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Synthesis, biological active molecular design, and molecular docking study of novel deazaflavin–cholestane hybrid compounds

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ABSTRACT

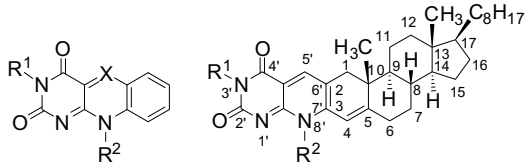
Novel deazaflavin–cholestane hybrid compounds, 3′,8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones, have been synthesized by condensation reaction between 6-(monosubstituted amino)-pyrimidin-2,4(1*H*,3*H*)-diones and 2-hydroxymethylenecholest-4-en-3-one in presence of *p*-toluenesulfonic acid monohydrate and diphenyl ether. The antitumor activities against human tumor cell lines (CCRF-HSB-2 and KB cells) have been investigated in vitro, and many of these compounds showed promising antitumor activities. Furthermore, molecular docking study using LigandFit within the software package Discovery Studio 1.7 was done for lead optimization of these compounds as potential PTK inhibitors. In general, all of the synthesized steroid-hybrid compounds showed good binding affinities into PTK (PDB code: 1t46).

1. Introduction

Design and synthesis of new type of pharmacologically interesting steroidal hybrid compounds have received much attention during the past two decades. Recently, we have reported the synthesis of new hybrid compounds such as androstanolone with 5-deazaflavins and their potential anticoccidiosis activities.[1] These compounds were synthesized with the rationale that steroids can penetrate the cell membrane, bind the cell nucleus and steroid receptors may serve to localize and concentrate appended drug species, mainly in hormones responsive cancers. Moreover, 5-deazaflavins (5-deazaisoalloxazines) have greatly attracted our interest.[2–7] Actually, the interest in 5-deazaflavin is by reason of their first synthesis as potential flavin antagonists or flavin models,[8] the discovery that they can serve as co-factors for several flavin-catalyzed reactions[9] and having the potent broad-spectrum activity against coccidiosis.[10]

It has been reported that the redox active coenzyme Factor 420 (F_{420}), which has 5-deazaflavin skeleton and absorbance maximum at 420 nm, was isolated from methanogenic bacteria.[11] Also, 5-deazaflavins have been known for their strong oxidizing ability which can oxidize alcohols and amines to the corresponding carbonyl compounds, and they behave as autorecycling turnover catalysts in the redox reaction.[12,13] In addition, our previous study on the selective protein kinase C (PKC) inhibitory activities of 5-deazaflavins, 10-substituted-2-deoxo-2-phenyl-5–deazaflavins {2-phenylpyrimido[4,5-*b*]quinolin-4(10*H*)-ones} and flavin-5-oxides have revealed that they can exhibit significant potential antitumor activities against NCI-H 460, HCT 116, A 431, CCRF-HSB-2, and KB cells.[2] (Scheme 1).

Molecular docking methodologies are powerful tools in drug design, which avail to predict the best mode by which a given compound will fit into a binding site of a macromolecular target.[14] The in silico-based docking methods are of practical importance for lead-compound generation in drug discovery.[15] Molecular docking enables one to rapidly screen large chemical databases and thereby identify promising candidate compounds for further experimental processing.[16] Many docking programs such as DOCK,[17] FlexX,[18] GOLD,[19] AutoDock,[20] GLIDE,[21] QXP,[22] and ICM[23] have been developed to achieve the purpose. In this study, we present an approach of flexible docking of molecules using the docking program



X= CH: 5-Deazaflavin
X= N: Flavin

3′,8′-Disubstituted-5′-deaza-cholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones

Scheme 1. Flavin, 5-deazaflavin and 3′,8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-g]pteridine-2′,4′(3′*H*,8′*H*)-diones.

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LigandFit within the software package Discovery Studio 1.7. LigandFit[24] is said to provide a rapid accurate protocol for docking small molecule ligands into protein active sites by considering shape complimentarity between the ligand and the protein active site. Ligand docking is a method in which the conformational selection of compounds is of high impact for the feasibility of the proposed binding poses. The program processes compound collections in two steps viz. the docking step, which uses combination of a shape-based filter with a Monte-Carlo conformational search and the scoring step, which can be performed with one or several scoring functions.[25]

In the present study, we describe the novel synthesis of new archetype of deazaflavin–steroid hybrids which are biologically and pharmacologically different active compounds. As a new trial for the active molecular design of the drug, we have selected cholesterol as the steroid moiety. The target molecule is composed of a six-membered ring system having deazaflavin moiety incorporated onto the A-ring of cholesterol. Also, the compounds were docked into the binding pocket of PTK using LigandFit within the software package Discovery Studio 1.7.

2. Result and discussion

2.1. Chemistry

The requisite precursors, 6-(monosubstituted amino)-3-methylpyrimidin-2,4(1*H*,3*H*)-diones (**4a–j**), 6-(monosubstituted amino) -pyrimidin-2,4(1*H*,3*H*)-diones (**6a–i**), and 6-(monosubstituted amino)-3-phenylpyrimidin-2,4(1*H*,3*H*)-diones (**8a–g**), were prepared by direct fusion of 6-chloro-3-methylpyrimidin-2,6(1*H*, 3*H*)-dione,[26] 6-chloropyrimidin-2,6(1*H*,3*H*)-dione[26] and 6-chloro-3-phenylpyrimidin-2,6(1*H*,3*H*)-dione[27] with appropriate alkyl or aryl amino derivatives, respectively. The 6-chloro-3-methylpyrimidin-2,6(1*H*,3*H*)-dione was obtained by reaction of 1-methylurea and malonic acid in presence of acetic acid and acetic anhydride,[28] followed by chlorination with phosphoryl chloride. The 6-chloropyrimidin-2,6(1*H*,3*H*)-dione was obtained by chlorination of barbituric acid, first forming 2,4,6-trichloropyrimidine and then hydrolysis by sodium hydroxide. And the 6-chloro-3-phenylpyrimidin-2,6(1*H*,3*H*)-dione was obtained by reaction of 1-phenylurea and diethyl malonate in presence of sodium ethoxide, forming 1-phenylbarbituric acid.[29] After chlorination by phosphoryl chloride, three chloro derivatives of phenyl barbituric acid were obtained, from which 6-chloro-3-phenylpyrimidin-2,6(1*H*,3*H*)-dione was isolated following the method as described in the literature.[30] The amino derivatives of the corresponding starting compounds were prepared from appropriate alkyl or aryl amines by direct fusion reaction. The derivatives containing aryl amines could be prepared by the fusion of appropriate amines with corresponding starting compounds at higher temperature (180–200 °C) for approximately 10–15 min, whereas the preparation of the derivatives containing alkyl amines having the lower boiling point such as methylamine and ethylamine, requires rather strenuous conditions and is carried out in a stainless steel bomb. Usually reaction of alkyl amines needs longer time and those having lower molecular weight and boiling points require more than 15 h for the completion of the reaction (Scheme 2).

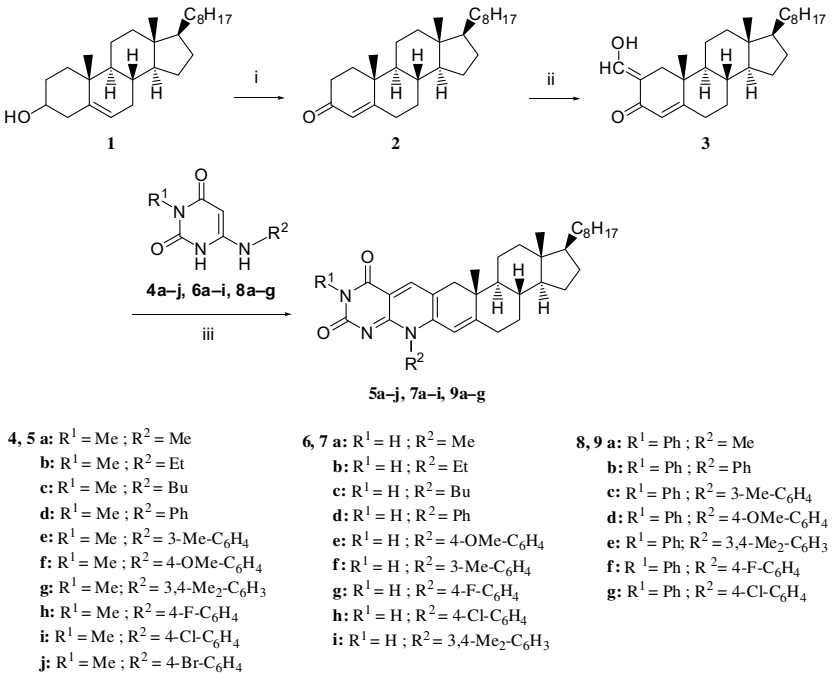
The key intermediate, 2-hydroxymethylenecholestenone (**3**), was synthesized by treating cholest-4-en-3-one (**2**), with ethyl formate in the presence of sodium hydride.[31] The precursor, cholest-4-en-3-one (**2**) was prepared by the oxidation of cholesterol using aluminum isopropoxide as the oxidizing agent and acetone as proton acceptor as described by Oppenauer.[32] The target hybrid compound was then prepared by heating the key intermediate, 2-hydroxymethylenecholest-4-en-3-one (**3**) (1 equiv) with 3,6-disubstituted-pyrimidin-2,6(1*H*,3*H*)-diones (**4**, **6**, and **8**) (0.9 equiv) and *p*-toluenesulfonic acid monohydrate (0.01 equiv) in diphenyl ether (1 mL) under an environment of nitrogen with constant stirring at 180 °C for 45 min. It has been found that temperature higher than the 180 °C could lead to the formation of other side products, and lower temperature causes the reduction in the overall yield of the product. Also, diphenyl ether has been found to be appropriate solvent for the reaction than other usual solvents like dioxane, dimethylformamide, and acetic anhydride, acetic acid, etc. The products (**5**, **7**, and **9**) thus obtained were separated by flash column chromatography on silica gel (230–400 mesh, Fuji Silysia Co. Ltd, BW-300; eluent: ethyl acetate).

UV–vis, IR, NMR spectra, and elemental analyses were used for the determination and identification of the synthesized novel steroidal hybrid compounds. The structures of the compounds, in particular, were confirmed by ^{1}H NMR and ^{13}C NMR, where cholesterol moiety and deazaflavin moiety both showed their characteristic peaks. Particularly, the characteristic singlet signal of $C_{5'}$-H at the lower field at δ_H 8.2–8.6 strongly supports the presence of deazaflavin moiety in the skeleton of the hybrid compounds. The appearance of the peak at such downfield region indicates that the methine at $C_{5'}$-position is more electron-deficient than other methine. It has been found that the aryl substituents in the 8′-position of the hybrid compound cause the deshielding of the $C_{5'}$-proton, exhibiting the signal at δ_H8.35–8.46. This may probably be due to the electron-withdrawing inductive effect of the aromatic ring. Steroidal moiety of the hybrid compound had appeared at the upper field at δ_H 0.7–3.3, showing the presence of characteristic cholesterol peaks. The geminal protons at the C_1-position of cholestane-2,4-diene appeared in the region δ_H 2.6–3.0 with the coupling constant value of 15.6–16.2 Hz, in agreement with usually found value for geminal coupling constant (12–18 Hz). The downfield doublet was assigned for axial proton (1α-H), and the upfield doublet was assigned for equatorial proton (1β-H). It is also noteworthy that substituents at the $C_{3'}$-position have less effect on the shifting of the peaks than the substituents at the $C_{8'}$-position. Moreover, aryl substituents at the 8′-position were generally found to be deshielding the protons, causing the appearance of the peaks at downfield region than that of corresponding compounds possessing alkyl substituents. In ^{13}C-NMR, the characteristic peak of $C_{5'}$-H appeared in the downfield region of δ_C 140–142, exhibiting the high electrophilicity of the carbon.

The structural identity of the synthesized hybrid compounds was verified by UV–vis absorption spectra. Generally, all of the synthesized compounds showed three absorption maxima at 210–235, 253–291, and 421–457 nm. In case of 3′-methyl-8′-(alkyl or aryl)-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**5**), the absorption peaks appeared in the longer wavelength region than 8′-(alkyl or aryl)-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**7**) and 3′-phenyl-8′-(alkyl or aryl)-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**9**). An aryl substituent at 8′-position was found to cause the blue shifting (hypsochromic shift) of the absorption peaks in the region of 210–235 nm and red shifting (bathochromic shift) in the region of 421–457 nm. It is due to the presence of absorption maxima at the region of 421–457 nm that all of the hybrid compounds are colored, ranging from yellow to red.

