF-HSB-2 and KB tumor cells was fairly good and showed almost same correlation coefficients (R^2) of 0.64 as shown in Figures 3 and 4.

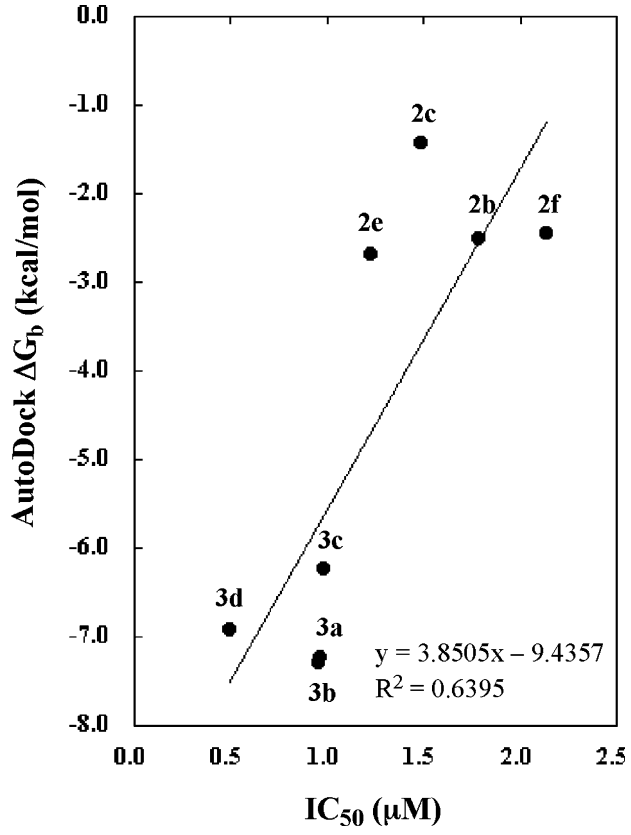


Figure 3. Correlation between the binding free energy (ΔG_b) and IC_{50} (μM) of 2-deoxo-2-phenyl-5-deazaflavins (**2b**, **c**, **e**, **f**) and 2-deoxo-2-phenylflavin-5-oxides (**3a**–**d**) against human T-cell acute lymphoblastic leukemia (CCRF-HSB-2).

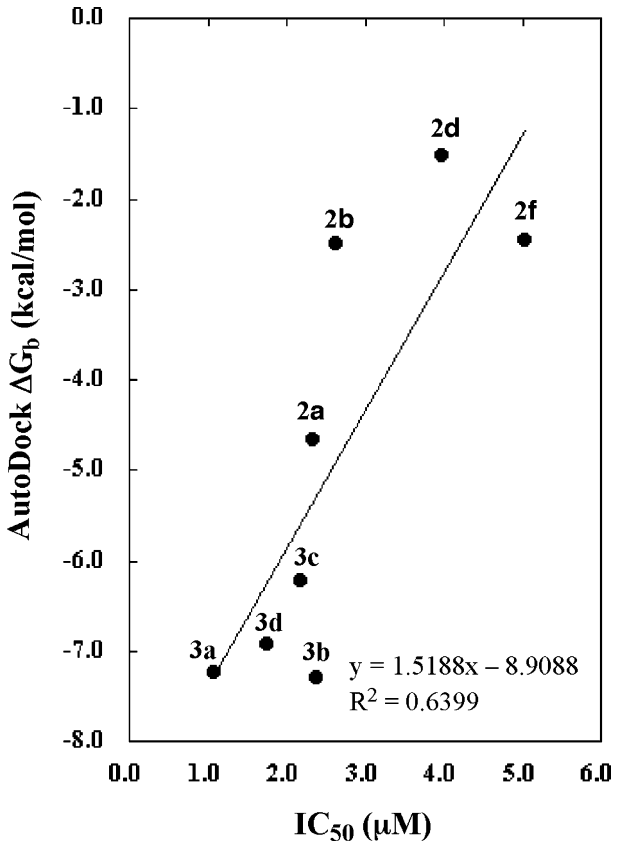


Figure 4. Correlation between the binding free energy (ΔG_b) and IC_{50} of 2-deoxo-2-phenyl-5-deazaflavins (**2a**, **b**, **d**, **f**) and 2-deoxo-2-phenylflavin-5-oxides (**3a**–**d**) against human oral epithelial carcinoma (KB).

Figure 5 shows the differential docking mode of three flavin analogs. Interestingly, the flavin-5-oxide (**3b**), whose ΔG_b was −7.30 kcal/mol, got deeply embedded within the groove of the binding pocket. This is highly correlated to its potent inhibition of the proliferation of human T-cell acute lymphoblastic leukemia cells (CCRF-HSB-2, IC_{50}: 0.96 μM). Whereas the 2-deoxo-5-deazaflavin (**2b**), whose ΔG_b and IC_{50} was −2.50 kcal/mol and 1.79 μM against CCRF-SB2, respectively, was docked at the same position as the flavin isomer (**4b**), whose ΔG_b was −2.27 kcal/mol. Both flavin (**4b**) and 5-deazaflavin (**2b**) appeared to be superimposed onto each other. Moreover, the flavin-5-oxide (**3b**) exhibited three true hydrogen bonds with Arg 12, Arg 32, and Glu 35 of bond angles being 143.0°, 175.9°, and 152.3°, respectively. Another the false hydrogen bond (1.95 Å) with Cys 42 was omitted because of the angle through a hydrogen between C_4–O of **3b** and N of Cys 42 was 86°. While compounds **2b** and **4b** did not exhibit any hydrogen bond. As cited in the literature,[19] the compound with the higher number of hydrogen bonds, with the lowest binding energy, and with the smallest RMSD (root mean square deviation) is generally said to be a reasonable candidate for potent inhibitors against the enzyme. Actually the docking results showed that there was no significant difference between flavins and 2-deoxo-5-deazaflavins with respect to docking affinities into the binding site as shown in Table 4 and

