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Synthesis of New 2,4-Pteridinediones and Their Application to Fluorescence Derivatization Reagents

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ABSTRACT: 3-(*p*-Chlorocarbonyl)phenyl-1-methyl-2,4(1*H*,3*H*)-pteridinedione (**6**) was synthesized through 4 steps from commercially available methyl 3-amino-2-pyrazinecarboxylate. 3-(*p*-Chlorocarbonyl)phenyl-6-(*p*-methoxy)phenyl-2,4(1*H*,3*H*)-pteridinedione (**11**) was also synthesized through 5 steps from methyl 3-amino-2-pyrazinecarboxylate. The derivatization of phenethylamine and cholesterol with pteridinedione (**6**) was carried out to give the derivatized products (**12**) and (**13**). The derivatization of phenethylamine with pteridinedione (**11**) was also carried out to give the derivatized product (**14**), which emitted a strong fluorescence. Absorption and emission maxima of the derivatized products (**12**) and (**13**) were observed at around 330 and 380 nm in MeCN, respectively. Absorption and emission maxima of the derivatized product (**14**) were observed at 291 and 369 nm, and 474 and 473 nm, respectively. Pteridinedione (**11**) was demonstrated to be applicable to a new fluorescence derivatization reagent for amines.

Keywords: 2,4(1*H*,3*H*)-pteridinedione, synthesis, fluorescence derivatization reagent, amine

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Introduction

There are many bioactive substances such as carboxylic acids, amines, amino acids, alcohols, and ketones in urine or blood, although the quantity of each substance is very small. Determining the quantity of these trace substances seemed to be indispensable for elucidation of many kinds of physiological functions in the body, diagnosis of sickness, explication of cause of disease, and medical treatment. Knowing accurate movement of drug in the body is required for effective treatment of each patient. From these backgrounds, the importance of quantitative analysis of biologically active substances and drugs has increased year by year.[1)]

Various kinds of amines are found in biological materials, and play physiologically important roles at trace levels. Parkinson's disease is a disease of nervous system characterized by a slowly spreading tremor, muscular weakness and rigidity, and a peculiar gait. Since 1,2,3,4-tetrahydroisoquinoline was found to be one of inductive substances for Parkinson's disease, pharmacological and biochemical studies on the derivatives has been intensively performed. Naturally occurring 2-phenylethylamine is a trace amine in body fluids and thought to be a neuromodulator in the central nervous system. An abnormal amount in urine is observed in patients with neurogical diseases such as schizophrenia and depression. Catecholamine is generic name of three kinds of amines, *viz.*, adrenaline, noradrenalin, and dopamine which contain the catechol skeleton. They are adrenocortical hormones and act as neurotransmitter and intercellular transmitter.[2)]

There are many kinds of alcohols like cholestanol, cholesterol, and so on. Cholestanol is a metabolite of cholesterol. In case of crebraltedoxanthma (CTX), the concentration of cholestanol raises although that of cholesterol is almost constant. The simultaneous quantitative analysis of these compounds serves to diagnose CTX. Therefore, the determination of these substances was required for biomedical and clinical treatment.[3)]

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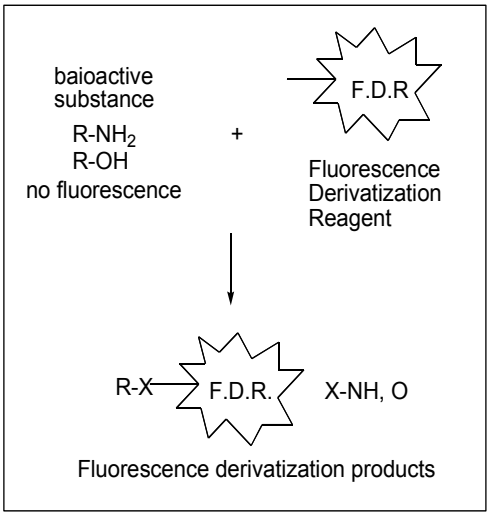
As mentioned above, a highly sensitive and selective determination of biologically active substances is quite important for their biological and clinical investigation. Unfortunately, biological substances have the following disadvantages; 1) they are high boiling points and polar compounds, and 2) they usually absorb light at shorter wavelength region below 250 nm. These two factors cause the low-sensitive determination by means of conventional gas chromatography (GC), GC-mass spectrometry, and ion-exchange chromatography. There are several high-performance liquid chromatography (HPLC) methods with electrochemical and spectrophotometric detection. However, these detections have a limit in sensitivity. At present, one of popular methods for the detection of biological substances is HPLC method with fluorescence detection. Accordingly, various fluorescence derivatization reagents with high sensitivity have been used for HPLC determination of biological substances as depicted below.