2.2. In vitro antitumor activities of 3′,8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones

The synthesized compounds were tested in vitro for growth inhibitory activities against various cultured tumor cell lines. Two human tumor cell lines viz, human T-cell acute lymphoblastoid leukemia cell line (CCRF-HSB-2) and human oral epidermoid carcinoma cell line (KB) were used for the test using the antitumor agent with cytosine arabinoside (Ara-C) as the positive control in



Scheme 2. General procedure for the preparation of 3′,8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**5a–j**, **7a–i**, **9a–g**). Reagents and conditions: (i) Al(*i*-PrO)$_3$, acetone, toluene, 85 °C, 8 h, 79%; (ii) HCOOEt, NaH, toluene, 3 days, rt, 92%; (iii) 0.01 equiv *p*-TsOH, Ph_2O, 180 °C, 45 min.

the study. The IC_{50} values [the concentration (μg/mL) required for 50% inhibition of cell growth] for these compounds are shown in Table 1.

As can be seen in Table 1, many deazaflavin–cholestane hybrids derivatives have been found to show fairly good antitumor activities. Although the tested steroidal hybrid compounds exhibited poor antitumor activities than that of the control, Ara-C (IC_{50}: 0.017 μg/mL), appreciably many of the compounds showed the 2.1–4.3 μg/mL potential growth inhibitory activities against CCRF-HSB-2 cell line revealing their promising potential inhibitory activities and less cytotoxicities. Among them, the compounds **7e** and **9c** are found to be better inhibitors with less than 2.5 μg/mL (IC_{50}). Other compounds **7f–h** also exhibited promising antitumor activities of 2.7 μg/mL (IC_{50}). Similarly, in case of KB cell line, they exhibited good growth inhibitory activities of about one-third antitumor potency of Ara-C (IC_{50}: 0.73 μg/mL), that is, the IC_{50} value of the compounds **7e**, **7g**, **7h**, and **9c** were 1.7, 2.1, 1.8, and 1.9 μg/mL, respectively. Moreover, almost all of the compounds exhibited reasonable potential growth inhibitory activities of ca. 4.0–8.0 μg/mL (IC_{50}) against CCRF-HSB-2 cell line. Also, almost all of the compounds exhibited appreciable potential growth inhibitory activities of ca. 4.0–8.0 μg/mL (IC_{50}) against KB cell line. The SAR revealed that the substituents at the 3′-position of the hybrid compounds figure out the potential inhibitory activities of the compounds towards tumor cell lines. In general, any substitution at the 3′-position was found to reduce the antitumor activities of the compounds. It has been revealed that compounds possessing methyl substituent at the 3′-position exhibited less potent antitumor activities than those possessing phenyl substituent or no substituents. The compounds with aryl groups at the 8′-position also showed more efficient antitumor activities than those with alkyl groups in case of both CCRF-HSB-2 and KB cell lines.

2.3. Molecular docking study

The in silico automated docking studies were performed using the molecular docking software LigandFit within the software package Discovery Studio 1.7. The docking study of

Table 1
Growth inhibitory activities of 3′,8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**5**, **7**, and **9**) against human tumor cell lines (CCRF-HSB-2 and KB)

Compound	Inhibitory activity [IC_{50} (μg/mL)]				Compound	Inhibitory activity [IC_{50} (μg/mL)]			
	R_1	R_2	CCRF-HSB-2	KB		R_1	R_2	CCRF-HSB-2	KB
5a	Me	Me	36.8	40.9	**7e**	H	4-OMe-C_6H_4	2.1	1.7
5b	Me	Et	>100.0	>100.0	**7f**	H	3-Me-C_6H_4	2.7	4.4
5c	Me	Bu	10.0	7.5	**7g**	H	4-F-C_6H_4	2.7	2.1
5d	Me	Ph	6.2	7.6	**7h**	H	4-Cl-C_6H_4	2.7	1.8
5e	Me	3-Me-C_6H_4	5.4	7.4	**7i**	H	3,4-Me-C_6H_3	4.7	7.8
5f	Me	4-OMe-C_6H_4	4.5	7.4	**9a**	Ph	Me	5.5	7.4
5g	Me	3,4-Me-C_6H_3	5.6	8.3	**9b**	Ph	Ph	2.4	1.9
5h	Me	4-F-C_6H_4	6.9	8.4	**9c**	Ph	3-Me-C_6H_4	5.4	8.0
5i	Me	4-Cl-C_6H_4	7.6	9.2	**9d**	Ph	4-OMe-C_6H_4	7.5	8.7
5j	Me	4-Br-C_6H_4	14.0	14.2	**9e**	Ph	3,4-Me-C_6H_3	7.7	9.1
7a	H	Me	8.8	8.4	**9f**	Ph	4-F-C_6H_4	5.5	8.4
7b	H	Et	37.9	40.3	**9g**	Ph	4-Cl-C_6H_4	9.0	9.3
7c	H	Bu	4.3	5.7	Ara-C			0.017	0.73
7d	H	Ph	5.4	7.9					

3′,8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**5**, **7**, and **9**) was carried out, which were docked within the protein tyrosine kinase (PDB code: 1t46). Protein tyrosine kinases (PTKs) are enzymes, which catalyze the phosphorylation of tyrosine residues, and these enzymes are involved in cellular signaling pathways and regulate key cell functions such as proliferation, differentiation, antiapoptotic signaling, and neuritis outgrowth. Unregulated activation of these enzymes can lead to various forms of cancer as well as benign proliferative conditions.[33] Therefore, efforts have been concentrated to develop potential inhibitors which can inhibit the activity of tyrosine kinase and thus could lead to the development of a new class of therapeutics of cancers as well as other proliferative diseases. In the present study, we have studied the in silico binding affinities of the synthesized steroid-hybrids, 3′,8′-disubstituted-5′-deaza-cholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**5, 7**, and **9**) into PTK.

2.3.1. Validation of the accuracy and performance of LigandFit

As a general rule, if the best-docked conformation of a ligand resembles the bound native ligand in the experimental crystal structure, the used scoring function is said to be successful. According to the literature,[34] the successful scoring function is the one in which the RMSD (root mean square deviation) of the best docked conformation is $\leqslant$2.0 Å from the experimental one. Therefore, before doing actual docking simulation experiment with synthesized compounds, the validation of the function achieved in LigandFit/Discovery Studio 1.7 was validated by docking of the native ligand into its binding site. Then the docked result was compared with the crystal structure of the bound ligand–protein complex. It has been confirmed that the accuracy and performance of the LigandFit/Discovery Studio 1.7 was highly satisfactory, as shown in Figure 1. In the study, the STI-571 ligand [4-[(4-methylpiperazin-1-yl)methyl]-*N*-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino]-phenyl]-benzamide, commonly known as Imatinib or Gleevec was docked into its C-kit receptor PTK (PDB code: 1t46). From our previous studies,[2,3] we have found that the deazaflavin derivatives were favorably fitted into the binding pocket of PTK which was comparable with the native ligand STI-571. Therefore, the STI-571 was selected as a reference ligand for the comparative study of binding action of the synthesized compounds in this study, where we used deazaflavin–cholestane hybrid molecules as ligands.

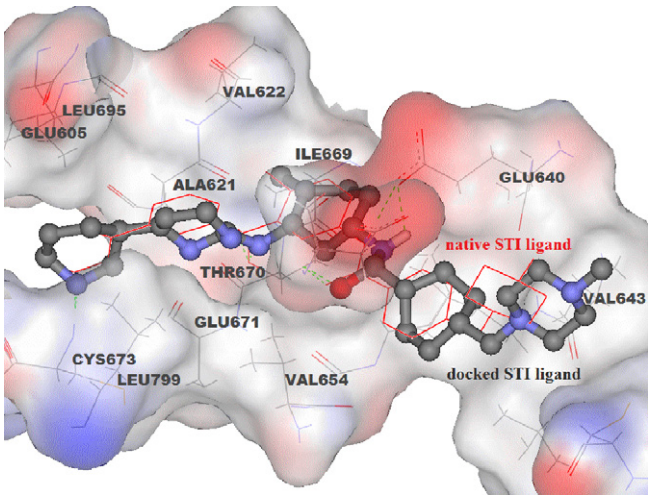


Figure 1. The validation of accuracy and performance of LigandFit. The docked STI ligand (ball and stick, colored by elements) nearly superimposed on the native STI ligand (red line) exhibiting same number of hydrogen bonds (green dotted lines) with same amino acids involved by the native one. ΔG_b: −10.42 kcal/mol; RMSD: 1.15 Å.

As can be seen in Figure 1, the docked ligand (shown in ball and stick, colored by element) has been exactly superimposed on the native bound one (shown in a red line) with same number of hydrogen bonds. It has been revealed that the docked ligand formed four hydrogen bondings with the same four amino acids viz., Cys 673, Thr 670, Glu 640, and Asp 810, involved in the native bound ligand. The RMSD of the docked ligand was 1.15 Å and the calculated binding free energy (ΔG_b) of the docked ligand was −10.42 kcal/mol. These results exhibited the high accuracy and success of the LigandFit simulation in comparison with the biological methods.

2.3.2. Calculation of binding affinities of the synthesized deazaflavin–cholestane hybrid compounds into PTK

The binding affinity was evaluated by the binding free energies (ΔG_b, kcal/mol), inhibition constant, hydrogen bonding and RMSD values. The synthesized deazaflavin–cholestane hybrid compounds are composed to two parts, one having more hydrophilic deazaflavin moiety and another having highly lipophilic cholestane moiety. Due to the presence of bulky lipophilic cholestane moiety, the main interaction involved in the docking simulation was van der Waals forces. The hydrophilic interaction by hydrogen bonding was contributed by deazaflavin moiety, and most of the compounds exhibited only one hydrogen bonding with the PTK receptor. Almost all compounds exhibited similar binding affinities comparable with the native bound STI-571 ligand. Some compounds exhibited lower binding energies than STI-571 ligand, such as **5j**, **7h**, and **9c**, and **9c** exhibited binding free energies as lower as −11.72 kcal/mol in comparison with the STI-571 ligand, that is, −10.42 kcal/mol. This shows that the deazaflavin–cholestane hybrid compounds have possessed better affinities towards PTK than the native bound one. This affinity of the hybrid compounds may be attributed to the high lipophilicity of the steroid moiety of the compound, due to which better van der Waals interaction was prospective between ligand and the receptor. In Table 2, the compounds with higher binding affinities, that is, lower binding energies and their hydrogen bond interactions into the target macromolecules are listed. As shown in the Table 2, two compounds **7c** and **9f** exhibited multiple hydrogen bonding; compound **7c** (ΔG_b: − 9.59 kcal/mol) exhibited two hydrogen bonds with Thr 670 and Val 668 and compound **9f** (ΔG_b: −8.75 kcal/mol) also exhibited two hydrogen bonds with two NH moieties of Arg 684.

The molecular docking study revealed that the majority of the compounds docked into the c-Kit receptor PTK (PDB code: 1t46) with its bound ligand STI exhibited hydrogen bonds via their $C_{4'}$ and $C_{2'}$-oxo groups as illustrated for compounds **7b** and **7c** in Figure 2 and for compounds **5a**, **7e**, and **9a** in Figure 3.

The study of correlation between the growth inhibitory activities (IC_{50}, μg/mL) of the synthesized 3′,8′-disubstituted-5′deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones against tumor cells and binding affinities predicted by LigandFit showed fairly good correlation for some compounds. In case of the growth inhibition against CCRF-HSB-2 cells, the fairly good correlation was obtained for compounds **5c**, **d**, **f**–**i**, **7c**, **d**, **h**, **9a**, **b**, **d**, **f**, and **9g** with correlation coefficient (R^2) of 0.5185, as shown in Figure 4. Similarly, the growth inhibition against KB cells exhibited also similar correlation with LigandFit binding free energies for compounds **5c**–**e**, **h**, **7c**–**f**, **h**, **i**, and **9b**, **d**, **f**, **g** with correlation coefficient (R^2) value of 0.572 as shown in Figure 5.