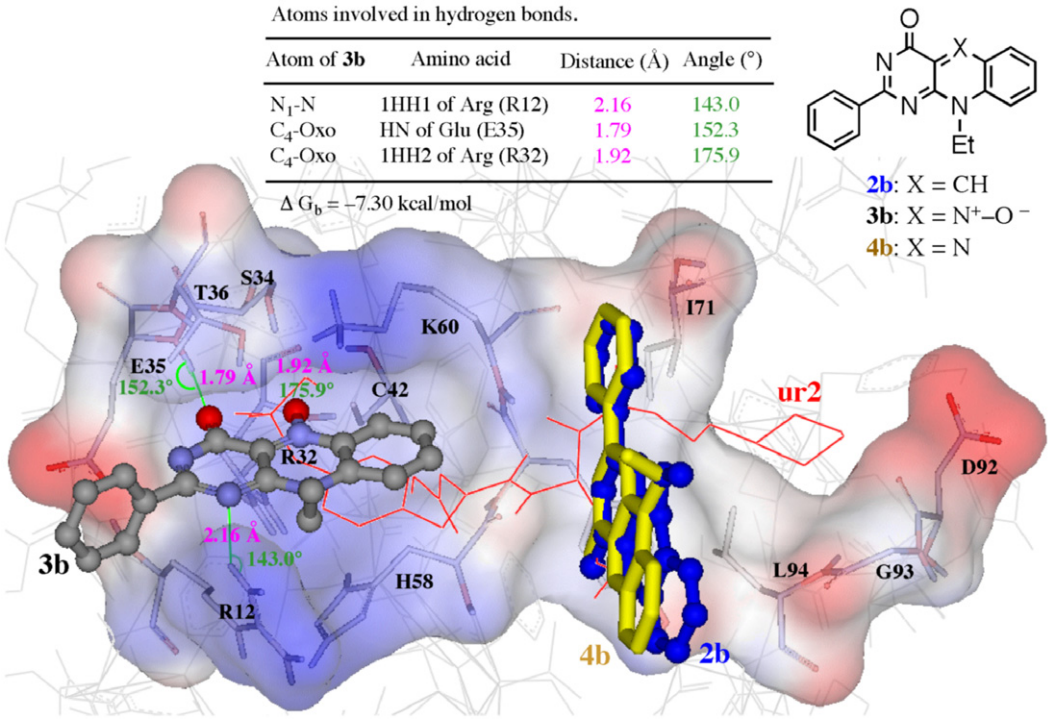


Figure 5. The differential docking mode of flavin analogs (**2b**, **3b**, and **4b**) and the binding pocket shown in transparent solid surface with labeled amino acids, and the ur2 ligand is shown as a red line.

Figure 5. This may be explained as CH and N are equivalent bioisosteres with similar size and comparable electronic properties.

2.5. Mode of interaction of inhibitors within the binding pocket

Most docked inhibitors interacted by the same mode of the co-crystallized ur2 ligand within the PTK binding site. They exhibited up to three hydrogen bonds involving Arg 32 and Glu 35, which are also involved in ur2 ligand, and Arg 12 and Lys 60. Also these inhibitors were involved in a hydrophobic interaction with only three amino acids, that is, Tyr 59, Ile 71, and Asp 92. Therefore, the docked inhibitors exhibited reasonable RMSD values as shown in Figure 6, where compound **3e** was bound, nearly superimposed on the ur2 ligand exhibiting ΔG_b being −7.44 kcal/mol, three true hydrogen bonds with Arg 12, Arg 32, and Glu 35

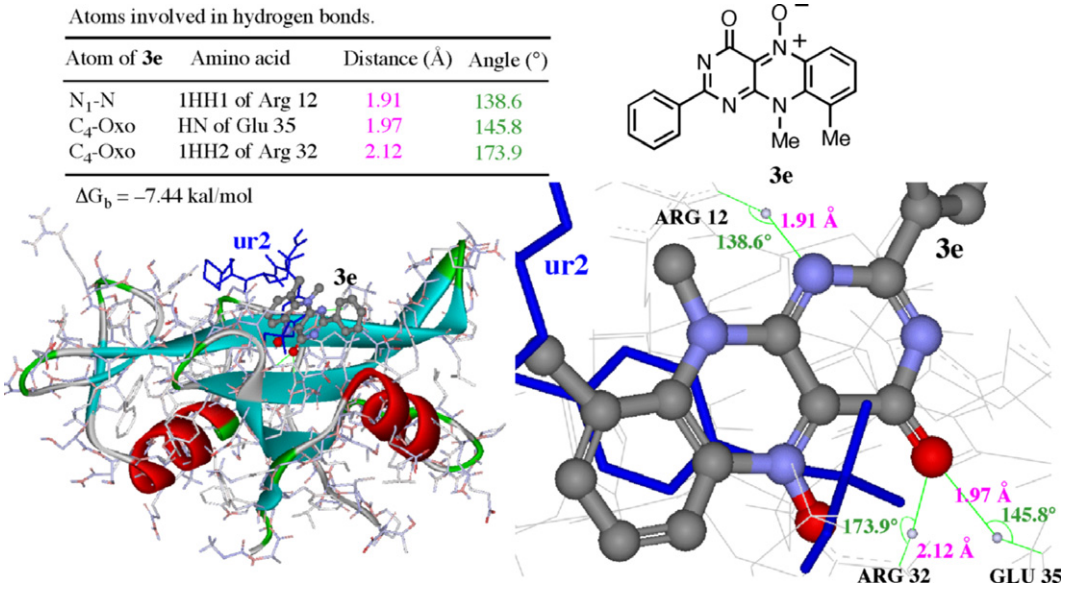


Figure 6. AutoDock binding affinity of 2-deoxo-9,10-dimethyl-2-phenylflavin-5-oxide (**3e**) shown as ball and stick colored by element. The binding pocket of the $pp60^{c\text{-}src}$ shown as solid backbone ribbon with the ur2 ligand (blue stick).

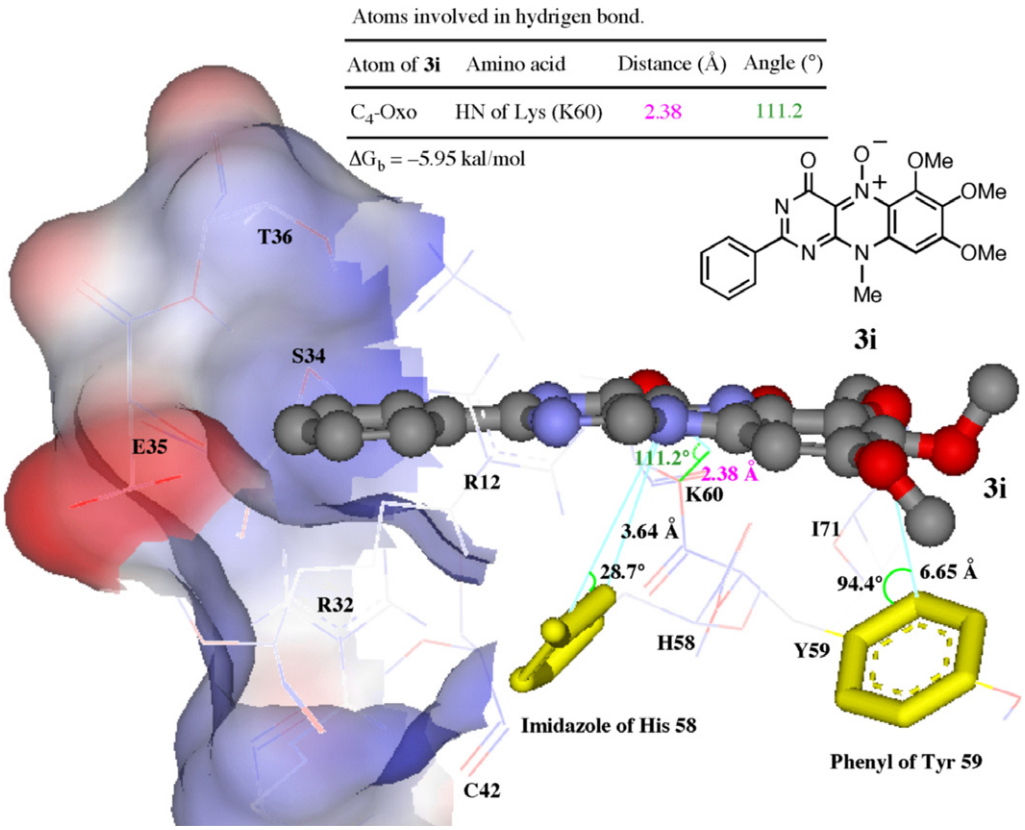


Figure 7. Interaction of compound **3i** (ball and stick, colored by element), docked into the binding pocket of PTK whose amino acids are shown as labeled lines.

of angles being 138.6°, 173.9°, 145.8°, respectively, and RMSD being 6.94 Å.