The following points are required for a highly sensitive and selective fluorescence derivatization reagent; 1) it emits strong fluorescence at region of longer wavelength, 2) the difference in the excitation and emission wavelength is large, 3) the reaction proceeds rapidly and quantitatively under mild conditions, and 4) the derivatization reagent and the resulting product are stable and the molecular size of the derivatization reagent is small.

In the past, a variety of fluorescence derivatization reagents such as OPA[4], NDA[5], Dansyl-Cl[6], NBD-Cl[7], DMEQ-COCl[8], FMOC-Cl[9], AEOC[10], and AOCD[11] for amines have been developed. However, these reagents have some disadvantages. For example, the use of the naphthalene-2,3-dicarbaldehyde (NDA) was limited to primary amines. (2-Phthalimidyl)benzoyl chloride (PIB-Cl) was not suitable for aromatic amines. At the same time, the stability of the derivatized compounds is not so high, and thus the decomposition was sometimes observed during analysis. In cases of 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) and 9-(2-acridone)oxyethylcarbonylimidazole (AOCD), their Stokes' shifts are not so large. In a case of (9-fluorenyl)methyl chloroformate (FMOC-Cl), its emission wavelength is short.

A variety of fluorescence derivatization reagents such as DMEQ-CON_3[12], MOC-CON_3[13], MOC-COCl[14], and DACB-NCO[15] for alcohols have also been developed. However, these reagents also have some disadvantages; 1) they are unstable toward moisture, 2) the reaction of them with alcohols requires heating for complete derivatization, and 3) they are expensive. Thus, the development of more efficient and cheap fluorescence derivatization reagents is still desired.

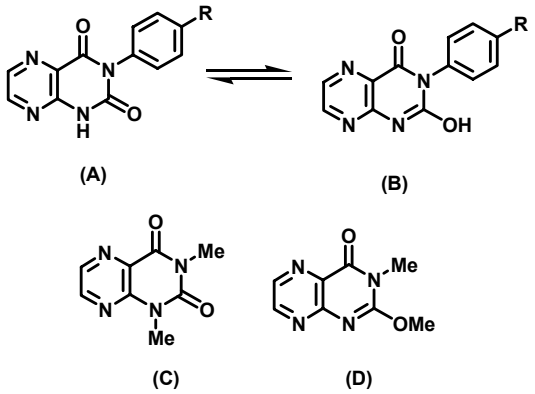
In our laboratory, synthesis of 3-(aryl)substituted-2,4(1*H*,3*H*)-pteridinediones (**A**) and their UV-VIS and fluorescence spectroscopic characteristics have been reported.[16] Compound (A, R=H) showed two absorption bands at 320 and 390 nm in MeCN, the latter having a small molar absorption coefficient, and at 273 and 371 nm in EtOH. Similar spectra were obtained in case of pteridinedione (A, R=OCH_3). (Table 1) It is well know that 3-substituted 2,4(1*H*,3*H*)-pteridinedione exists in tautomeric equilibrium between lactam (**A**) and lactim (**B**) as shown in the next page. Then, the date of 3-substituted 2,4-pteridinediones were compared with those of model compounds for these two tautomers, 1,3-dimethyl-2,4(1*H*,3*H*)-pteridinedione (**C**) and 2-methoxy-3-methyl-4(3H)-pteridinedione (**D**).[17] The absorption bands around 320 nm in MeCN and 270 nm in EtOH were observed on the spectra of model compounds (**C**) and (**D**), respectively, suggesting that 3-(aryl) substituted 2,4(1*H*,3*H*)-pteridinediones predomi- nantly exist in the lactam from (**A**) in MeCN and in the lactim from (**B**) in EtOH. In the fluorescence spectra, pteridinediones showed two emission bands at around 380 and 460 nm in MeCN. On the other hand, pteridinedines

showed a emission band at around 470 nn when the excitation wavelength of 320 nm was applied.