3. Conclusion

In this study, various novel deazaflavin–cholestane hybrid compounds, 3′,8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**5a–j**, **7a**–**i**, and **9a–g**) have been

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

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 (°)	
5a	−9.60	0.12	$C_{4'}$-Oxo	HN of Lys 623	2.28	122.60	8.75
5b	−9.66	0.11	$C_{4'}$-Oxo	HN of Lys 623	2.01	126.40	8.71
5c	−8.81	0.45	$C_{4'}$-Oxo	HN of Cys 673	1.75	178.10	13.76
5f	−10.15	0.05	Ar-OCH_3	HN of Ile 789	2.18	133.70	6.12
5j	−11.58	4.40E−03	C_2-Oxo	HN of Lys 593	1.76	147.50	9.68
7a	−10.29	0.04	$C_{2'}$-Oxo	HN of Lys 593	1.77	150.70	8.60
7b	−9.23	0.22	$C_{4'}$-Oxo	HN of Lys 623	1.75	130.90	10.26
7c	−9.59	0.12	$C_{4'}$-Oxo	O of Thr 670	2.13	151.40	3.64
			3′NH	O of Val 668	2.08	157.20	
7e	−9.77	0.09	Ar-OCH_3	HN of Ile 789	2.20	132.60	6.06
7g	−8.85	0.42	Ar-F	HN of Ile 571	2.30	157.90	8.56
7h	−11.60	4.30E−03	$C_{2'}$-Oxo	HN of Lys 593	1.75	148.20	10.20
9a	−9.81	0.08	—	—	—	—[d]	11.78
9b	−10.04	0.06	$C_{4'}$-Oxo	HN of Asn 680	2.50	102.40	10.49
9c	−11.72	3.50E−03	$C_{2'}$-Oxo	HN of Lys 593	1.80	147.90	10.49
9d	−9.16	0.25	Ar-OCH_3	HN of Arg 684	2.45	115.70	11.01
9e	−10.42	0.03	$C_{4'}$-Oxo	HN of Asn 680	2.45	106.80	10.74
9f	−8.75	0.49	Ar-F	HN of Arg 684	2.02	133.20	11.15
			Ar-F	HN of Arg 684	2.27	128.90	
STI[e]	−10.42	0.03	N_5-N	HN of Cys 673	2.03	146.00	1.15
			NH (H56)	O of Thr 670	1.87	116.50	
			NH (H66)	O of Glu 640	2.18	113.10	
			036	HN of Asp 810	1.79	145.40	

[a] Binding free energy.
[b] Inhibition constant.
[c] Root mean square deviation.
[d] No hydrogen bond.
[e] The docked ligand (STI-571) of PTK (PDB code: lt46).

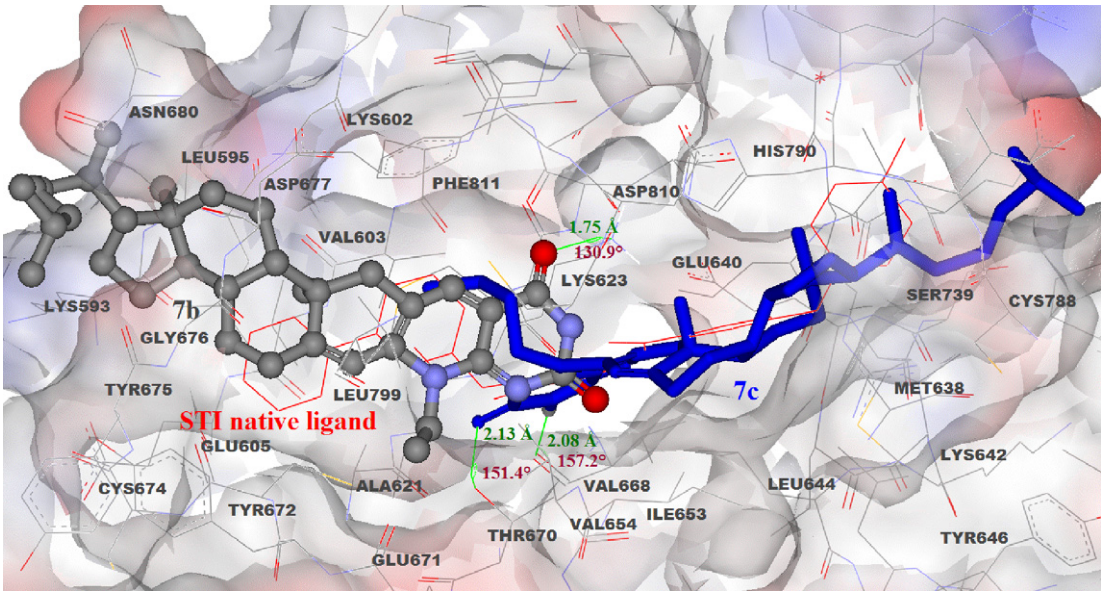


Figure 2. Compound (**7b**) (colored by elements, ball and stick) is bound into the PTK receptor site via hydrophobic interaction with Asn 680, Lys 593, Leu 595, Asp 677, Val 603, Gly 676, Leu 799, Tyr 672, Ala 621, and Phe 811 in addition with one hydrogen bond (green line) between C_4-oxo and NH of Lys 623, while compound (**7c**) (blue stick) exhibited two hydrogen bonds with Thr 670 and Val 668. The binding pocket is shown in the solid surface with labeled amino acids, and the STI ligand is shown as a red line.

synthesized by condensation reaction between 6-(monosubstituted amino)-pyrimidin-2,4(1*H*,3*H*)-diones (**4a–j, 6a–i**, and **8a–g**) and 2-hydroxymethylenecholest-4-en-3-one (**3**) in presence of *p*-toluenesulfonic acid monohydrate and diphenyl ether. Compounds 5a–**j** were synthesized in better yield than compounds **7a–i** and **9a–g**. Our strategy for the study was to synthesize three parallel derivatives of 3-substituted deazaflavin–cholestane hybrid compounds and the comparative study of their antitumor activity potential and in silico binding affinities. From the study it has been revealed that the 3-unsubstituted hybrid compounds (**7a–i**) possessed better antitumor potencies than 3-methyl and 3-phenyl derivatives. Also, 3-methyl derivatives (**5a–j**) exhibited the least potential antitumor activities in vitro against CCRF-HSB-2 and KB cells. Compound **7e** exhibited best antiproliferative potencies with IC_{50} value of 2.1 μg/mL against CCRF-HSB-2 and 1.7 μg/mL against KB cells. Other derivatives like **7f–h** exhibited fairly similar activities with IC_{50} value of 2.7 μg/mL against CCRF-HSB-2 and 1.8–4.4 μg/mL against KB cells. These results showed that

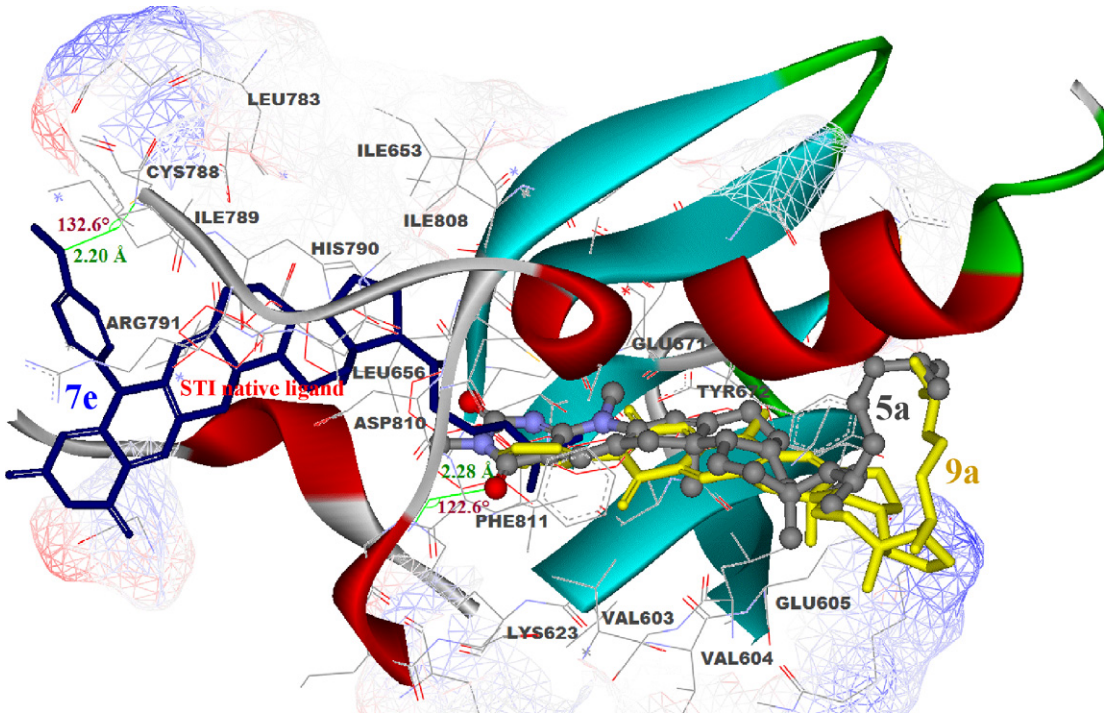


Figure 3. Stereo view for comparative binding affinities of 3′,8′-dimethyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**5a**, ball and stick), 8′-(4-methoxyphenyl)-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**7e**, blue stick), and 8′-methyl-3-phenyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**9a**, yellow stick). Compounds **5a** and **7e** exhibited one hydrogen bond shown as a green line, respectively, and compound **9a** exhibited no hydrogen bond. The binding pocket of PTK is shown in the wire mesh surface with labeled amino acids, the PTK protein structure is shown in solid ribbon view, and the STI ligand is shown as a red line.

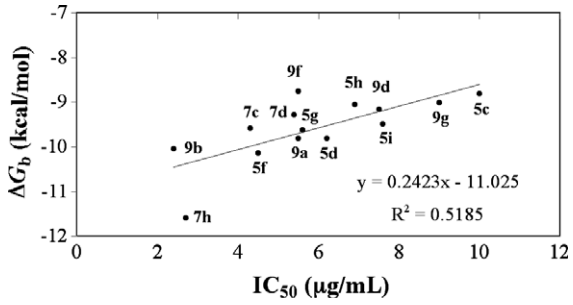


Figure 4. Correlation between the binding energy (ΔG_b) and IC_{50} (μg/mL) of 3′, 8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**5c**, **d**, **f–i**, **7c**, **d**, **h**, and **9a**, **b**, **d**, **f**, **g**) against human T-cell acute lymphoblastioid leukemia cells (CCRF-HSB-2).

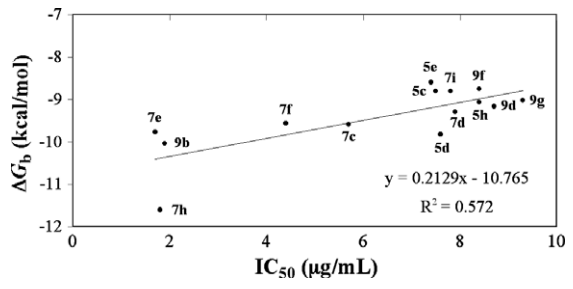


Figure 5. Correlation between the binding energy (ΔG_b) and IC_{50} (μg/mL) of 3′,8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (**5c–e**, **h**, **7c–f**, **h**, **i**, and **9b**, **d**, **f**, **g**) against human oral epithelial carcinoma cells (KB).

3-unsubstituted deazaflavin–cholestane hybrid compounds **7c–i** could be the potential candidate for antiproliferative activities with better antitumor activities with less cytotoxicities.

The in silico docking study of the synthesized deazaflavin–cholestane derivatives (**5a–j**, **7a–i**, and **9a–g**) was carried out for lead optimization using the software LigandFit within the software package Discovery Studio 1.7. The synthesized hybrid compounds were docked into the c-kit protein tyrosine kinase. Almost all of the compounds exhibited good binding affinities within the PTK receptor. The overall correlation between the growth inhibitory activities of the synthesized deazaflavin–cholestane hybrid compounds against tumor cells and the binding affinities predicted by LigandFit was fairly good for some compounds. In case of the growth inhibition against CCRF-HSB-2 cells, fairly good correlation was obtained for compounds **5c, d, f–i, 7c**, **d**, **h**, **9a**, **b**, **d**, **f**, and **9g** with correlation coefficient (R^2) of 0.5185. Similarly, the growth inhibition against KB cells also exhibited similar correlation with LigandFit binding free energies for compounds **5c–e, h, 7c–f, h, i, 9b, d, f** and **9g** with correlation coefficient (R^2) value of 0.572.

Thus, the overall studies by computational design, chemical synthesis, and biological investigation revealed that the deazaflavin–cholestane hybrid compounds are the new archetype of hybrid compounds with promising antiproliferative activities with less cytotoxicities. Further, the in silico binding affinity investigation revealed that due to the higher lipophilicity of the hybrid compounds, they possess good binding affinities within the protein tyrosine kinase.

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. The specific optical rotations were measured by JASCO DIP-1000 digital polarimeter,

and absolute ethanol was used as the solvent. IR spectra were recorded on a JASCO FT/IR-200 spectrophotometer as Nujol mulls. ^{1}H and ^{13}C NMR spectra were obtained using a Varian VXR 300 and 75 MHz spectrophotometer, respectively, and chemical shift values are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as internal standard. Coupling constants are given in Hertz. UV spectra were measured in absolute EtOH using Beckman DU-68S UV spectrophotometers and absorption values in italics refers to wave lengths at which shoulders or inflexions occur in the absorption. All reagents were of commercial quality and were used without further purification. Organic solvents were dried in the presence of appropriate drying 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 3′,8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-diones (5, 7, and 9)

A mixture of 2-hydroxymethylenecholest-4-en-3-one (**3**) (1.0 g) and 3,6-disubstituted-pyrimidin-2,6-(1*H*,3*H*)-diones (**4, 6**, and **8**) (0.9 equiv) in diphenyl ether (1 mL) in the presence of *p*-toluenesulfonic acid monohydrate (0.01 equiv) was refluxed with constant stirring under an environment of nitrogen at 180 °C for 45 min. After cooling, the reaction mixture was diluted with ethanol, and the products were separated using flash column chromatography on silica gel (230–400 mesh, Fuji Silysia Co. Ltd, BW-300; eluent: ethyl acetate). The yellow or red crystals thus obtained were recystallized from an appropriate solvent to afford the corresponding product as yellow or red needles in 30–55% yield.