Figure 7 illustrates the hydrogen bond and van der Waals contacts similarly to the ur2 ligand with the Src SH2 domain. Instead of the binding mode for the phenyl phosphate group of ur2 within the pTyr (pY) pocket, the C_2-phenyl moiety of **3i** assumes the same position but has a different relative orientation, when compared with the phosphate of other Src SH2 ligand complexes. The benzene ring of **3i** is parallel to the phenyl ring of Tyr 59 within a distance of 6.65 Å and angle of 94.4°, while the imidazole ring of His 58 is oriented perpendicularly to the planar benzopteridine ring within a distance of 3.64 Å and angle of 28.7°. Exceptionally, the same pY pocket side chains are involved in the hydrogen-bonding scheme of the C_4-oxo group with Lys 60. Similarly to ur2, Arg 12 does not make any hydrogen bond with **3i**, while Arg 12 does in other structures **3b** and **3e** discussed previously in Figures 5 and 6, respectively. Also **3i** interacted hydrophobically with Tyr 59, Ile 71, and Asp 92.

3. Conclusion

In this study, ten 2-deoxo-5-deazaflavins (**2a**–**j**) and nine flavin-5-oxides (**3a**–**i**) were synthesized to investigate their biological activities. In vitro growth inhibitory activities of compounds **2a**–**j** and **3a**–**i** against NCI-H 460, HCT 116, A 431, CCRF-HSB-2, and KB cells showed significant potential antitumor activities. Interestingly, the activities of some compounds **2a**, **2c**, **3a**, and **3f** against HCT 116 cells were higher than that of cisplatin. Especially, compound **3f** (IC_{50}: 0.72 μM) exhibited 3-fold more potent antitumor activity than cisplatin (IC_{50}: 2.33 μM). Approximately similar inhibitory activities (IC_{50}: 0.5–2.0 μM) to Ara-C (IC_{50}: 0.15 μM) against CCRF-HSB-2 cells were observed in many compounds. Additional results of promising antitumor activity were obtained in compounds showing potential activities of 0.5–5.0 μM (IC_{50}) against KB cells. Furthermore, in vivo antitumor activity of 2-deoxo-10-methyl-2-phenyl-5-deazaflavin (**2a**) against human adenocarcinoma A 431 cells implanted subcutaneously into mice was undertaken. As the result, the tumor volume was reduced to 57.2% after 17 days of the intratumoral administration (25 mg/kg), to 53.4% after 10 days of the intraperitoneal administration (100 mg/kg) and to 51.8% after 18 days of the oral administration (150 mg/kg).

The AutoDock study of 2-deoxo-2-phenyl-5-deazaflavins (**2a**–**j**), 2-deoxo-2-phenylflavin-5-oxides (**3a**–**j**), and 2-deoxo-2-phenylflavins (**4a**–**j**) was carried out, and they were docked within the protein tyrosine kinase (PTK $pp60^{c\text{-}src}$, PDB code: 1skj). All flavin-5-oxides (**3a**–**j**) exhibited the lowest binding free energies between −7.84 and −5.95 kcal/mol. Namely, they possessed the highest potential binding affinity into the target site.

The correlation between the binding free energy (ΔG_b, kcal/mol) and IC_{50} (μM) values against CCRF-HSB-2 and KB tumor cells showed fairly good correlation coefficients. 2-Deoxo-10-ethyl-2-phenylflavin-5-oxide (**3b**), whose ΔG_b was −7.30 kcal/mol, got deeply embedded within the groove of the binding pocket, and is highly correlated to its potent inhibition of the proliferation of human T-cell acute lymphoblastic leukemia cells (CCRF-HSB-2, IC_{50}: 0.96 μM).

Thus, SAR studies by computational design, chemical synthesis, and biological investigation revealed new structural requirements for the modified flavins that would enhance the binding characteristics within the protein tyrosine kinase. The most effective molecules for flavin and 5-deazaflavins as antitumor activities were elucidated that a phenyl substituent at the 2-position is necessary.

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. ^1H and ^{13}C NMR spectra were obtained using a Varian VXR 300 and 75 MHz spectrophotometer, respectively, and chemical shift values were expressed in δ values (ppm) relative to tetramethylsilane (TMS) as internal standard. Coupling constants are given in Hz. UV spectra were measured in absolute EtOH using Hitachi U-2001 and Beckman DU-68S UV spectrophotometers and absorption values in italic 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 6-(*N*-alkylanilino)-2-phenylpyrimidin-4(3*H*)-ones (1a–j). A mixture of 6-chloro-2-phenylpyrimidin-4(3*H*)-one[13] (3.5 g, 17 mmol) and *N*-alkylaniline (0.051–0.085 mol) was heated under nitrogen atmosphere at 180–200 °C for 0.5–5 h. After cooling, the resulting solid was crushed with diethyl ether to get powdery crystals, which were filtered off, washed with water, dried, and recrystallized from an appropriate solvent with charcoal to afford colorless needles.

4.1.2. 6-(*N*-Methylanilino)-2-phenylpyrimidin-4(3*H*)-one (1a). Yield, 4.47 g (95%); mp 248 °C (EtOH) (lit.[13] mp 248 °C); ^1H NMR ($CDCl_3$), δ 3.56 (3H, s, 6-NMe), 5.27 (1H, s, 5-H), 7.28–7.33 (3H, m, N–Ph–*o*,*p*H), 7.42–7.54 (5 H, m, N–Ph–*m*H, 2-Ph–*m*,*p*H), 8.17–8.21 (2H, m, 2–Ph-*o*H), 12.60 (1 H, br s, 3-NH); IR (cm^{-1}) 3240 (NH), 1650 (C=O).

4.1.3. 6-(*N*-Ethylanilino)-2-phenylpyrimidin-4(3*H*)-one (1b)[13]. Yield, 4.01 g (81%); mp 268 °C (lit.[13] mp 265 °C); ^1H NMR ($CDCl_3$), δ 1.27 (3H, t, J = 7.2 Hz, 6-NCH_2–CH_3), 4.06 (2H, q, J = 7.2 Hz, 6-NCH_2–CH_3), 5.10 (1H, s, 5-H), 7.24–7.27 (2H, m, N–Ph–*o*H), 7.31–7.37 (1H, m, N–Ph–*p*H), 7.42–7.45 (1H, m, N–Ph–*m*H), 7.48–7.50 (3H, m, 2-Ph–*m*,*p*H), 8.16–8.19 (2H, m, 2-Ph–*o*H), 12.37 (1H, br s, 3-NH); IR (cm^{-1}) 3210 (NH), 1635 (C=O).