Table 1 UV and fluorescence properties of pteridinediones (A)

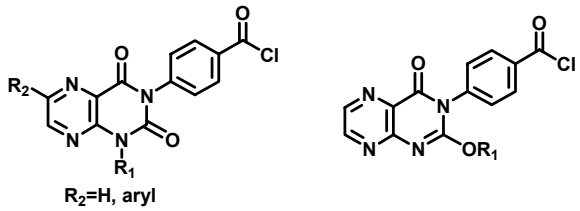
R	UV/λ_{max} nm (ε)		Fluorescence/λ_{max} nm	
	in MeCN	in EtOH	in MeCN	in EtOH
H	320(3900)	273(10300)	376	467
	389 (260)	371 (3300)	450	
OCH_3	317(7900)	274(19500)	377	469
	382 (360)	374 (6000)	471	

From these results, it was suggested that pteridinediones have the possibility as new fluorescent derivatization reagents if pteridinediones having a reactive group at the *para* position of the phenyl group at C-3 position and high solubility in organic solvents are synthesized.



The chlorocarbonyl and sulfonyl chloride groups are widely used as reactive groups on the derivatization reaction of amines and alcohols. It has already been reported that 9-fluorenylmethyl chloroformate having a chlorocarbonyl group reacts with primary and secondary amines in the borate buffer solution (pH 8) and the reaction completes within 2 minutes at room temperature. Chlorocarbonyl, carbonyl azide, isocyanate, and carbonyl nitrile groups are also used as reactive groups in derivatization reagents for alcohols. Among these reagents, the chlorocarbonyl group has been widely and conventionally used for a long time.

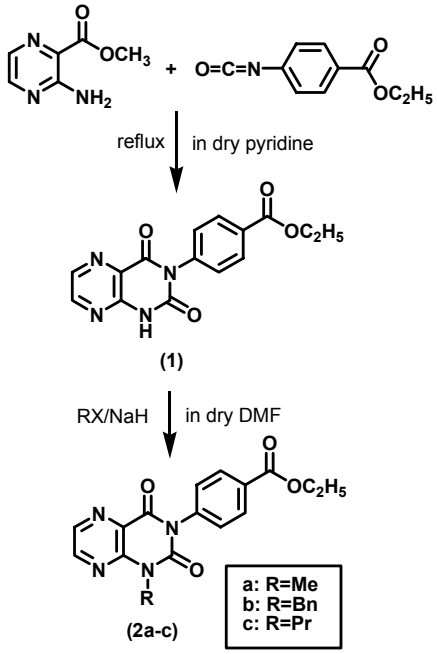
We have reported the application of heterocyclic compounds to fluorescence derivatization reagents.[18] As extensive studies, we would like to describe here 1) the synthesis of *N*-alkylated and *O*-alkylated pteridinedione derivatives bearing the *p*-(chlorocarbonyl)phenyl group at C-3 position, 2) synthesis of 6-(aryl) substituted pteridinediones bearing the *p*-(chlorocarbonyl)phenyl group at C-3 position, and 3) the evaluation of the possibility of synthetic compounds as new fluorescence derivatization reagents for amines and alcohols.



Results and Discussion

Synthesis of *N*-alkylated pteridinediones

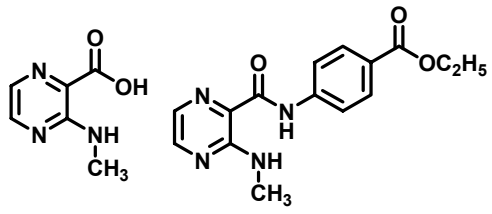
A solution of commercially available methyl 3-amino-2-pyrazinecarboxylate and ethyl 4-isocyanato-benzoate in dry pyridine was refluxed for 9 h to give 3-(*p*-ethoxycarbonyl)phenyl-2,4(1*H*,3*H*)-pteridinedione (**1**) in 73% yield. (Scheme 1) The structural assignment of 3-(*p*-ethoxycarbonyl)phenyl-2,4-(1*H*,3*H*)-pteridinedione (**1**) was carried out by means of ^{1}H-NMR and IR spectroscopies. The signals assignable to 6-H and 7-H on the pteridinedione ring were observed at δ 8.59 and 8.72 ppm, respectively, in DMSO-d_6. The absorption bands due to NH and two C=O stretching vibrations were observed at 3426, 1717 and 1677 cm^{-1}, respectively.