4.1.2. 3′,8′-Dimethyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (5a)

Yield, (570 mg, 44%); mp 250–252 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −30.60°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 235 (4.24), 289 (4.26), 422 (4.19); IR (ν_{max}/cm^{-1}): 1688, 1638 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.73 (3H, s, 18-CH_3), 0.88 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 0.98 (3H, s, 19-CH_3), 1.11–2.11 (22H, m), 2.55 (2H, m, 6-CH_2), 2.67 (1H, d, J = 15.6 Hz, 1β-H), 2.87 (1H, d, J = 15.6 Hz, 1α-H), 3.45 (3H, s, 3′-NCH_3), 4.04 (3H, s, 8′-CH_3), 6.40 (1H, s, 4-CH), 8.26 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$)[35]: δ_C 11.92 (C_{18}), 17.56 (C_{21}), 18.65 (C_{19}), 21.62 (C_{11}), 22.56 (C_{26}), 22.82 (C_{27}), 23.80 (C_{23}), 24.26 (C_{15}), 28.01 (3′-NCH_3), 28.15 (C_{16}), 31.18 (C_{25}), 33.49 (C_6 and C_7), 35.73 (8′-NCH_3), 35.76 (C_{22}), 36.09 (C_8 and C_{20}), 38.95 (C_{12}), 39.47 (C_{24}), 39.54 (C_{10}), 40.32 (C_1), 42.40 (C_{13}), 53.69 (C_{14}), 55.71 (C_9), 56.10 (C_{17}), 111.26 (C_4), 112.75 ($C_{6'}$), 117.52 ($C_{4'a}$), 140.18 ($C_{5'}$), 147.90 ($C_{7'}$), 155.62 ($C_{2'}$=O), 157.37 (C_5), 162.71 ($C_{4'}$=O), 168.99 ($C_{8'a}$). Anal. Calcd for $C_{34}H_{49}N_3O_2\cdot0.3H_2O$: C, 76.02; H, 9.31; N, 7.82. Found: C,76.04; H, 9.13; N, 7.83.

4.1.3. 8′-Ethyl-3′-methyl-5′-deazacholest-2, 4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (5b)

Yield, (680 mg, 51%); mp 275–277 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −28.44°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 235 (4.36), 289 (4.38), 421 (4.31); IR (ν_{max}/cm^{-1}): 1686, 1637 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 0.98 (3H, s, 19-CH_3), 1.11–2.11 (22H, m), 1.40 (3H, t, J = 6.9 Hz, 8′-NCH$_2$$CH_3$), 2.55 (2H, m, 6-$CH_2$), 2.68 (1H, d, J = 15.3 Hz, 1β-H), 2.88 (1H, d, J = 15.3 Hz, 1α-H), 3.45 (3H, s, 3′-NCH_3), 4.54 (1H, br s, 8′-NCH_aH$_b$CH$_3$), 4.85 (1H, br s, 8′-NCH$_a$$H_bCH_3$), 6.37 (1H, s, 4-CH), 8.27 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.24 (C_{18}), 14.25 (8′-NCH$_2$$CH_3$), 17.89 ($C_{21}$), 18.96 ($C_{19}$), 21.93 ($C_{11}$), 22.87 ($C_{26}$), 23.13 ($C_{27}$), 24.11 ($C_{23}$), 24.57 ($C_{15}$), 28.32 (3′-N$CH_3$), 28.46 ($C_{16}$), 31.48 ($C_{25}$), 33.73 ($C_6$ and C_7), 36.08 (C_{22}), 36.39 (C_8 and C_{20}), 39.18 (C_{12}), 39.79 (C_{24}), 39.86 (C_{10}), 40.77 (C_1), 41.36 (8′-NCH_2CH$_3$), 42.71 (C_{13}), 53.99 (C_{14}), 56.03 (C_9), 56.41 (C_{17}), 111.71 (C_4), 112.76 ($C_{6'}$), 118.13 ($C_{4'a}$), 140.48 ($C_{5'}$), 147.50 ($C_{7'}$), 155.29 ($C_{2'}$=O), 157.91 (C_5), 163.10 ($C_{4'}$=O), 168.99 ($C_{8'a}$). Anal. Calcd for $C_{35}H_{51}N_3O_2$: C, 77.02; H, 9.42; N, 7.70. Found: C,77.09; H, 9.24; N, 7.69.

4.1.4. 8′-Butyl-3′-methyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (5c)

Yield, (745 mg, 53%); mp 128–130 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −24.80°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 255 (4.20), 289 (4.14), 422 (4.05); IR (ν_{max}/cm^{-1}): 1685, 1633 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.73 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 0.98 (3H, s, 19-CH_3), 0.99 (3H, t, J = 7.5 Hz, 8′-NCH$_2$CH$_2$CH$_2$$CH_3$), 1.11–2.11 (22H, m), 1.44 (2H, m,J = 7.5 Hz, 8′-NCH$_2$$CH_2CH_2CH_3$) 2.55 (2H, m, 6-$CH_2$), 2.68 (1H, d, J = 15.3 Hz, 1β-H), 2.88 (1H, d, J = 15.3 Hz, 1α-H), 3.45 (3H, s, 3′-NCH_3), 4.45 (1H, br s, 8′-NCH_aH$_b$CH$_2$CH$_2$CH$_3$), 4.79 (1H, br s, 8′-NCH$_a$$H_bCH_2CH_2CH_3$), 6.34 (1H, s, 4-CH), 8.27 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.08 (C_{18}), 13.93 (8′-NCH$_2$CH$_2$CH$_2$$CH_3$), 17.69 ($C_{21}$), 18.81 ($C_{19}$), 20.09 (8′-NCH$_2CH_2$$CH_2CH_3$), 21.78 ($C_{11}$), 22.72 ($C_{26}$), 22.97 ($C_{27}$), 23.95 ($C_{23}$), 24.42 ($C_{15}$), 28.16 (3′-N$CH_3$), 28.30 ($C_{16}$), 30.81 (8′-NCH$_2$$CH_2CH_2CH_3$), 31.32 ($C_{25}$), 33.67 ($C_6$ and C_7), 35.92 (C_{22}), 36.25 (C_8 and C_{20}), 39.03 (C_{12}), 39.63 (C_{24}), 39.71 (C_{10}), 40.64 (C_1), 42.56 (C_{13}), 45.72 (8′-NCH_2CH$_2$CH$_2$CH$_3$), 53.82 (C_{14}), 55.88 (C_9), 56.26 (C_{17}), 111.52 (C_4), 112.77 ($C_{6'}$), 118.37 ($C_{4'a}$), 140.37 ($C_{5'}$), 147.70 ($C_{7'}$), 155.28 ($C_{2'}$=O), 158.04 (C_5), 162.92 ($C_{4''}$=O), 169.03 ($C_{8'a}$). Anal. Calcd for $C_{37}H_{55}N_3O_2\cdot0.5H_2O$: C, 76.24; H, 9.68; N, 7.21. Found: C, 76.32; H, 9.38; N, 7.25.

4.1.5. 3′-Methyl-8′-phenyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (5d)

Yield, (720 mg, 50%); mp 197–199 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −18.12°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 234 (4.45), 291 (4.38), 428 (4.34); IR (ν_{max}/cm^{-1}): 1684, 1623 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.06–2.32 (24H, m), 2.73 (1H, d, J = 15.6 Hz, 1β-H), 2.93 (1H, d, J = 15.6 Hz, 1α-H), 3.41 (3H, s, 3′-NCH_3), 5.49 (1H, s, 4-CH), 7.13–7.24 (2H, m, 8′-Ar-*m*H), 7.53–7.58 (3H, m, 8′-Ar-*o*,*p*H), 8.38 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.15 (C_{18}), 18.11 (C_{21}), 18.88 (C_{19}), 21.86 (C_{11}), 22.78 (C_{26}), 23.04 (C_{27}), 24.01 (C_{23}), 24.45 (C_{15}), 28.23 (3′-NCH_3), 28.35 (C_{16}), 31.30 (C_{25}), 33.30 (C_6 and C_7), 35.96 (C_{22}), 36.31 (C_8 and C_{20}), 39.27 (C_{12}), 39.70 (C_{24}), 39.79 (C_{10}), 40.45 (C_1), 42.62 (C_{13}), 54.06 (C_{14}), 55.92 (C_9), 56.31 (C_{17}), 111.96 (C_4), 114.08 ($C_{6'}$), 117.38 ($C_{4'a}$), 127.93 (8′-$C_{3''}$) and ($C_{5'}$), 130.07 (8′-$C_{4''}$), 130.34 (8′-$C_{2''}$) and ($C_{5'}$), 137.06 (8′-$C_{1''}$), 141.75 ($C_{5'}$), 148.43 ($C_{7'}$), 157.09 ($C_{2'}$=O), 157.55 (C_5), 162.72 ($C_{4'}$=O), 168.11 ($C_{8'a}$). Anal. Calcd for $C_{39}H_{51}N_3O_{2.0}\cdot0.38H_2O$: C, 77.99; H, 8.68; N, 7.00. Found: C,77.85; H, 8.35; N, 7.39.

4.1.6. 3′-Methyl-8′-(3-methylphenyl)-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (5e)

Yield, (770 mg, 52%); mp 194–196 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −21.72°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 213 (4.41), 291 (4.24), 428 (4.21); IR (ν_{max}/cm^{-1}): 1686, 1631 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.06–2.32 (24H, m), 2.43 (3H, s, 8′-Ar-CH_3), 2.71 (1H, d, J = 15.6 Hz, 1β-H), 2.92 (1H, d, J = 15.6 Hz, 1α-H), 3.39 (3H, s, 3′-NCH_3), 5.51 (1H, s, 4-CH), 6.92–7.01 (2H, m, 8′-Ar-2″,5″H), 7.31–7.47 (2H, m, 8′-Ar-4″,6″H), 8.37 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.08 (C_{18}), 17.99 (C_{21}), 18.81 (C_{19}), 21.64 (C_{11}), 21.78 (8′-Ar-*m*CH_3), 22.71 (C_{26}), 22.96 (C_{27}), 23.94 (C_{23}), 24.38 (C_{15}), 28.16 (3′-NCH_3), 28.28 (C_{16}), 31.25 (C_{25}), 33.25 (C_6, C_7), 35.87 (C_{22}), 36.24

(C_8 and C_{20}), 39.20 (C_{12}), 39.63 (C_{24}), 39.72 (C_{10}), 40.40 (C_1), 42.55 (C_{13}), 54.02 (C_{14}), 55.86 (C_9), 56.24 (C_{17}), 111.81 (C_4), 114.07 ($C_{6'}$), 117.21 ($C_{4'a}$), 124.77 (8′-$C_{4''}$), 128.14 (8′-($C_{5'}$)), 130.13 (8′-$C_{3''}$), 130.88 (8′-$C_{6''}$), 136.95 (8′-$C_{2''}$), 140.49 (8′-$C_{1''}$), 141.54 ($C_{5'}$), 148.45 ($C_{7'}$), 157.03 ($C_{2'}$=O),157.45 (C_5), 162.71 ($C_{4'}$=O), 167.88 ($C_{8'a}$). Anal. Calcd for $C_{40}H_{53}N_3O_2{\cdot}H_2O$: C, 76.76; H, 8.86; N, 6.71. Found: C, 76.90; H, 8.67; N, 6.71.

4.1.7. 8′-(4-Methoxyphenyl)-3′-methyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (5f)

Yield, (530 mg, 35%); mp 185–188 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −26.84°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 226 (4.67), 291 (4.49), 429 (4.40); IR (ν_{max}/cm^{-1}): 1684, 1633 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.06–2.32 (24H, m), 2.71 (1H, d, J = 15.6 Hz, 1β-H), 2.91 (1H, d, J = 15.6 Hz, 1α-H), 3.41 (3H, s, 3′-NCH_3), 3.88 (3H, s, 8′-Ar-OCH_3), 5.59 (1H, s, 4-CH), 7.03–7.06 (2H, m, 8′-Ar-*m*H), 7.10–7.13 (2H, m, 8′-Ar-*o*H), 8.36 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.06 (C_{18}), 18.02 (C_{21}), 18.80 (C_{19}), 21.78 (C_{11}), 22.70 (C_{26}), 22.96 (C_{27}), 23.93 (C_{23}), 24.37 (C_{15}), 28.14 (3′-NCH_3), 28.28 (C_{16}), 31.24 (C_{25}), 33.23 (C_6 and C_7), 35.88 (C_{22}), 36.23 (C_8 and C_{20}), 39.21 (C_{12}), 39.62 (C_{24}), 39.70 (C_{10}), 40.41 (C_1), 42.53 (C_{13}), 53.98 (C_{14}), 55.67 (8′-Ar-OCH_3), 55.84 (C_9), 56.23 (C_{17}), 111.81 (C_4), 114.12 ($C_{6'}$), 115.41 (8′-$C_{3''}$) and ($C_{5'}$), 117.57 ($C_{4'a}$), 125.01 (8′-$C_{1''}$), 128.89 (8′-$C_{2''}$ and $C_{6''}$), 141.59 ($C_{5'}$), 148.95 ($C_{7'}$), 157.18 ($C_{2'}$=O), 157.79 (C_5), 160.43 (8′-$C_{4''}$), 162.65 ($C_{4'}$=O), 168.20 ($C_{8'a}$). Anal. Calcd for $C_{40}H_{53}N_3O_3$: C, 77.01; H, 8.56; N, 6.74. Found: C, 77.30; H, 8.23; N, 6.45.