4.1.4. 6-(*N*-Isopropylanilino)-2-phenylpyrimidin-4(3*H*)-one (1c). Yield, 2.12 g (41%); mp > 300 °C (MeOH); ^1H NMR ($CDCl_3$), δ 1.18 (6H, d, J = 6.6 Hz, 6-*N*–CH–$(CH_3)_2$), 4.78 (1H, s, 5-H), 5.40 (1H, quintet, J = 6.6 Hz, 6-*N*–C*H*–$(CH_3)_2$), 7.13–7.18 (2H, m, N–Ph–*o*H), 7.36–7.54 (6H, m, 2-Ph–*m*,*p*H and N–Ph–*m*,*p*H), 8.10–8.14 (2H, m, 2-Ph–*o*H), 11.59 (1H, br s, 3-NH); IR (cm^{-1}) 3200 (NH), 1635 (C=O); Anal. $C_{19}H_{19}N_3O$ requires C, 74.73; H, 6.27; N, 13.76. Found: C, 74.69; H, 6.39; N, 13.60.

4.1.5. 6-(*N*-Methyl-4-methylanilino)-2-phenylpyrimidin-4(3*H*)-one (1d). Yield, 4.75 g (96%); mp 265–267 °C (dioxane); ^1H NMR ($CDCl_3$), δ 2.40 (3H, s, *p*Me), 3.54 (3H, s, 6-NMe), 5.23 (1H, s, 5-H), 7.16 (2H, dd, $J_{2'',3''} = 9.0$ Hz, $J_{3'',5''} = 2.1$ Hz, N-Ar-*o*H), 7.24 (2H, dd, $J_{2'',3''} = 8.7$ Hz, $J_{2'',6''} = 2.1$ Hz, N-Ar-*m*H), 7.47–7.52 (3H, m, 2-Ph–*m*,*p*H), 8.16–8.20 (2H, m, 2-Ph–*o*H), 12.49 (1H, br s, 3-NH); IR (cm^{-1}) 3210 (NH), 1630 (C=O); Anal. $C_{18}H_{17}N_3O$ requires C, 74.20; H, 5.88; N, 14.42. Found: C, 74.00; H, 5.91; N, 14.36.

4.1.6. 6-(*N*-Methyl-2-methylanilino)-2-phenylpyrimidin-4(3*H*)-one (1e). Yield, 3.07 g (62%); mp 254–256 °C (EtOH–H_2O); ^1H NMR ($CDCl_3$), δ 2.20 (3H, s, *o*Me), 3.51 (3H, s, 6-NMe), 4.84 (1H, s, 5-H), 7.15–7.21 (1H, m, N-Ar-*o*H), 7.27–7.45 (3H, m, N-Ar-*m*,*p*H), 7.46–7.54 (3H, m, 2-Ph-*m*,*p*H), 8.15–8.30 (2H, m, 2-Ph–*o*H), 12.36 (1H, br s, 3NH); IR (cm^{-1}) 3210 (NH), 1640 (C=O); Anal. $C_{18}H_{17}N_3O$ requires C, 74.20; H, 5.88; N, 14.42. Found: C, 73.83; H, 5.94; N, 14.42.

4.1.7. 6-(*N*-Methyl-4-methoxyanilino)-2-phenylpyrimidin-4(3*H*)-one (1f). Yield, 4.86 g (93%); mp 273–275 °C (EtOH); ^1H NMR ($CDCl_3$), δ 3.52 (3H, s, 6-NMe), 3.85 (3H, s, OMe), 5.18 (1H, s, 5-H), 6.96 (2H, dd, $J_{2'',3''} = 9.0$ Hz, $J_{3'',5''} = 2.1$ Hz, N-Ar-*o*H), 7.19 (2H, d, $J_{2'',3''} = 9.0$ Hz, $J_{2'',6''} = 2.1$ Hz, N-Ar-*m*H), 7.46–7.52 (3H, m, 2-Ph–*m*,*p*H), 8.15–8.20 (2H, m, 2-Ph–*o*H), 12.29 (1H, br s, 3-NH); IR (cm^{-1}) 3210 (NH), 1630 (C=O); Anal. $C_{18}H_{17}N_3O_2$ requires C, 70.34; H, 5.58; N, 13.67. Found: C, 70.10; H, 5.66; N, 13.28.

4.1.8. 6-(*N*-Methyl-3-methoxyanilino)-2-phenylpyrimidin-4(3*H*)-one (1g). Yield, 3.87 g (74%); mp 235–237 °C (EtOH); ^1H NMR ($CDCl_3$), δ 3.55 (3H, s, 6-NMe), 3.83 (3H, s, OMe), 5.29 (1H, s, 5-H), 6.82–6.90 (3H, m, N-Ar-*o*,*p*H), 7.32–7.38 (1H, m, N-Ar-*m*H), 7.48–7.54 (3H, m, 2-Ph–*m*,*p*H), 8.14–8.19 (2H, m, 2-Ph-*o*H), 12.18 (1H, br s, 3-NH); IR (cm^{-1}) 3200 (NH), 1645

(C=O); Anal. $C_{18}H_{17}N_3O_2$ requires C, 70.34; H, 5.58; N, 13.67. Found: C, 70.09; H, 5.69; N, 13.62.

4.1.9. 6-(*N*-Ethyl-4-methylanilino)-2-phenylpyrimidin-4(3*H*)-one (1h). Yield, 4.77 g (92%); mp 295–297 °C (decomp., EtOH); ^{1}H NMR ($CDCl_3$), δ 1.25 (3H, t, J = 7.0 Hz, 6-NCH$_2$–CH_3), 2.40 (3H, s, *p*Me), 4.04 (2H, q, J = 7.0 Hz, 6-NCH_2–CH$_3$), 5.07 (1H, s, 5-H), 7.11 (2H, br d, J = 8.4 Hz, N-Ar-*o*H), 7.24 (2H, br d, J = 8.4 Hz, N-Ar-*m*H), 7.48–7.54 (3H, m, 2-Ph–*m*,*p*H), 8.15–8.19 (2H, m, 2-Ph–*o*H), 12.25 (1H, br s, 3-NH); IR (cm^{-1}) 3200 (NH), 1635 (C=O); Anal. $C_{19}H_{19}N_3O$ requires C, 74.73; H, 6.27; N, 13.76. Found: C, 74.66; H, 6.36; N, 13.94.

4.1.10. 6-(*N*-Ethyl-2-methylanilino)-2-phenylpyrimidin-4(3*H*)-one (1i). Yield, 3.01 g (58%); mp 249–251 °C (EtOH); ^{1}H NMR ($CDCl_3$), δ 1.25 (3H, t, J = 7.2 Hz, 6-NCH$_2$–CH_3), 2.19 (3H, s, *o*Me), 4.27 (2H, br s, 6-NCH_2–CH$_3$), 4.80 (1H, s, 5-H), 7.13–719 (2H, m, N-Ar-3″ and 6″H), 7.28–7.34 (2H, m, N-Ar-4″ and 5″-H), 7.46–7.54 (3H, m, 2-Ph–*m*,*p*H), 8.16–8.22 (2H, m, 2-Ph–*o*H), 11.91 (1H, br s, 3-NH); IR (cm^{-1}) 3200 (NH), 1640 (C=O); Anal. $C_{19}H_{19}N_3O{\cdot}1/10H_2O$ requires C, 74.29; H, 6.30; N, 13.68. Found: C, 74.13; H, 6.33; N, 13.46.