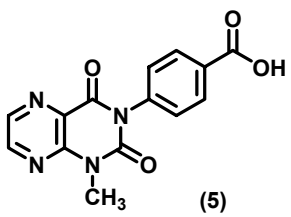
Scheme 1

Pteridinedione (**1**) was allowed to react with CH_3I in the presence of NaH in dry DMF at 80 ºC for 4 h to give 3-(*p*-ethoxycarbonyl)phenyl-1-methyl-2,4(1*H*,3*H*)-pteridinedione (**2a**) in 96% yield as yellow powders. The structural assignment of compound (**2a**) was carried out by means of ^{1}H-NMR, IR, and UV spectroscopies. The signal observed at δ 3.76 ppm in $CDCl_3$ was assignable to *N*-CH_3 or *O*-CH_3 protons, because compound (**1**) produced ambient anion when was treated with NaH. The structure of the product was determined by comparison of UV-VIS spectrum of the product with the model compounds. The absorption bands around 320 nm in MeCN and 270 nm in EtOH were observed on UV spectra of model compounds (**C**) and (**D**), respectively.[17] Actually, the absorption bands of compound (**2a**) were 329 nm in MeCN and 331 nm in EtOH, respectively. Therefore, the structure of compound (**2a**) was assigned to be *N*-methylated product. The alkylation of pteridinedione (**1**) with benzyl bromide and propyl iodide were carried out in the similar manner to give pteridinedione (**2b**) in 64% as white crystals and (**2c**) in 74 % yield as white powders, respectively.

The hydrolysis of pteridinedione (**2a**) was carried out using two solvent systems [MeOH:DMF(1:1) or MeOH]. At first, to a solution of compound (**2a**) in MeOH:DMF (1:1) mixture was added 1M NaOH and the mixture was stirred at room temperature for 4 h, but the desired 3-(*p*-carboxy)phenyl-1-methyl-2,4(1*H*,3*H*)-pteridinedione (**5**) could not be obtained. Instead, the ring-opening products (**3**) and (**4**) were isolated.



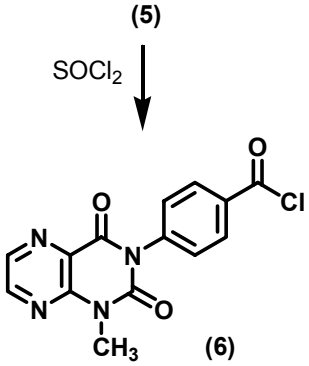
(3) (4)



(5)

On the other hand, to a solution of compound (**2a**) in MeOH was added 1M NaOH and the mixture was stirred at room temperature for 4 h to give the desired product (**5**) in 58% yield. The structural assignment of compounds (**3-5**) was carried out by means of ^{1}H-NMR and IR spectroscopies. In the case of compound (**3**), the signals assignable to 6-H and 7-H on the pteridinedione ring were shifted to upper magnetic field at δ 8.36 and 7.74 ppm in $CDCl_3$, respectively. The absorption bands due to COOH stretching vibration were observed at 2845 (broad) and 1679 cm^{-1}. In the case of compound (**4**), the signals due to protons of the ethyl ester were observed at δ 4.36 and 1.39 ppm, and two doublet signals owing to 5-H and 6-H on the pyrazine ring were observed at δ 8.00 and 7.99 ppm in $CDCl_3$, respectively. The absorption bands due to NH and two C=O stretching vibrations were observed at 3316, 1736 and 1695 cm^{-1}, respectively. In the case of compound (**5**), the signals assignable to protons of the ethyl ester group of the starting material (**2a**) could not be observed in CD_3OD. The absorption bands due to the COOH stretching vibration were observed at 3077 (broad) and 1718 cm^{-1}.