4.1.8. 3′-Methyl-8′-(3,4-dimethylphenyl)-5′-deazacholest-2, 4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (5g)

Yield, (650 mg, 43%); mp 208–210 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −41.48°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 212 (4.53), 291 (4.34), 428 (4.30), 453 (4.20); IR (ν_{max}/cm^{-1}): 1684, 1625 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71(3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.06–2.30 (24H, m), 2.31 (3H, s, 8′-Ar-3″CH_3), 2.34 (3H, s, 8′-Ar-4″CH_3), 2.71 (1H, d, J = 15.6 Hz, 1β-H), 2.91 (1H, d, J = 15.6 Hz, 1α-H), 3.41 (3H, s, 3′-NCH_3), 5.56 (1H, s, 4-CH), 6.82–6.94 (2H, m, 8′-Ar-2″, 5″H), 7.27–7.31 (1H, m, 8′-Ar-6″H), 8.36 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.08 (C_{18}), 18.06 (C_{21}), 18.80 (C_{19}), 19.82 (8′-Ar-*m*CH_3), 20.16 (8′-Ar-*p*CH_3), 21.78 (C_{11}), 22.71 (C_{26}), 22.96 (C_{27}), 23.94 (C_{23}), 24.38 (C_{15}), 28.16 (3′-NCH_3), 28.28 (C_{16}), 31.24 (C_{25}), 33.14 (C_6 and C_7), 35.87 (C_{22}), 36.24 (C_8 and C_{20}), 39.63 (C_{12}), 39.62 (C_{24}), 39.72 (C_{10}), 40.42 (C_1), 42.54 (C_{13}), 53.01 (C_{14}), 55.86 (C_9), 56.23 (C_{17}), 111.73 (C_4), 114.25 ($C_{6'}$), 117.29 ($C_{4'a}$), 124.86 (8′-($C_{5'}$)), 128.34 (8′-$C_{4''}$), 131.34 (8′-$C_{2''}$), 134.52 (8′-$C_{1''}$), 138.91 (8′-$C_{6''}$ and $C_{3''}$), 141.47 ($C_{5'}$), 148.67 ($C_{7'}$), 157.08 ($C_{2'}$=O), 157.56 (C_5), 162.79 ($C_{4'}$=O), 167.77 ($C_{8'a}$). Anal. Calcd for $C_{41}H_{55}N_3O_2$: C, 79.18; H, 8.91; N, 6.76. Found: C, 79.23; H, 8.86; N, 6.74.

4.1.9. 8′-(4-Fluorophenyl)-3′-methyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (5h)

Yield, (710 mg, 48%); mp 218–220 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −33.38°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 235 (4.33), 291 (4.22), 431 (4.17); IR (ν_{max}/cm^{-1}): 1698, 1634 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.06–2.32 (24H, m), 2.72 (1H, d, J = 15.6 Hz, 1β-H), 2.93 (1H, d, J = 15.6 Hz, 1α-H), 3.41 (3H, s, 3′-NCH_3), 5.52 (1H, s, 4-CH), 7.01–7.16 (2H, m, 8′-Ar-*m*H), 7.20–7.25 (3H, m, 8′-Ar-*o*H), 8.38 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.08 (C_{18}), 18.06 (C_{21}), 18.81 (C_{19}), 21.79 (C_{11}), 22.71 (C_{26}), 22.97 (C_{27}), 23.94 (C_{23}), 24.38 (C_{15}), 28.16 (3′-NCH_3 and C_{16}), 31.23 (C_{25}), 33.30 (C_6 and C_7), 34.48 (C_{22}), 35.88 (C_8 and C_{20}), 36.24 (C_{12}), 39.26 (C_{24}), 39.63 (C_{10}), 40.33 (C_1), 42.54 (C_{13}), 54.01 (C_{14}), 55.83 (C_9), 56.24 (C_{17}), 111.92 (C_4), 113.78 ($C_{6'}$), 117.45 ($C_{4'a}$), 8′-$C_{3''}$ and ($C_{5'}$), 129.89 (8′-$C_{2''}$ and $C_{6''}$), 132.68 (8′-$C_{1''}$), 141.91 ($C_{5'}$), 148.42 ($C_{7'}$), 157.53 ($C_{2'}$=O), 161.45 (C_5), 162.52 ($C_{4'}$=O), 164.77 (8′-$C_{4''}$), 168.67 ($C_{8'a}$). Anal. Calcd for $C_{39}H_{50}FN_3O_2$: C, 73.32; H, 8.36; N, 6.58. Found: C, 73.14; H, 8.09; N, 6.75.

4.1.10. 8′-(4-Chlorophenyl)-3′-methyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (5i)

Yield, (710 mg, 47%); mp 240–242 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −42.80°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 219 (4.40), *278* (4.15), 448 (4.06); IR (ν_{max}/cm^{-1}): 1684, 1627 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.06–2.32 (24H, m), 2.72 (1H, d, J = 15.6 Hz, 1β-H), 2.93 (1H, d, J = 15.6 Hz, 1α-H), 3.40 (3H, s, 3′-NCH_3), 5.52 (1H, s, 4-CH), 7.07–7.19 (2H, m, 8′-Ar-*m*H), 7.51–7.56 (3H, m, 8′-Ar-*o*H), 8.38 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.14 (C_{18}), 18.84 (C_{21}) and (C_{19}), 21.80 (C_{11}), 22.74 (C_{26}), 22.99 (C_{27}), 23.96 (C_{23}), 24.40 (C_{15}), 28.19 (3′-NCH_3 and C_{16}), 31.24 (C_{25}), 33.30 (C_6 and C_7), 34.68 (C_{22}), 35.90 (C_8 and C_{20}), 36.28 (C_{12}), 39.27 (C_{24}), 39.64 (C_{10} and C_1), 42.55 (C_{13}), 54.03 (C_{14}), 55.85 (C_9), 56.26 (C_{17}), 111.95 (C_4), 113.78 ($C_{6'}$), 117.32 ($C_{4'a}$), 129.39 (8′-$C_{3''}$) and ($C_{5'}$), 130.71 (8′-$C_{2''}$ and $C_{6''}$), 135.47 (8′-$C_{4''}$), 136.17 ($C_{1''}$), 141.98 ($C_{5'}$), 148.09 ($C_{7'}$), 157.17 ($C_{2'}$=O), 157.39 (C_5), 162.52 ($C_{4'}$=O), 168.66 ($C_{8'a}$). Anal. Calcd for $C_{39}H_{50}ClN_3O_2{\cdot}0.66H_2O$: C, 73.16; H, 8.08; N, 6.56. Found: C, 73.03; H, 7.84; N, 6.72.

4.1.11. 8′-(4-Bromophenyl)-3′-methyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (5j)

Yield, (550 mg, 33%); mp 247–249 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −64.44°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 220 (4.32), *283* (4.04), 455 (4.01); IR (ν_{max}/cm^{-1}): 1697, 1634 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.95 (3H, d, J = 6.3 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.06–2.32 (24H, m), 2.71 (1H, d, J = 15.6 Hz, 1β-H), 2.92 (1H, d, J = 15.6 Hz, 1α-H), 3.40 (3H, s, 3′-NCH_3), 5.46 (1H, s, 4-CH), 7.01–7.16 (2H, m, 8′-Ar-*m*H), 7.66–7.73 (3H, m, 8′-Ar-*o*H), 8.37 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.08 (C_{18}), 18.87 (C_{21}) and (C_{19}), 21.80 (C_{11}), 22.71 (C_{26}), 22.96 (C_{27}), 23.93 (C_{23}), 25.67 (C_{15}), 28.16 (3′-NCH_3 and C_{16}), 33.00 (C_{25}), 33.28 (C_6 and C_7), 34.65 (C_{22}), 35.88 (C_8 and C_{20}), 36.23 (C_{12}), 39.26 (C_{24}), 39.62 (C_{10} and C_1), 42.58 (C_{13}), 54.02 (C_{14}), 55.83 (C_9), 56.24 (C_{17}), 111.91 (C_4), 113.72 ($C_{6'}$), 117.99 ($C_{4'a}$), 124.29 (8′-$C_{4''}$), 129.63 (8′-$C_{3''}$) and ($C_{5'}$), 133.71 (8′-$C_{2''}$ and $C_{6''}$), 136.17 ($C_{1''}$), 142.26 ($C_{5'}$), 148.03 ($C_{7'}$), 158.21 ($C_{2'}$=O), 160.68 (C_5), 162.50 ($C_{4'}$=O), 168.68 ($C_{8'a}$). Anal. Calcd for $C_{39}H_{50}BrN_3O_2{\cdot}0.60H_2O$: C, 68.53; H, 7.55; N, 6.15. Found: C, 68.54; H, 7.34; N, 6.19.

4.1.12. 8′-Methyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (7a)

Yield, (510 mg, 40%); mp 295–297 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −15.20°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 220 (4.26), 258 (4.24), *304* (3.92), 421 (4.02); IR (ν_{max}/cm^{-1}): 3170 (NH), 1686, 1621 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.72 (3H, s, 18-CH_3), 0.88 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 0.99 (3H, s, 19-CH_3), 1.11–2.12 (22H, m), 2.56 (2H, m, 6-CH_2), 2.68 (1H, d, J = 15.6 Hz, 1β-H), 2.86 (1H, d, J = 15.6 Hz, 1α-H), 4.05 (3H, s, 8′-NCH_3), 6.42 (1H, s, 4-CH), 8.26 (1H, s, 5′-CH), 8.46 (1H, s, exchangeable with D_2O, 3′-N*H*); ^{13}C NMR ($CDCl_3$): δ_C11.96 (C_{18}), 17.58 (C_{21}), 18.66 (C_{19}), 21.61 (C_{11}), 22.56 (C_{26}), 22.79 (C_{27}), 23.80 (C_{23} and C_{15}), 27.99 (C_{16}), 31.19 (C_{25}), 33.93 (C_6 and C_7), 35.75 (8′-NCH_3 and C_{22}), 36.05 (C_8 and C_{20}), 39.01 (C_{12}), 39.49 (C_{24} and C_{10}), 40.25 (C_1), 42.40 (C_{13}), 53.68 (C_{14}), 55.68 (C_9), 56.10 (C_{17}), 111.69 (C_4), 112.69 ($C_{6'}$), 117.94 ($C_{4'a}$), 141.06 ($C_{5'}$), 148.64 ($C_{7'}$), 156.89 ($C_{2'}$=O), 157.57 (C_5), 162.59 ($C_{4'}$=O), 168.88 ($C_{8'a}$). Anal. Calcd for $C_{33}H_{47}N_3O_2$: C, 76.77; H, 9.15; N, 8.12. Found: C, 76.62; H, 8.82; N, 8.12.

4.1.13. 8′-Ethyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (7b)

Yield, (450 mg, 35%); mp 270–272 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −19.80°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 235 (4.54), 289 (4.52), 420 (4.44); IR (ν_{max}/cm^{-1}): 3160 (NH), 1682, 1637 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.70 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.91 (3H, d, J = 6.3 Hz, 21-CH_3), 0.96 (3H, s, 19-CH_3), 1.11–2.10 (22H, m), 1.39 (3H, t, J = 6.9 Hz, 8′-NCH$_2CH_3$), 2.54 (2H, m, 6-CH_2), 2.65 (1H, d, J = 15.6 Hz, 1β-H), 2.88 (1H, d, J = 15.6 Hz, 1α-H), 4.52 (1H, br s, 8′-NC$H_aH_bCH_3$), 4.82 (1H, br s, 8′-NCH$_a$H$_b$CH$_3$), 6.35 (1H, s, 4-CH), 8.22 (1H, s, 5′-CH), 8.58 (1H, s, exchangeable with D_2O, 3′-N*H*); ^{13}C NMR ($CDCl_3$): δ_C 11.94 (C_{18}), 13.86 (8′-NCH$_2CH_3$), 17.59 (C_{21}), 18.68 (C_{19}), 21.62 (C_{11}), 22.58 (C_{26}), 22.83 (C_{27}), 23.81 (C_{23}), 24.27 (C_{15}), 28.15 (C_{16}), 31.18 (C_{25}), 33.51 (C_6 and C_7), 35.76 (C_{22}), 36.11 (C_8,C_{20} and C_{12}), 38.91 (C_{24}), 39.56 (C_{10}), 40.42 (C_1), 41.68 (8′-NCH$_2$CH$_3$), 42.41 (C_{13}), 53.68 (C_{14}), 55.72 (C_9), 56.12 (C_{17}), 111.79 (C_4), 112.41 ($C_{6'}$), 118.19 ($C_{4'a}$), 139.92 ($C_{5'}$), 147.89 ($C_{7'}$), 156.92 ($C_{2'}$=O), 157.09 (C_5), 162.70 ($C_{4'}$=O), 169.52 ($C_{8'a}$). Anal. Calcd for $C_{34}H_{49}N_3O_2\cdot0.25H_2O$: C, 76.15; H, 9.30; N, 7.84. Found: C, 76.18; H, 9.10; N, 7.93.