4.1.11. 6-(*N*-Methyl-3,4,5-trimethoxyanilino)-2-phenylpyrimidin-4(3*H*)-one (1j). Yield, 5.93 g (95%); mp 294 °C (decomp., EtOH); ^{1}H NMR ($CDCl_3$), δ 3.55 (3H, s, 6-NMe), 3.85 (6H, s, N-Ar-3″,5″-diOMe), 3.89 (3H, s, N-Ar-4″-OMe), 5.23 (1H, s, 5-H), 6.50 (2H, s, N-Ar-2″H and 6″H), 7.45–7.60 (3H, m, 2-Ph–*m*,*p*H), 8.17–8.24 (2H, m, 2-Ph–*o*H), 12.54 (1H, br s, 3-NH) ; IR (cm^{-1}) 3210 (NH), 1635 (C=O); Anal. $C_{20}H_{21}N_3O_4$ requires C, 65.38; H, 5.76; N, 11.44. Found: C, 64.98; H, 5.79; N, 11.06.

4.1.12. General procedure for the preparation of 2-deoxo-2-phenyl-5-deazaflavin derivatives (2a–j). A mixture of 6-(*N*-alkylanilino)- or 6-anilino-2-phenylpyrimidin-4(3*H*)-ones[15] (**1**, 0.05 mol) and phosphoryl chloride (77 g, 0.5 mol) in *N*,*N*-dimethylformamide (100 mL) was heated under stirring at 90 °C for 1–4 h. Then, the reaction mixture was poured onto ice and neutralized with aqueous ammonia (pH 7). The yellow crystals, which separated, were filtered off, washed with water, dried, and recrystallized from an appropriate solvent. All compounds were obtained as yellow (**2a**–**g**) or pale yellow needles (**2h**–**j**).

4.1.13. 10-Methyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (2a). Yield, 13.5 g (94%); mp 293–295 °C (EtOH) (lit.[13] mp 290 °C); ^{1}H NMR ($CDCl_3$), δ 4.45 (3H, s, 10-Me), 7.34–7.49 (3H, m, 6-, 7-, and 9-H), 7.54–7.61 (1H, m, 8-H), 7.84–7.89 (1H, m, Ph–*p*H), 7.95–8.01 (2H, m, Ph–*m*H), 8.53–8.59 (2H, m, Ph–*o*H), 9.27 (1H, s, 5-H); IR (cm^{-1}) 1645 (C=O).

4.1.14. 10-Ethyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (2b)[13]. Yield, 12.21 g (81%); mp > 300 °C (decomp., EtOH) (lit.[13] mp 303 °C); ^{1}H NMR ($CDCl_3$), δ 1.64 (3H, t, J = 7.2 Hz, 10-CH$_2$–CH_3), 5.16 (2H, q, J = 7.2 Hz, 10-CH_2–CH$_3$), 7.42–7.54 (3H, m, 6-, 7-, and 9-H), 7.57–7.65 (1H, m, 8-H), 7.87–7.92 (1H, m, Ph–*p*H), 7.96–8.07 (2H, m, Ph–*m*H), 8.63–8.68 (2H, m, Ph–*o*H), 9.34 (1H, s, 5-H); IR (cm^{-1}) 1640 (C=O).

4.1.15. 7,10-Dimethyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (2c). Yield, 10.25 g (68%); mp 295–297 °C (decomp., DMF); ^{1}H NMR [$(CD_3)_3SO$], δ 2.44 (3H, s, 7-Me), 4.46 (3H, s, 10-Me), 7.43–7.66 (3H, m, 6-, 8-, and 9-H), 7.88–8.22 (3H, m, Ph–*m*,*p*H), 8.43–8.61 (2H, m, Ph–*o*H), 9.29 (1H, s, 5-H); IR (cm^{-1}) 1650 (C=O); Anal. $C_{19}H_{15}N_3O$ requires C, 75.73; H, 5.02; N, 13.94. Found: C, 75.31; H, 5.18; N, 13.57.

4.1.16. 9,10-Dimethyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (2d). Yield, 9.19 g (61%); mp 262–264 °C (DMF); ^{1}H NMR [$(CD_3)_3SO$], δ 2.98 (3H,s, 9-Me), 4.57 (3H, s, 10-Me), 7.53–7.61 (4H, m, 7-H and Ph–*m*,*p*H), 7.92 (1H, br d, $J_{7,8}$ = 7.8 Hz, 8-H), 8.23 (1H, d, $J_{6,7}$ = 7.8 Hz, Ph–6H), 8.49–8.54 (2H, m, Ph–*o*H), 9.36 (1H, s, 5-H); IR (cm^{-1}) 1640 (C=O); Anal. $C_{19}H_{15}N_3O{\cdot}1/5\ H_2O$ requires C, 74.83; H, 5.09; N, 13.78. Found: C, 74.80; H, 5.21; N, 13.74.

4.1.17. 7-Methoxy-10-methyl-2-phenylpyrimido[4,5-*b*]-quinolin-4(10*H*)-one (2e). Yield, 15.08 g (95%); mp 279–281 °C (decomp., DMF); ^{1}H NMR [$(CD_3)_3SO$], δ 3.95 (3H, s, 7-OMe), 4.51 (3H, s, 10-Me), 7.50–7.60 (3H, m, Ph-*m*,*p*H), 7.78 (1H, dd, $J_{6,8}$ = 2.4 Hz, $J_{8,9}$ = 9.0 Hz, 6-H), 7.92 (1H, d, $J_{6,8}$ = 2.4 Hz, 8-H), 8.20 (1H, d, $J_{8,9}$ = 9.0 Hz, 9-H), 8.52–8.57 (2H, m, Ph–*o*H), 9.36 (1H, s, 5-H); IR (cm^{-1}) 1620 (C=O); Anal. $C_{19}H_{15}N_3O_2{\cdot}1/10\ H_2O$ requires C, 71.51; H, 4.80; N, 13.17. Found: C, 71.27; H, 4.97; N, 13.42.

4.1.18. 8-Methoxy-10-methyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (2f). Yield, 14.60 g (92%); mp 265–267 °C (decomp., EtOH); ^{1}H NMR [$(CD_3)_3SO$], δ 4.10 (3H, s, 8-OMe), 4.45 (3H, s, 10-Me), 7.35 (1H, dd, $J_{6,7}$ = 9.0 Hz, $J_{7,9}$ = 2.1 Hz, 7-H), 7.44 (1H, d, $J_{7,9}$ = 2.1 Hz, 9-H), 7.25–7.62 (3H, m, Ph–*m*,*p*H), 8.31 (1H, d, $J_{6,7}$ = 9.0 Hz, 6-H), 8.50–8.58 (2H, m, Ph–*o*H), 9.31 (1H, s, 5-H); IR (cm^{-1}) 1625 (C=O); Anal. $C_{19}H_{15}N_3O_2{\cdot}3/10\ H_2O$ requires C, 70.71; H, 4.87; N, 13.02. Found: C, 70.30; H, 4.89; N, 12.88.