The conversion of the carboxyl into chlorocarbonyl group was attempted. A solution of pteridinedione (**5**) in $SOCl_2$ was refluxed for 8 h to give 3-(*p*-chlorocarbonyl)phenyl-1-methyl-2,4(1*H*,3*H*)-pteridinedione (**6**). (Scheme 2)



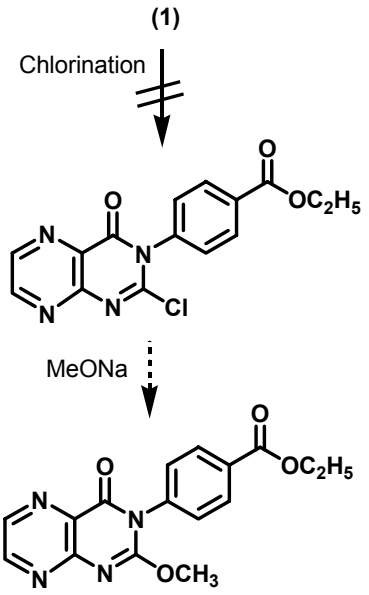
Scheme 2

The absorption band typical for the C=O stretching vibration of the COCl group was observed at 1770 cm^{-1}.

An attempt to synthesize the *O*-alkylated pteridinone

At first, synthesis of *O*-alkylated pteridinone was attempted according to Scheme 3. The chlorination of pteridinedione (**1**) was carried out using three methods (method A: thionyl chloride; method B: phosphorus oxychloride; method C: a mixture of phosphorus oxychloride and phosphorus pentachloride). In the case of method A, a solution of pteridinedione (**1**) and $SOCl_2$ in

dry dioxane and DMF was stirred at 90 ºC for 5 h, but the starting material (**1**) was recovered.



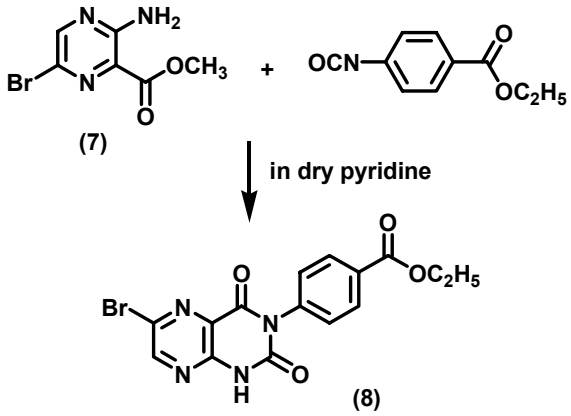
Scheme 3

In the case of method B, a solution of pteridinedione (**1**) in $POCl_3$ was refluxed for 4 days. Again, the desired product could not be obtained. In the case of method C, a solution of pteridinedione (**1**), $POCl_3$, and PCl_5 in $CHCl_3$ was refluxed for 2 days, and then heated at 120 ºC for 40 h in a sealed tube. However, the desired product again could not be obtained.

Next, the preparation of *O*-alkylated pteridinone was attempted according to the reported method.[19] However, any trials are unsuccessful. From these results, the synthesis of *O*-alkylated pteridinone was abandoned.

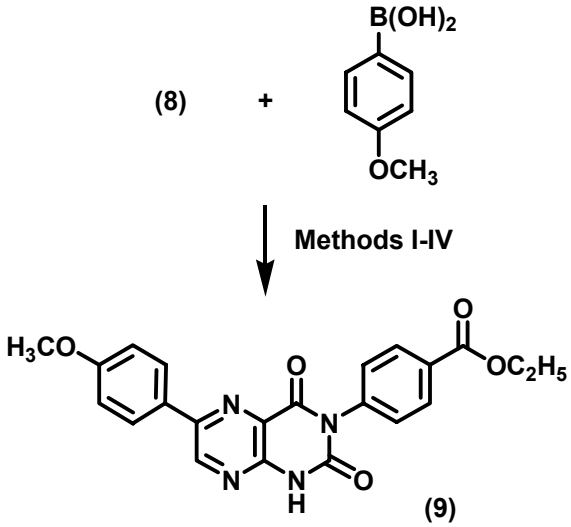
Synthesis of 6-(aryl) substituted pteridinedione