4.1.14. 8′-Butyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-*dione* (7c)

Yield, (460 mg, 34%); mp 233–235 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −22.32°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 232 (4.12), 289 (4.08), 425 (3.98); IR (ν_{max}/cm^{-1}): 3145 (NH), 1651, 1596 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.73 (3H, s, 18-CH_3), 0.88 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 0.98 (3H, s, 19-CH_3), 0.99 (3H, t, J = 7.5 Hz, 8′-NCH$_2$CH$_2$CH$_2CH_3$), 1.50 (2H, m,J = 7.5 Hz, 8′-NCH$_2CH_2$CH$_2$CH$_3$), 1.11–2.12 (22H, m), 2.57 (2H, m, 6-CH_2), 2.67 (1H, d, J = 15.6 Hz, 1β-H), 2.86 (1H, d, J = 15.6 Hz, 1α-H), 4.40 (1H, m, 8′-NCH_aH_bCH$_2$CH$_2$CH$_3$), 4.74 (1H, m, 8′-NCH$_a$H$_b$CH$_2$CH$_2$CH$_3$), 6.34 (1H, s, 4-CH), 8.25 (1H, s, 5′-CH), 8.42 (1H, s, exchangeable with D_2O, 3′-N*H*); ^{13}C NMR ($CDCl_3$): δ_C 12.06 (C_{18}), 13.93 (8′-NCH$_2$CH$_2$CH$_2CH_3$), 17.76 (C_{21}), 18.78 (C_{19}), 20.11 (8′-NCH$_2$CH$_2CH_2$CH$_3$), 21.76 (C_{11}), 22.73 (C_{26}), 22.96 (C_{27}), 23.97 (C_{23}), 24.42 (C_{15}), 28.17 (C_{16}), 30.75 (8′-NCH$_2CH_2$CH$_2$CH$_3$), 31.33 (C_{25}), 33.74 (C_6 and C_7), 35.90 (C_{22}), 36.23 (C_8, C_{20} and C_{12}), 39.07 (C_{24}), 39.64 (C_{10}), 40.59 (C_1), 42.56 (C_{13}), 46.28 (8′-NCH$_2$CH$_2$CH$_2$CH$_3$), 53.81 (C_{14}), 55.86 (C_9), 56.25 (C_{17}), 111.91 (C_4), 112.74 ($C_{6'}$), 118.57 ($C_{4'a}$), 140.07 ($C_{5'}$), 148.27 ($C_{7'}$), 157.23 ($C_{2'}$=O), 157.57 (C_5), 162.95 ($C_{4'}$=O), 169.67 ($C_{8'a}$). Anal. Calcd for $C_{36}H_{53}N_3O_2$: C, 77.24; H, 9.54; N, 7.51. Found: C, 77.23; H, 9.42; N, 7.21.

4.1.15. 8′-Phenyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (7d)

Yield, (470 mg, 33%); mp 227–229 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −23.24°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 220 (4.11), 275 (4.00), 485 (3.70); IR (ν_{max}/cm^{-1}): 3190 (NH), 1617, 1560 (CO); ^{1}H NMR ($CDCl_3$): δ H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 1.06–2.33 (24H, m), 2.72 (1H, d, J = 15.6 Hz, 1β-H), 2.93 (1H, d, J = 15.6 Hz, 1α-H), 5.51 (1H, s, 4-CH), 7.13–7.23 (2H, m, 8′-Ar-*m*H), 7.55–7.61 (3H, m, 8′-Ar-*o*,*p*H), 8.37 (1H, s, 5′-CH), 8.39 (1H, s, exchangeable with D_2O, 3′-N*H*); ^{13}C NMR ($CDCl_3$): δ_C 11.89 (C_{18}), 18.63 (C_{21} and C_{19}), 21.40 (C_{11}), 22.61 (C_{26}), 22.78 (C_{27}), 23.82 (C_{23} and C_{15}), 27.98 (C_{16}), 32.85 (C_{25}), 33.15 (C_6 and C_7), 35.73 (C_{22}), 36.06 (C_8 and C_{20}), 37.81 (C_{12}), 39.11 (C_{24}), 39.47 (C_{10}), 40.17 (C_1), 42.45 (C_{13}), 53.83 (C_{14}), 55.70 (C_9), 56.11 (C_{17}), 112.10 (C_4), 115.47 ($C_{6'}$), 117.50 ($C_{4'a}$), 127.48 (8′-$C_{3''}$) and ($C_{5'}$), 130.18 (8′-$C_{4''}$), 130.26 (8′-$C_{2''}$) and ($C_{5'}$), 136.39 (8′-$C_{1''}$), 141.17 ($C_{5'}$), 148.91 ($C_{7'}$), 156.46 ($C_{2'}$=O), 156.82 (C_5), 162.36 ($C_{4'}$=O), 168.66 ($C_{8'a}$). Anal. Calcd for $C_{38}H_{49}N_3O_2$: C, 78.72; H, 8.52; N, 7.25. Found: C, 78.69; H, 8.47; N, 7.32.

4.1.16. 8′-(4-Methoxyphenyl)-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (7e)

Yield, (510 mg, 34%); mp 219–221°C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −38.80°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^{3}mol^{-1} cm^{-1}): 223 (4.23), 262 (4.14), 456 (3.97); IR (ν_{max}/cm^{-1}): 3195 (NH), 1683, 1618 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.72 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.95 (3H, d, J = 6.3 Hz, 21-CH_3), 1.07 (3H, s, 19-CH_3), 1.06–2.33 (24H, m), 2.94 (1H, d, J = 16.2 Hz, 1β-H), 3.05 (1H, d, J = 16.2 Hz, 1α-H), 3.88 (3H, s, 8′-Ar-OCH_3), 6.61 (1H, s, 4-CH), 6.99–7.07 (4H, m, 8′-Ar-H), 8.45 (1H, s, 5′-CH), 8.56 (1H, s, exchangeable with D_2O, 3′-N*H*); ^{13}C NMR ($CDCl_3$): δ_C 12.04 (C_{18}), 18.84 (C_{21} and C_{19}), 21.53 (C_{11}), 22.71 (C_{26}), 22.98 (C_{27}), 23.95 (C_{23} and C_{15}), 28.15 (C_{16}), 32.99 (C_{25}, C_6 and C_7), 35.82 (C_{22}), 36.21 (C_8 and C_{20}), 37.93 (C_{12}), 39.18 (C_{24}), 39.61 (C_{10}), 39.96 (C_1), 42.57 (C_{13}), 49.60 (C_{14}), 55.75 (8′-Ar-OCH_3), 56.07 (C_9), 56.52 (C_{17}), 115.49 (8′-$C_{3''}$) and ($C_{5'}$), 115.69 ($C_{4''}$ and $C_{6''}$), 120.10 ($C_{4'a}$), 121.44 (8′-$C_{1''}$), 128.90 (8′-$C_{2''}$ and $C_{6''}$), 141.83 ($C_{5'}$), 146.89 ($C_{7'}$), 152.60 ($C_{2'}$=O), 156.72 (C_5), 159.49 (8′-$C_{4''}$), 160.76 ($C_{4'}$=O), 162.05 ($C_{8'a}$). Anal. Calcd for $C_{39}H_{51}N_3O_3\cdot H_2O$: C, 74.61; H, 8.51; N, 6.69. Found: C, 74.96; H, 8.58; N, 6.74.

4.1.17. 8′-(3-Methylphenyl)-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (7f)

Yield, (470 mg, 32%); mp 213–215 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −31.24°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 225 (4.43), 279 (4.39), 444 (4.07); IR (ν_{max}/cm^{-1}): 3178 (NH), 1624, 1540 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.92 (3H, d, J = 6.3 Hz, 21-CH_3), 1.12 (3H, s, 19-CH_3), 1.06–2.33 (24H, m), 2.40 (3H, s, 8′-Ar-CH_3), 2.93 (1H, d, J = 16.5 Hz, 1β-H), 3.03 (1H, d, J = 16.5 Hz, 1α-H), 5.51 (1H, s, 4-CH), 6.92–7.03 (2H, m, 8′-Ar-2″, 5″H), 7.32–7.45 (2H, m, 8′-Ar-4″, 6″H), 8.35 (1H, s, 5′-CH), 8.44 (1H, s, exchangeable with D_2O, 3′-N*H*); ^{13}C NMR ($CDCl_3$): δ_C 11.90 (C_{18}), 18.60 (C_{21} and C_{19}), 21.49 (C_{11} and 8′-Ar-mCH_3), 22.51 (C_{26}), 22.62 (C_{27}), 23.79 (C_{23} and C_{15}), 28.06 (C_{16}), 32.87 (C_{25}), 33.13 (C_6 and C_7), 35.81 (C_{22},C_8 and C_{20}), 37.81 (C_{12}), 39.11 (C_{24}), 39.48 (C_{10}), 39.78 (C_1), 42.43 (C_{13}), 53.81 (C_{14}), 56.07 (C_9 and C_{17}), 112.04 (C_4), 115.41 ($C_{6'}$), 117.67 ($C_{4'a}$), 124.34 (8′-$C_{4''}$), 127.71 (8′-($C_{5'}$)), 130.30 (8′-$C_{3''}$), 131.27 (8′-$C_{6''}$), 136.29 (8′-$C_{2''}$), 139.278 (8′-$C_{1''}$), 140.46 ($C_{5'}$), 149.02 ($C_{7'}$), 156.69 ($C_{2'}$=O),157.12 (C_5), 161.85 ($C_{4'}$=O), 168.69 ($C_{8'a}$). Anal. Calcd for $C_{36}H_{53}N_3O_2$: C, 78.88; H, 8.66; N, 7.08. Found: C, 78.71; H, 8.58; N, 7.11.

4.1.18. 8′-(4-Fluorophenyl)-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (7g)

Yield, (470 mg, 32%); mp 227–229 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −58.84°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 216 (3.99), 253 (3.87), 457 (3.67); IR (ν_{max}/cm^{-1}): 3180 (NH), 1683, 1601 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71(3H, s, 18-CH_3), 0.88 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 1.06–2.34 (24H, m),2.72 (1H, d, J = 15.6 Hz, 1β-H), 2.91 (1H, d, J = 15.6 Hz, 1α-H), 6.52 (1H, s, 4-CH), 7.12–7.14 (2H, m, 8′-Ar-*m*H), 7.22–7.28 (3H, m, 8′-Ar-*o*H), 8.37 (1H, s, 5′-CH), 8.49 (1H, s, exchangeable with D_2O, 3′-N*H*); ^{13}C NMR ($CDCl_3$): δ_C12.08 (C_{18}), 18.12 (C_{21}), 18.81 (C_{19}), 21.46 (C_{11}), 22.71 (C_{26}), 22.96 (C_{27}), 23.94 (C_{23} and C_{15}), 28.16 (C_{16}), 31.21 (C_{25}), 33.38 (C_6 and C_7), 35.87 (C_{22}), 36.22 (C_8 and C_{20}), 37.99 (C_{12}), 39.32 (C_{24}), 39.63 (C_{10}), 40.27 (C_1), 42.59 (C_{13}), 55.83 (C_{14}), 56.26 (C_9), 56.52 (C_{17}), 112.27 (C_4), 113.90 ($C_{6'}$), 117.94 ($C_{4'a}$), 8′-$C_{3''}$ and ($C_{5'}$), 129.74 (8′-$C_{2''}$ and $C_{6''}$), 135.26 (8′-$C_{1''}$), 141.81 ($C_{5'}$), 149.16 ($C_{7'}$), 157.41 ($C_{2'}$=O), 161.47 (C_5), 162.45 ($C_{4'}$=O), 164.80 (8′-$C_{4''}$), 169.54 ($C_{8'a}$). Anal. Calcd for $C_{38}H_{48}FN_3O_2\cdot0.2H_2O$: C, 75.89; H, 8.11; N, 6.99. Found: C, 76.02; H, 8.38; N, 6.74.