4.1.19. 10-Ethyl-7-methyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (2g). Yield, 11.8 g (75%); mp 291–293 °C (decomp., DMF); ^{1}H NMR [$(CD_3)_3SO$], δ 1.51 (3H, t, J = 7.0 Hz, 10-CH$_2$–CH_3), 2.54 (3H, s, 7-Me), 5.18 (2H, q, J = 7.0 Hz, 10-CH_2–CH$_3$), 7.50–7.60 (3H, m, 6-H, 8-H, and Ph–*p*H), 7.98 (1H, d, $J_{8,9}$ = 9.3 Hz, 9-H), 8.17–8.23 (2H, m, Ph–*m*H), 8.50–8.58 (2H, m, Ph–*o*H), 9.33 (1H, s, 5-H); IR (cm^{-1}) 1640 (C=O); Anal. $C_{20}H_{17}N_3O$ requires C, 76.17; H, 5.43; N, 13.32. Found: C, 75.82; H, 5.65; N, 13.22.

4.1.20. 2-Phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (2h). Yield, 11.75 g (86%); mp > 300 °C (DMF); ^{1}H NMR [$(CD_3)_3SO$], δ 7.5–7.76 (4H, m, 6-H,7-H, and Ph–*m*H), 7.94 (1H, ddd, $J_{6,8}$ = 1.5 Hz, $J_{7,8}$ = 8.7 Hz, $J_{8,9}$ = 8.7 Hz, 8-H), 8.09 (1H, dd, $J_{7,9}$ = 1.5 Hz, $J_{8,9}$ = 8.7 Hz, 9-H), 8.24–8.32 (3H, m, Ph–*o*,*p*H), 9.30

(1H, s, 5-H), 12.64 (1H, br s, 10-NH) ; IR (cm^{-1}) 3170 (NH), 1680 (C=O); Anal. $C_{17}H_{11}N_3O$ requires C, 74.71; H, 4.06; N, 15.38. Found: C, 74.36; H, 4.31; N, 15.24.

4.1.21. 9-Methyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (2i). Yield, 9.77 g (68%); mp > 300 °C (DMF); ^1H NMR [$(CD_3)_3SO$], δ 2.78 (3H, s, 9-Me), 7.45–7.70 (4H, m, 6-H, 7-H, and Ph-*m*H), 7.75–7.82 (1H, m, 8-H), 8.10–8.19 (1H, m, Ph–*p*H), 8.25–8.30 (2H, m, Ph–*o*H), 9.24 (1H, s, 5-H), 12.63 (1H, br s, 10-NH) ; IR (cm^{-1}) 3160 (NH), 1670 (C=O); Anal. $C_{18}H_{13}N_3O$ requires C, 75.25; H, 4.56; N, 14.63. Found: C, 74.85; H, 4.82; N, 14.78.

4.1.22. 8-Methoxy-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (2j). Yield, 11.68 g (77%); mp > 300 °C (DMF); ^1H NMR [$(CD_3)_3SO$], δ 3.99 (3H, s, 8-OMe), 7.31 (1H, dd, $J_{6,7}$ = 7.5 Hz, $J_{7,9}$ = 2.1 Hz, 7-H), 7.42 (1H, d, $J_{7,9}$ = 2.1 Hz, 9-H), 7.55–7.72 (3H, m, Ph–*m*,*p*H), 8.14 (1H, d, J = 7.8 Hz, 6-H), 8.24–8.29 (1H, m, Ph–*o*H), 9.16 (1H, s 5-H), 12.57 (1H, br s, 10-NH) ; IR (cm^{-1}) 3160 (NH), 1680 (C=O); Anal. $C_{18}H_{13}N_3O_2 \cdot 1/10\ H_2O$ requires C, 71.28; H, 4.32; N, 13.85. Found: C, 71.11; H, 4.54; N, 13.54.

4.1.23. Methylation of 2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (2h). A mixture of 2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (**2h**, 0.5 g, 1.83 mmol), methyl iodide (1.30 g, 9.16 mmol), and anhydrous potassium carbonate (1.01 g, 7.31 mmol) in DMF (50 mL) was stirred at room temperature for 10 h. After the reaction was complete, the precipitated potassium carbonate was filtered off. The residue obtained by concentration in vacuo was subjected to column chromatography on silica gel using a mixture of *n*-hexane and ethyl acetate (1:1) as eluting solvent to isolate the corresponding 10-methyl-2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-one (**2a**, 0.425 g, 80%), which was identical to the product (**2a**) prepared by the reaction of **1a** with Vilsmeier reagent in all spectral data as described above.

4.1.24. General procedure for the preparation of 2-deoxo-2-phenylflavin-5-oxide derivatives (3a–i). To a stirring solution of 6-(*N*-alkylanilino)-2-phenylpyrimidin-4(3*H*)-ones (**1**, 10 mmol) in acetic acid (5–15 mL) at 10–15 °C was added sodium nitrite (20–40 mmol) by portions, and the mixture was stirred for 2–5 h at room temperature. The solid deposited was collected by suction filtration and washed with water. Then, the solid dried was recrystallized from an appropriate solvent. All compounds were obtained as red needles.

4.1.25. 4,10-Dihydro-10-methyl-4-oxo-2-phenylbenzo[*g*]-pteridine-5-oxide (3a). Yield, 2.19 g (72%); mp > 300 °C (DMF); ^1H NMR [$(CD_3)_3SO$], δ 4.29 (3H, s, 10-Me), 7.50–7.60 (3H, m, Ph–*m*,*p*H), 7.73 (1H, ddd, $J_{6,7}$ = 7.8 Hz, $J_{7,8}$ = 7.8 Hz, $J_{7,9}$ = 1.5 Hz, 7-H), 8.09 (1H, ddd, $J_{6,8}$ = 1.5 Hz, $J_{7,8}$ = 7.8 Hz, $J_{8,9}$ = 7.8 Hz, 8-H), 8.21 (1H, dd, $J_{7,9}$ = 1.5 Hz, $J_{8,9}$ = 7.8 Hz, 9-H), 8.45 (1H, dd, $J_{6,7}$ = 8.7 Hz, $J_{6,8}$ = 1.5 Hz, 6-H), 8.47–8.51 (2H, m, Ph–*o*H); IR (cm^{-1}), 1540 (N–O), 1650 (C=O); Anal. $C_{17}H_{12}N_4O_2$ requires C, 67.10; H, 3.97; N, 18.41. Found: C, 67.11; H, 4.20; N, 18.62.

4.1.26. 10-Ethyl-4,10-dihydro-4-oxo-2-phenylbenzo[*g*]-pteridine-5-oxide (3b). Yield, 2.42 g (76%); mp 262–264 °C (decomp., EtOH); ^1H NMR [$(CD_3)_3SO$], δ 1.52 (3H, t, J = 6.9 Hz, 10-CH_2–CH_3), 4.98 (2H, q, J = 6.9 Hz, 10-CH_2-CH_3), 7.49–7.61 (3H, m, Ph–*m*,*p*H), 7.72 (1H, ddd, $J_{6,7}$ = 7.8 Hz, $J_{7,8}$ = 7.8 Hz, $J_{7,9}$ = 1.5 Hz, 7-H), 8.07 (1 H, ddd, $J_{6,8}$ = 1.5 Hz, $J_{7,8}$ = 7.8 Hz, $J_{8,9}$ = 7.8 Hz, 8 H), 8.23 (1H, dd, $J_{7,9}$ = 1.5 Hz, $J_{8,9}$ = 7.8 Hz, 9-H), 8.44–8.50 (2H, m, Ph–*o*H), 8.56 (1H, dd, $J_{6,7}$ = 7.8 Hz, $J_{6,8}$ = 1.5 Hz, 6-H); IR (cm^{-1}), 1540 (N–O), 1640 (C=O); Anal. $C_{18}H_{14}N_4O_2$ requires C, 67.91; H, 4.43; N, 17.60. Found: C, 67.94; H, 4.62; N, 17.58.

4.1.27. 4,10-Dihydro-10-isopropyl-4-oxo-2-phenylbenzo[*g*]-pteridine-5-oxide (3c). Yield, 2.66 g (80%); mp 267–269 °C (MeOH); ^1H NMR [$(CD_3)_3SO$], δ 1.85 (6H, d, J = 6.9 Hz, 10-CH–(CH_3)$_2$), 6.19 (1 H, br s, 10-C*H*–(CH_3)$_2$), 7.56–7.64 (3H, m, Ph-*m*,*p*H), 7.71 (1H, ddd, $J_{6,7}$ = 7.8 Hz, $J_{7,8}$ = 7.8 Hz, $J_{7,9}$ = 1.5 Hz, 7-H), 8.04 (1H, ddd, $J_{6,8}$ = 1.5 Hz, $J_{7,8}$ = 7.8 Hz, $J_{8,9}$ = 7.8 Hz, 8-H), 8.37 (1H, dd, $J_{7,9}$ = 1.5 Hz, $J_{8,9}$ = 7.8 Hz, 9-H), 8.44–8.50 (3H, m, 6, Ph–*o*H) ; IR (cm^{-1}), 1540 (N–O), 1640 (C=O); Anal. $C_{19}H_{16}N_4O_2$ requires C, 68.66; H, 4.85; N, 16.86. Found: C, 68.45; H, 4.94; N, 16.69.

4.1.28. 4,10-Dihydro-7,10-dimethyl-4-oxo-2-phenylbenzo[*g*]-pteridine-5-oxide (3d). Yield, 2.16 g (68%); mp > 300 °C (decomp., DMF); ^1H NMR [$(CD_3)_3SO$], δ 2.51 (3H, s, 7-Me), 4.27 (3H, s, 10-Me), 7.49-7.61 (3H, m, Ph–*m*,*p*H), 7.92 (1H, dd, $J_{6,8}$ = 1.5 Hz, $J_{8,9}$ = 8.7 Hz, 8-H), 8.12 (1H, d, $J_{8,9}$ = 8.7 Hz, 9-H), 8.25 (1H, d, $J_{6,8}$ = 1.5 Hz, 6-H), 8.45–8.48 (2H, m, Ph–*o*H); IR (cm^{-1}), 1540 (N–O), 1640 (C=O); Anal. $C_{18}H_{14}N_4O_2$ requires C, 67.91; H, 4.43; N, 17.60. Found: C, 68.12; H, 4.56; N, 17.51.

4.1.29. 4,10-Dihydro-9,10-dimethyl-4-oxo-2-phenylbenzo[*g*]-pteridine-5-oxide (3e). Yield, 2.61 g (82%); mp > 300 °C (DMF); ^1H NMR [$(CD_3)_3SO$], δ 1.91 (3H, s, 9-Me), 4.52 (3H, s, 10-Me), 7.63–7.84 (4H, m, 7-H and Ph–*m*,*p*H), 8.08 (1H, dd, $J_{6,8}$ = 1.5 Hz, $J_{7,8}$ = 7.5 Hz, 8-H), 8.41–8.48 (3H, m, 6-H and Ph–*o*H); IR (cm^{-1}), 1540 (N–O), 1655 (C=O); Anal. $C_{18}H_{14}N_4O_2$ requires C, 67.91; H, 4.43; N, 17.60. Found: C, 68.16; H, 4.68; N, 17.63.

4.1.30. 4,10-Dihydro-7-methoxy-10-methyl-4-oxo-2-phenylbenzo[*g*]pteridine-5-oxide (3f). Yield, 2.67 g (80%); mp > 300 °C (DMF); ^1H NMR [$(CD_3)_3SO$], δ 4.07 (3H, s, 7-OMe), 4.55 (3H, s, 10-Me), 7.65–7.73 (2H, m, Ph–*m*H), 7.75–7.80 (1H, m, Ph–*p*H), 7.92 (1H, d, $J_{6,8}$ = 1.5 Hz, 6-H), 8.01 (1H, d, J = 9.0 Hz, 9-H), 8.41–8.47 (2H, m, Ph–*o*H), 8.50 (1H, dd, $J_{6,8}$ = 1.5 Hz, $J_{8,9}$ = 9.0 Hz, 8-H); IR (cm^{-1}), 1539 (N–O), 1645 (C=O); Anal. $C_{18}H_{14}N_4O_3$ requires C, 64.66; H, 4.22; N, 16.76. Found: C, 64.69; H, 4.47; N, 16.85.

4.1.31. 4,10-Dihydro-8-methoxy-10-methyl-4-oxo-2-phenylbenzo[*g*]pteridine-5-oxide (3g). Yield, 2.71 g (81%); mp > 300 °C (DMF); ^{1}H NMR [$(CD_3)_3SO$], δ 4.17 (3H, s, 8-OMe), 4.48 (3H, s, 10-Me), 7.57–7.70 (4H, m, 9-H and Ph–*m*,*p*H), 7.77 (1H, d, $J_{6,7}$ = 7.5 Hz, 6-H), 8.42–8.47 (2H, m, Ph–*o*H), 8.52 (1H, dd, $J_{6,7}$ = 8.7 Hz, $J_{7,9}$ = 1.5 Hz, 7-H); IR (cm^{-1}), 1540 (N–O), 1640 (C═O); Anal. $C_{18}H_{14}N_4O_3$ requires C, 64.66; H, 4.22; N, 16.76. Found: C, 64.68; H, 4.56; N, 16.82.

4.1.32. 10-Ethyl-4,10-dihydro-7-methyl-4-oxo-2-phenylbenzo[*g*]pteridine-5-oxide (3h). Yield, 2.56 g (77%); mp 268–269 °C (MeOH); ^{1}H NMR [$(CD_3)_3SO$], δ 1.48 (3H, t, J = 7.2 Hz, 10-CH_2–CH_3), 2.49 (3H, s, 7-Me), 4.97 (2H, q, J = 7.2 Hz, 10-CH_2–CH_3), 7.51–7.59 (3H, m, Ph–*m*,*p*H), 7.92 (1H, dd, $J_{6,8}$ = 9.0 Hz, $J_{8,9}$ = 1.5 Hz, 8-H), 8.17 (1 H, d, J = 9.0 Hz, 9-H), 8.28 (1H, d, $J_{6,8}$ = 1.5 Hz, 6-H), 8.44–8.49 (2H, m, Ph–*o*H) ; IR (cm^{-1}), 1540 (N–O), 1650 (C═O); Anal. $C_{19}H_{16}N_4O_2$ requires C, 68.66; H, 4.85; N, 16.86. Found: C, 68.54; H, 4.96; N, 16.78.