4.1.19. 8′-(4-Chlorophenyl)-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (7h)

Yield, (470 mg, 31%); mp 223–225 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −35.92°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 221 (4.53),

252 (4.41), 289 (4.27), 452 (4.24); IR (ν_{max}/cm^{-1}): 3195 (NH), 1683, 1620 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.93 (3H, d, J = 6.3 Hz, 21-CH_3), 1.05 (3H, s, 19-CH_3), 1.06–2.16 (24H, m), 2.93 (1H, d, J = 16.5 Hz, 1β-H), 3.07 (1H, d, J = 16.5 Hz, 1α-H), 6.50 (1H, s, 4-CH), 7.04–7.08 (2H, m, 8′-Ar-*m*H), 7.52–7.55 (3H, m, 8′-Ar-*o*H), 8.41 (1H, s, 5′-CH), 8.88 (1H, s, exchangeable with D_2O, 3′-N*H*); ^{13}C NMR ($CDCl_3$): δ_C 11.81 (C_{18}), 18.22 (C_{21}), 18.32 (C_{19}), 21.35 (C_{11}), 22.59 (C_{26}), 22.76 (C_{27}), 23.80 (C_{23}), 23.98 (C_{15}), 27.97 (C_{16}), 32.83 (C_{25}, C_6 and C_7), 35.67 (C_{22}), 36.05 (C_8 and C_{20}), 37.82 (C_{12}), 39.01 (C_{24}), 39.45 (C_{10}), 39.69 (C_1), 42.43 (C_{13}), 49.40 (C_{14}), 55.92 (C_9), 56.35 (C_{17}), 115.45 (C_4), 119.98 ($C_{6'}$), 120.55 ($C_{4'a}$), 128.97 (8′-$C_{3''}$) and ($C_{5'}$), 130.57 (8′-$C_{2''}$ and $C_{6''}$), 134.71 (8′-$C_{4''}$), 136.49 ($C_{1''}$), 141.96 ($C_{5'}$), 145.93 ($C_{7'}$), 152.77 ($C_{2'}$=O), 156.33 (C_5), 159.04 ($C_{4'}$=O), 161.64 ($C_{8'a}$). Anal. Calcd for $C_{38}H_{48}ClN_3O_2{\cdot}0.4H_2O$: C, 73.44; H, 7.91; N, 6.76. Found: C, 73.58; H, 7.96; N, 6.79.

4.1.20. 8′-(3,4-Dimethylphenyl)-5′-deazacholest-2,4- dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (7i)

Yield, (480 mg, 32%); mp 197–199 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −19.08°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 219 (4.46), 286 (4.28), 428 (4.11); IR (ν_{max}/cm^{-1}): 3195 (NH), 1697, 1623 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.87 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.93 (3H, d, J = 6.3 Hz, 21-CH_3), 1.00 (3H, s, 19-CH_3), 2.29 (3H, s, 8′-Ar-3″CH_3), 1.06–2.29 (24H, m), 2.33 (3H, s, 8′-Ar-4″CH_3), 2.71 (1H, d, J = 15.9 Hz, 1β-H), 2.90 (1H, d, J = 15.9 Hz, 1α-H), 5.58 (1H, s, 4-CH), 6.84–6.94 (2H, m, 8′-Ar-2″, 5″H), 7.27–7.29 (1H, m, 8′-Ar-6″H), 8.35 (1H, s, 5′-CH), 8.61 (1H, s, exchangeable with D_2O, 3′-N*H*); ^{13}C NMR ($CDCl_3$): δ_C11.88 (C_{18}), 17.92 (C_{21}), 18.68 (C_{19}), 19.64 (8′-Ar-*m*CH_3), 20.04 (8′-Ar-*p*CH_3), 21.62 (C_{11}), 22.57 (C_{26}), 22.84 (C_{27}), 23.80 (C_{23}), 24.22 (C_{15}), 28.02 (C_{16}), 31.06 (C_{25}), 33.05 (C_6, C_7), 35.71 (C_{22}), 36.08 (C_8 and C_{20}), 37.78 (C_{12}), 39.08 (C_{24}), 39.47 (C_{10}), 40.20 (C_1), 42.38 (C_{13}), 53.81 (C_{14}), 55.69 (C_9), 56.09 (C_{17}), 111.99 (C_4), 114.14 ($C_{6'}$), 117.55 ($C_{4'a}$), 124.53 (8′-($C_{5'}$)), 128.14 (8′-$C_{4''}$), 131.10 (8′-$C_{2''}$), 134.43 (8′-$C_{1''}$), 138.73 (8′-$C_{6''}$ and $C_{3''}$), 141.06 ($C_{5'}$), 149.18 ($C_{7'}$), 157.07 ($C_{2'}$=O), 158.79 (C_5), 162.45 ($C_{4'}$=O), 168.42 ($C_{8'a}$). Anal. Calcd for $C_{40}H_{53}N_3O_2$: C, 79.03; H, 8.79; N, 6.91. Found: C, 79.06; H, 8.66; N, 6.75.

4.1.21. 8′-Methyl-3-phenyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (9a)

Yield, (450 mg, 31%); mp 218–220 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −16.44°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 220 (4.15), 286 (4.09), 420 (3.90); IR (ν_{max}/cm^{-1}): 1698, 1635 (CO); ^{1}H NMR ($CDCl_3$): δ_H0.73 (3H, s, 18-CH_3), 0.88 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 0.99 (3H, s, 19-CH_3), 1.11–2.11 (22H, m), 2.56 (2H, m, 6-CH_2), 2.69 (1H, d, J = 15.9 Hz, 1β-H), 2.89 (1H, d, J = 15.9 Hz, 1α-H), 4.07 (3H, s, 8′-NCH_3), 6.43 (1H, s, 4-CH), 7.24 (2H, d, J = 7.8 Hz, 3′-Ar-*m*H), 7.41 (1H, m, 3′-Ar-*p*H), 7.48 (2H, d, J = 7.8 Hz, Ar-*o*H), 8.26 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.08 (C_{18}), 17.78 (C_{21}), 18.83 (C_{19}), 21.80 (C_{11}), 22.72 (C_{26}), 22.99 (C_{27}),23.97 (C_{23}), 24.43 (C_{15}), 28.17 (C_{16}), 31.35 (C_{25}, C_6 and C_7), 33.66 (C_{22}), 33.87 (C_8 and C_{20}), 35.91 (8′-NCH_3), 36.25 (C_{12}), 39.17 (C_{24}), 39.63 (C_{10}), 40.46 (C_1), 42.56 (C_{13}), 53.86 (C_{14}), 55.87 (C_9), 56.26 (C_{17}), 111.95 (C_4), 112.98 ($C_{6'}$), 117.98 ($C_{4'a}$), 128.47 (3′-$C_{3''}$, $C_{5''}$ and $C_{4''}$),129.43 (3′-$C_{2''}$ and $C_{6''}$), 136.75 (3′-$C_{1''}$), 140.74 ($C_{5'}$), 148.48 ($C_{7'}$), 156.32 ($C_{2'}$=O), 157.01 (C_5), 162.93 ($C_{4'}$=O), 169.68 ($C_{8'a}$). Anal. Calcd for $C_{39}H_{51}N_3O_2$: C, 78.88; H, 8.66; N, 7.08. Found: C, 79.05; H, 8.74; N, 7.22.

4.1.22. 3′-8′-Diphenyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (9b)

Yield, (550 mg, 34%); mp 216–218 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −29.60°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 210 (4.29), 258 (4.14), *303* (3.93), 446 (3.97); IR (ν_{max}/cm^{-1}): 1698, 1646 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.88 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.93 (3H, d, J = 6.3 Hz, 21-CH_3), 1.07 (3H, s, 19-CH_3), 1.11–2.33 (24H, m), 2.94 (1H, d, J = 16.2 Hz, 1β-H), 3.06 (1H, d, J = 16.2 Hz, 1α-H), 6.51 (1H, s, 4-CH), 7.20–7.23 (4H, m, 3′, 8′-Ar-*m*H), 7.34–7.39 (1H, m, 3′-Ar-*p*H), 7.42–7.48 (2H, m, 3′-Ar-*o*H), 7.55–7.58 (3H, m, 8′-Ar-*o*,*p*H), 8.47 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.04 (C_{18}), 18.38 (C_{21}), 18.82 (C_{19}), 21.53 (C_{11}), 22.67 (C_{26}), 22.93 (C_{27}), 23.94 (C_{23} and C_{15}), 28.14 (C_{16}), 33.02 (C_{25}, C_6 and C_7), 35.85 (C_{22}), 36.24 (C_8 and C_{20}), 37.96 (C_{12}), 39.62 (C_{24} and C_{10}), 39.96 (C_1), 42.59 (C_{13}), 49.65 (C_{14}), 56.07 (C_9), 56.32 (C_{17}), 115.73 (C_4), 119.98 ($C_{6'}$), 121.13 ($C_{4'a}$), 127.67 (8′-$C_{3'''}$ and $C_{5'''}$),128.41 (3′-$C_{4''}$), $C_{3''}$ and ($C_{5'}$), 129.42 (3′-$C_{2''}$ and $C_{6''}$), 130.45 (8′-$C_{4'''}$), 130.62 (8′-$C_{2'''}$ and $C_{6'''}$), 136.45 (3′ − $C_{1''}$), 142.40 ($C_{5'}$), 146.08 ($C_{7'}$), 152.49 (8′-$C_{1'''}$), 156.35 ($C_{2'}$=O), 157.91 (C_5), 162.13 ($C_{4'}$=O), 168.57 ($C_{8'a}$). Anal. Calcd for $C_{44}H_{53}N_3O_2{\cdot}H_2O$: C, 78.42; H, 8.23; N, 6.24. Found: C, 78.76; H, 8.09; N, 6.35.

4.1.23. 8′-(3-Methylphenyl)-3′-phenyl-5′- deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (9c)

Yield, (580 mg, 35%); mp 214–216 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −32.00°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 214 (4.45), 260 (4.23), 454 (3.92); IR (ν_{max}/cm^{-1}): 1698, 1624 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.72 (3H, s, 18-CH_3), 0.88 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.93 (3H, d, J = 6.3 Hz, 21-CH_3), 1.13 (3H, s, 19-CH_3), 1.17–2.33 (24H, m), 2.42 (3H, s, 8′-Ar-*C*H_3), 2.94 (1H, d, J = 15.6 Hz, 1β-H), 3.12 (1H, d, J = 15.6 Hz, 1α-H), 5.15 (1H, s, 4-CH), 6.91–7.17 (2H, m, 8′-Ar-2″,5″H), 7.21 (2H, m, 3′-Ar-*m*H), 7.36–7.40 (3H, m, 3′-Ar-*p*H, 8′-Ar-4″,6″H), 7.45 (2H, m, 3′-Ar-*o*H) 8.49 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 11.82 (C_{18}), 18.27 (C_{21}), 18.65 (C_{19}), 21.43 (C_{11} and Ar-*m*CH_3), 22.52 (C_{26}), 22.58 (C_{27}), 23.77 (C_{23}), 23.98 (C_{15}), 27.99 (C_{16}), 32.85 (C_{25}, C_6 and C_7), 35.27 (C_{22}), 35.66 (C_8 and C_{20}), 37.04 (C_{12}), 39.12 (C_{24}), 39.45 (C_{10}), 39.79 (C_1), 42.43 (C_{13}), 49.51 (C_{14}), 55.92 (C_9), 56.37 (C_{17}), 115.46 (C_4), 120.06 ($C_{6'}$), 124.52 ($C_{4'a}$), 128.22 (3′-$C_{4''}$), $C_{3''}$ and ($C_{5'}$), 129.04 (8′-$C_{4'''}$), 129.30 (3′-$C_{2''}$ and $C_{6''}$), 135.21 (8′-$C_{5'''}$), 136.08 (3′ − $C_{1''}$), 137.30 (8′-$C_{6'''}$), 139.14 (8′-$C_{2''}$), 140.56 ($C_{5'}$), 142.39 (8′-$C_{1'''}$), 146.14 ($C_{7'}$), 151.25 (8′-$C_{3'''}$), 156.57 ($C_{2'}$=O), 157.62 (C_5), 161.94 ($C_{4'}$=O), 164.66 ($C_{8'a}$). Anal. Calcd for $C_{45}H_{55}N_3O_2{\cdot}0.5H_2O$: C, 79.61; H, 8.31; N, 6.19. Found: C, 79.51; H, 8.54; N, 6.19.