4.1.33. 4,10-Dihydro-6,7,8-trimethoxy-10-methyl-4-oxo-2-phenylbenzo[*g*]pteridine-5-oxide (3i). Yield, 3.19 g (81%); mp > 300 °C (DMF); ^{1}H NMR [$(CD_3)_3SO$], δ 3.94 (3H, s, 7-OMe), 4.18 (3H, s, 8-OMe), 4.21 (3H, s, 6-OMe), 4.43 (3H, s, 10-Me), 7.25 (1H, s, 9-H), 7.45–7.60 (3H, m, Ph–*m*,*p*H), 8.53–8.56 (2H, m, Ph–*o*H); IR (cm^{-1}), 1550 (N–O), 1645 (C═O); Anal. $C_{20}H_{18}N_4O_5{\cdot}7/10H_2O$ requires C, 59.02; H, 4.80; N, 13.77. Found: C, 59.15; H, 4.67; N, 13.40.

4.2. Growth inhibitory activities of 2-deoxo-2-phenyl-5-deazaflavins (2) and 2-deoxo-2-phenylflavin-5-oxides (3) against human tumor cell lines

AraC was a commercial product of Yamasa Corporation (Choshi, Japan) and 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO). Five human tumor cell lines of NCI-H 460 (human lung cancer), HCT 116 (human colon carcinoma), A 431 (human adenocarcinoma), CCRF-HSB-2 (human T-cell acute lymphoblastoid leukemia), and KB (human oral epidermoid carcinoma) were used in this study. Cell lines were maintained in RPMI 1640 medium (Life Technologies, Rockville, MD) supplemented with 10% heat-inactivated fetal bovine serum (Moregate Laboratories, Melbourne, Australia) and 60 μg/mL of kanamycin. The MTT assay developed by Mosmann[20] was modified[21] and used to determine the inhibitory effects of test compounds on cell growth in vitro. Briefly, medium containing 5×10^3 cells (NCI-H 460, HCT 116, A 431, CCRF-HSB-2 or KB cells) was seeded into each well of a 96-well microplate, with the compound solution added simultaneously to triplicate wells, before the final volume was made up to 100 μL. The plate was incubated at 37 °C for 72 h in a humidified atmosphere of 5% CO_2. After the incubation, 10 μL of an MTT solution (5 mg/mL in phosphate-buffered saline lacking calcium and magnesium) was added to each well. After a further incubation for 4 h at 37 °C, 100 μL of 0.02 *N* HCl/50% *N*,*N*-dimethylformamide/20% SDS was added to solubilize any MTT-formazan that had formed. The optical density of each well was measured at 570 nm (OD_{570}) with a microplate reader and the inhibition of cell growth (%) was calculated as $(1 - T/C) \times 100$, where C is the mean OD_{570} of the control group and T is that of the treated group. The IC_{50} was determined from the dose–response curve.

4.3. In vivo antitumor activities of 2-deoxo-2-phenyl-5-deazaflavins (2a and 2b)

Antitumor activity of 2-deoxo-2-phenyl-5-deazaflavins (**2a** and **2b**) against subcutaneously transplanted human adenocarcinoma A 431 was tested according to the modified method developed by Inaba et al.[22] The A 431 human adenocarcinoma was subcutaneously transplanted into mice skin of neonate (BALB/c nude mice), and the administration of test compounds was started on the day (day 1) when the mice show tumor growth in the 100–300 mm^3 range. Staurosporine as a positive control was used at 1 mg/kg and compounds **2a** and **2b** were used at 25 mg/kg for intratumoral administration (it), at 100 mg/kg for intra-abdominal administration (ip), and at 75 and 150 mg/kg for oral administration (po). Since the test compounds showed poor solubility in water, the administrations of these suspended in 0.5% gum arabic were done into intra-tumor (it), intraperitoneally (ip) or orally (po). The administration schedule for it was five consecutive daily injections per week for 3 weeks (days 1–5, 8–12, and 15–19). Administration schedules for ip and po were consecutive daily administration for 10 days (days 1–10). The tumor volume (T/C %) was measured on the 17 days (it), 10 days (ip), and 18 days (po) after the treatment of chemicals. The length (a) and width (b) of the tumor mass were measured twice or three times a week, and the tumor volume was calculated according to the following formula: tumor volume (mm^3) = $(a \times b^2)/2$. Relative tumor volume (T/C %) was calculated by the Battele Columbus formula, T/C % = $(T_n/T_1) \times 100/(C_n/C_1)$, in which C_n = tumor volume of control animals on day n and T_n = tumor volume of actively treated animals on day n of the treatment.

4.4. Docking study

AutoDock 3.05,[18] a grid-based docking program, was used for docking study. The crystal structure of protein tyrosine kinase $pp60^{c\text{-}src}$ (PDB code: 1skj),[23] which is a c-src tyrosine kinase of Rous sarcoma virus, was used in this docking study. The 3D structure of 1skj was reported by Plummer et al.[23] using X-ray diffraction technique with a resolution of 2.10 Å. The 1skj was retrieved from the Brookhaven protein database: URL*http://www.rcsb.org/pdb/Wecome.do accessed on January 10, 2006, as a complex bound with the inhibitor ur2: 4-[3-carboxymethyl-3-(4-phosphonooxybenzyl)ureido]-4-[(3-cyclohexylpropyl)-methylcarbamoyl]butyric acid. For the docking study, water molecules and ur2 ligand were removed from the protein, then the polar hydrogens and united atom *Kollman* charges were assigned for the protein. Then, various inhibitors were docked within the prepared protein (1skj). The mode

of interaction of ur2 ligand against 1skj tyrosine was used a standard docked model, the one used for the calculation of the root mean square deviation (RMSD) of the docked inhibitors.

4.4.1. Docking parameters. Prior to the AutoDock, AutoGrid was carried out for the preparation of the grid map using a grid box with a npts (number of points in *xyz*) of 60–60–60 Å box and 120–120–120 Å box, which encloses the original ligand ur2. The box spacing was 0.3 Å and grid center was designated at dimensions (*x*, *y*, *z*): −5.143, 62.487 and −8.776. A scoring grid was calculated from the original ligand structure (ur2) to minimize the computation time. Finally AutoDock was run using maximum number of retries and generations of 10,000 and 27,000, respectively. The genetic algorithm with local search (GALS) was used for calculation of the docking possibilities. The complexes obtained by AutoDock were minimized using a maximum 300 iterations and the hybrid GALS runs with max of 250 cycles using different random number seeds to obtain score convergence.

4.4.2. Preparation of small molecules. ChemDraw ultra 8.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2003)] was used for construction of compounds which were converted to 3D structures using Chem3D ultra 8.0 software and the constructed 3D structures were energetically minimized by using MOPAC (semi-empirical quantum mechanics) with AM1 mozyme geometry, 100 iterations and minimum RMS gradient of 0.10.

4.4.3. Evaluation of docked results. Accelrys DS modeling 1.5 software [DS modeling 1.5; Accelrys Inc., San Diego, CA (2005)] was utilized for molecular modeling for the evaluation of hydrogen bonds in ligand–receptor interaction. The correct hydrogen bonds were considered if their geometry angle within 110–180° according to Murray-Rust et al.[24] While the false hydrogen bonds between *N*-oxide moiety and Cys42 were omitted because the bond angles were less than 90° which are not matched with the considered parameters. Also DS modeling was used for measurement of RMSD, which was computed and expressed in Å as a structural comparison of two molecules in terms of distance. That is, it was measured as distance between the centroid of the docked inhibitor and the original ur2 ligand.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.09.063.

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