4.1.24. 8′-(4-Methoxyphenyl)-3′-phenyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (9d)

Yield, (610 mg, 36%); mp 202–204 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −23.04°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}): 222 (4.38), 282 (4.15), 428 (3.88); IR (ν_{max}/cm^{-1}): 1697, 1636 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.11–2.33 (24H, m), 2.73 (1H, d, J = 15.6 Hz, 1β-H), 2.94 (1H, d, J = 15.6 Hz, 1α-H), 3.88 (3H, s, 8′-Ar-OCH_3), 5.64 (1H, s, 4-CH), 7.07 (2H, d, J = 6.9 Hz, 8′-Ar-*m*H), 7.24 (2H, d, J = 6.9 Hz, 8′-Ar-*o*H), 7.25–7.28 (2H, m, 3′-Ar-*o*H), 7.35–7.39 (1H, m, 3′-Ar-*p*H), 7.44–7.48 (2H, m, 3′-Ar-*o*H), 8.40 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 11.95 (C_{18}), 18.65 (C_{21} and C_{19}), 21.64 (C_{11}), 22.56 (C_{26}), 22.82 (C_{27}), 23.77 (C_{23} and C_{15}), 27.99 (C_{16}), 31.11 (C_{25}), 33.13 (C_6 and C_7), 35.72 (C_{22}), 36.06 (C_{12}, C_8 and C_{20}), 39.11 (C_{24} and C_{10}), 39.47 (C_1), 42.39 (C_{13}), 53.85 (C_{14}), 55.68 (C_9, 8′-Ar-*p*OCH_3), 56.07 (C_{17}), 112.12 (C_4), 113.96 ($C_{6'}$), 115.22 (8′-$C_{3''}$) and ($C_{5'}$), 117.68 ($C_{4'a}$), 128.28 (3′-$C_{4''}$), $C_{3''}$ and ($C_{5'}$), 129.03 (8′-$C_{2'''}$ and $C_{6'''}$), 129.24 (3′-$C_{2''}$ and $C_{6''}$), 135.41 (8′-$C_{1'''}$), 136.45 (3′-$C_{1''}$), 141.72 ($C_{5'}$), 149.14 ($C_{7'}$),157.01 ($C_{2'}$=O), 157.52 (C_5), 160.36 (8′-$C_{4'''}$), 162.50 ($C_{4'}$=O), 168.53 ($C_{8'a}$). Anal. Calcd for $C_{45}H_{55}N_3O_3$: C, 78.79; H, 8.08; N, 6.13. Found: C, 79.10; H, 8.17; N, 6.13.

4.1.25. 8′-(3,4-Dimethylphenyl)-3′-phenyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (9e)

Yield, (740 mg, 44%); mp 197–199 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −36.68°; UV (EtOH): λ_{max}/nm ($\log\varepsilon$/dm^3 mol^{-1} cm^{-1}):

208 (4.30), 290 (4.02), 431 (3.85); IR (ν_{max}/cm^{-1}): 1697, 1645 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.88 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.94 (3H, d, J = 6.3 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 1.11–2.26 (24H, m), 2.31 (3H, s, 8′-Ar-3″CH_3), 2.35 (3H, s, 8′-Ar-4″CH_3), 2.73 (1H, d, J = 15.6 Hz, 1β-H), 2.94 (1H, d, J = 15.6 Hz, 1α-H), 5.61 (1H, s, 4-CH), 6.91–7.04 (2H, m, 8′-Ar-2″,5″H), 7.22–7.25 (2H, m, 3′-Ar-*m*H),7.25–7.33 (1H, m, 8′-Ar-6″H), 7.35–7.41 (1H, m, 3′-Ar-*p*H), 7.44–7.46 (2H, m, 3′-Ar-*o*H), 8.40 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 11.87 (C_{18}), 17.89 (C_{21}), 18.66 (C_{19}), 19.72 (8′-Ar-*m*CH_3), 20.12 (8′-Ar-*p*CH_3), 21.77 (C_{11}), 22.59 (C_{26} and C_{27}), 23.79 (C_{23} and C_{15}), 28.00 (C_{16}), 33.13 (C_{25}, C_6 and C_7), 35.73 (C_{22}), 36.06 (C_{12}, C_8 and C_{20}), 39.11 (C_{24} and C_{10}), 39.49 (C_1), 42.39 (C_{13}), 53.85 (C_{14}), 56.09 (C_9 and C_{17}), 112.11 (C_4), 114.21 ($C_{6'}$), 117.62 ($C_{4'a}$), 124.71 (8′-$C_{5'''}$), 128.30 (3′ – $C_{4''}$), $C_{3''}$ and ($C_{5'}$), 129.25 (8′-$C_{2'''}$ and $C_{6'''}$), 131.08 (8′-$C_{2''}$), 134.30 (8′-$C_{1'''}$), 136.48 (3′ – $C_{1''}$), 138.83 (8′-$C_{3''}$ and $C_{6''}$), 141.73 ($C_{5'}$), 148.93 ($C_{7'}$), 151.15 (8′-$C_{3'''}$), 151.49 (8′-$C_{4'''}$), 157.03 ($C_{2'}$=O), 157.63 (C_5), 162.57 ($C_{4'}$=O), 168.34 ($C_{8'a}$). Anal. Calcd for $C_{46}H_{57}N_3O_2\cdot0.33H_2O$: C, 80.07; H, 8.42; N, 6.09. Found: C, 80.15; H, 8.68; N, 6.03.

4.1.26. 8′-(4-Fluorophenyl)-3′-phenyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (9f)

Yield, (580 mg, 35%); mp 212–214 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −41.80°; UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 220 (4.33), 282 (4.18), 433 (3.97); IR (ν_{max}/cm^{-1}): 1697, 1636 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.88 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.93 (3H, d, J = 6.3 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.06–2.33 (24H, m), 2.72 (1H, d, J = 15.9 Hz, 1β-H), 2.93 (1H, d, J = 15.9 Hz, 1α-H), 5.54 (1H, s, 4-CH), 7.19–7.23 (2H, m, 8′-Ar-*m*H), 7.24–7.28 (3H, m, 3′-Ar-*m*H), 7.36–7.39 (1H, m, 3′-Ar-*p*H), 7.42–7.47 (2H, m, 8′-Ar-*o*H), 8.40 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 11.94 (C_{18}), 17.93 (C_{21}), 18.67 (C_{19}), 21.65 (C_{11}), 22.56 (C_{26}), 22.82 (C_{27}), 23.79 (C_{23}), 24.23 (C_{15}), 28.02 (C_{16}), 31.09 (C_{25}), 33.22 (C_6 and C_7), 35.72 (C_{22}), 36.10 (C_8 and C_{20}), 37.82 (C_{12}), 39.16 (C_{24}), 39.48 (C_{10}), 40.15 (C_1), 42.40 (C_{13}), 53.87 (C_{14}), 55.69 (C_9), 56.10 (C_{17}), 112.25 (C_4), 113.67 ($C_{6'}$), 117.26 ($C_{4'a}$), 117.61 (8′-$C_{3'''}$ and $C_{5'''}$), 128.27 (3′ – $C_{4''}$), $C_{3''}$ and ($C_{5'}$), 129.25 (3′-$C_{2''}$ and $C_{6''}$), 129.82 (8′-$C_{2'''}$ and $C_{6'''}$), 132.57 (8′-$C_{1'''}$), 136.40 (3′ – $C_{1''}$), 142.11 ($C_{5'}$), 148.64 ($C_{7'}$),157.44 ($C_{2'}$=O), 158.36 (C_5), 162.37 ($C_{4'}$=O), 164.66 (8′-$C_{4'''}$), 169.05 ($C_{8'a}$); *Anal.* Calcd for $C_{44}H_{52}FN_3O_2\cdot0.4H_2O$: C, 77.59; H, 7.81; N, 6.17. Found: C, 77.74; H, 7.44; N, 6.22.

4.1.27. 8′-(4-Chlorophenyl)-3′-phenyl-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*,8′*H*)-dione (9g)

Yield, (650 mg, 39%); mp 200–202 °C (from EtOH); $[\alpha]_D^{28}$ (EtOH): −31.52°; UV (EtOH): λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1}): 217 (4.42), 294 (4.16), 432 (4.14); IR (ν_{max}/cm^{-1}): 1697, 1637 (CO); ^{1}H NMR ($CDCl_3$): δ_H 0.71 (3H, s, 18-CH_3), 0.87 (6H, d, J = 6.6 Hz, 25-CH(CH_3)$_2$), 0.93 (3H, d, J = 6.3 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 1.11–2.33 (24H, m), 2.72 (1H, d, J = 15.6 Hz, 1β-H), 2.93 (1H, d, J = 15.6 Hz, 1α-H), 5.54 (1H, s, 4-CH), 7.19–7.24 (4H, m, 3′,8′-Ar-*m*H), 7.37–7.39 (1H, m, 8′-Ar-*p*H), 7.43–7.46 (2H, m, 3′-Ar-*o*H), 7.54–7.57 (3H, m, 8′-Ar-*o*H), 8.39 (1H, s, 5′-CH); ^{13}C NMR ($CDCl_3$): δ_C 12.11 (C_{18}), 18.10 (C_{21}), 18.82 (C_{19}), 21.79 (C_{11}), 22.73 (C_{26}), 23.00 (C_{27}), 23.96 (C_{23}), 24.40 (C_{15}), 28.17 (C_{16}), 31.26 (C_{25}), 33.33 (C_6 and C_7), 35.89 (C_{22}), 36.10 (C_{12}, C_8 and C_{20}), 39.30 (C_{24}), 39.65 (C_{10}), 40.28 (C_1), 42.56 (C_{13}), 54.03 (C_{14}), 55.85 (C_9), 56.26 (C_{17}), 112.41 (C_4), 113.83 ($C_{6'}$), 117.41 ($C_{4'a}$), 128.25 (8′-$C_{3'''}$ and $C'_{5''}$), 128.46 (3′ – $C_{4''}$), $C_{3''}$ and ($C_{5'}$), 129.37 (3′-$C_{2''}$ and $C_{6''}$), 130.66 (8′-$C_{2'''}$ and $C_{6'''}$), 135.39 (8′-$C_{4'''}$), 136.14 (8′-$C_{1'''}$), 136.69 (3′ – $C_{1''}$), 142.17 ($C_{5'}$), 148.42 ($C_{7'}$),156.60 ($C_{2'}$=O), 157.59 (C_5), 162.59 ($C_{4'}$=O), 168.92 ($C_{8'a}$); *Anal.* Calcd for $C_{44}H_{52}ClN_3O_2\cdot H_2O$: C, 74.60; H, 7.68; N, 5.93. Found: C, 74.43; H, 7.48; N, 5.93.

4.2. Growth inhibitory activities of 3′,8′-disubstituted-5′-deazacholest-2,4-dieno[2,3-*g*]pteridine-2′,4′(3′*H*, 8′*H*)-diones (5a–j, 7a–i and 9a–g) against human tumor cell lines

The procedure was carried out using the modified MTT assay[36] to determine the inhibitory effects of test compounds on cell growth in vitro as mentioned in detail in our previous work.[2] Two human tumor cell lines of human T-cell acute lymphoblastoid leukemia (CCRF-HSB-2) and human oral epidermoid carcinoma (KB) were used in this study.

4.3. Protocol for molecular docking study

The docking software LigandFit within the software package Discovery Studio 1.7 was used to evaluate and predict the in silico binding free energy of the inhibitors within the macromolecules. First, water molecules and phosphate group of the receptor molecule were removed, hydrogen was added under a condition pH 7.40, and the alternative conformations were corrected. A binding pocket of the native STI-571 ligand was selected as the binding site for the study and expanded to make the cavity. The cavity was partitioned into 10 partition sites, and the number of points of the cavity was 5461. The forcefield PLP1 function was used to roughly search the conformations when compounds dock into the PTK. Then the conformations were optimized using a forcefield function, CHARMm and 'Adopted Basis NR' was chosen for the algorithm to optimize conformations. At last, Ludi Energy Estimate 3 was used to estimate the binding free energies of the ligands. Among the docked conformations, the pose with highest value of Ludi-3 was selected for the calculation of binding free energy (ΔG_b) and inhibition constant (K_i).

4.3.1. Preparation of ligands and target tyrosine kinase and analysis of docked results

The compounds involved in this study as ligands including **5a–j**, **7a–i**, and **9a–g** were studied for their binding activities into protein tyrosine kinase. The three-dimensional structures of the aforementioned compounds were constructed using Chem 3D ultra 8.0 software [Chemical Structure Drawing Standard; Cambridge Soft Corporation, USA (2003)]. The crystal structures of protein tyrosine kinase (1t46) complex with (STI) were retrieved from the RCSB Protein Data Bank http://www.rcsb.org/pdb/home/home.do (PDB code: 1t46). All bound waters and ligands were removed from the protein, and the polar hydrogens were added to the proteins. Accelrys, DS Studio 1.7 (Accelrys, San Diego CA, www.accelrys.com) was used for evaluating the hydrogen bonding, for determining binding orientations, for molecular modeling, and for measuring RMSD, which was measured as distance between the centroids of the docked inhibitor and the native ligand. The native ligand (STI-571) within PTK was used as a standard docked model for the study of mode of interaction of the ligands with receptor and for the calculation of RMSD.

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