A solution of commercially available methyl 3-amino-2-pyrazinecarboxylate and an excess of Bu_4NBr_3 in a mixture of dry $CHCl_3$ and pyridine was refluxed for 15 h to give methyl 3-amino-6-bromopyrazine-2-carboxylate (**7**) in 49% yield as yellow powders. A solution of compound (**7**) and ethyl *p*-isocyanatobenzoate in dry pyridine was refluxed for 11 h to give 3-(*p*-ethoxycarbonyl)phenyl-6-bromo-2,4(1*H*,3*H*)-pteridinedione (**8**) in 58% yield as brown crystals. (Scheme 4) The signal typical for 7-H proton on the pteridinedione ring was observed at δ 8.70 ppm in $CDCl_3$. The absorption band due to C-Br stretching vibration was observed at 517 cm^{-1}. Further, the absorption bands due to two kinds of C=O stretching vibrations were observed at 1739 and 1693 cm^{-1}.



Scheme 4

The Suzuki coupling of compound (**8**) with *p*-methoxyphenylboronic acid was attempted according to Scheme 5.



Scheme 5

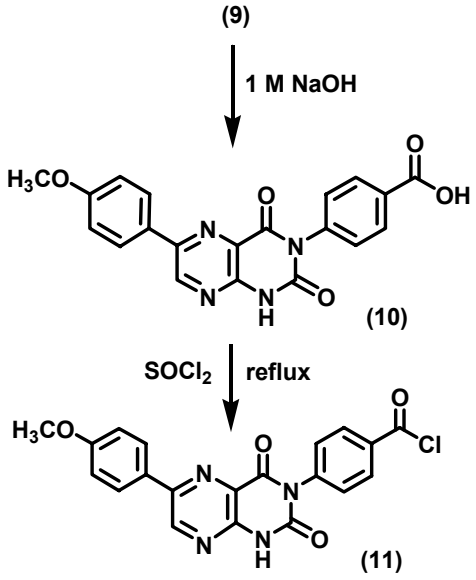
Four methods were applied to optimize the Suzuki coupling as summarized in Table 2. However, the yield of pteridinedione (**9**) could not be improved. The structural assignment of compound (**9**) was carried out by means of ^{1}H-NMR and IR spectroscopies. The signals assignable to 3'', 5''-H and 2'', 6''-H on the benzene ring and protons of the methoxy group at C-6 position were observed at δ 8.08 and 7.04, and 3.89 ppm, respectively, in $CDCl_3$. The signals due to 7-H and N-H protons were observed at δ 9.03 and 8.64 ppm, respectively. Two

signals due to ethyl ester were also observed at δ 1.41 and 4.42 ppm. In IR spectrum, the absorption bands due to three kinds of C=O stretching vibrations were observed at 1735, 1712, and 1690 cm^{-1}.

Table 2 Reaction conditions and yields of compound (9)

Method	Base	Conditions	Yield(%)
I	Na_2CO_3	dioxane/reflux (10 h)	20
II	Na_2CO_3	benzene / reflux (15 h)	0
III	K_3PO_4	DMF / 80-130 ºC (69 h)	30
IV	K_2CO_3	DMF/120 ºC (7 days)	25

To a solution of pteridinedione (**9**) in MeOH was added 1M NaOH. The reaction mixture was stirred at room temperature for 5 days and at 60 ºC for another 3 days to give the corresponding carboxylic acid (**10**) as yellow solids. (Scheme 6) The signals assignable to the protons of ethyl ester of the starting material (**9**) disappeared in DMSO-d_6. The absorption band due to the O-H and C=O stretching vibrations of COOH was observed at 3060 (broad) and 1692 cm^{-1}, respectively.



Scheme 6

The conversion of –COOH into –COCl was attempted as follows. A solution of pteridinedione (**10**) in $SOCl_2$ was refluxed for 4 h to give 3-(*p*-chlorocarbonyl) phenyl-6-(*p*-methoxy)phenyl-2,4(1*H*,3*H*)-pteridinedione (**11**) which was used for the next reaction without further purification. The absorption band typical for the C=O stretching vibration of COCl was observed at 1796 cm^{-1}.

Measurement of UV-VIS and fluorescence spectra of pteridinediones

UV-VIS and fluorescence spectral data of compounds (**2a-c** and **9**) are summarized in Table 3. Absorption maxima of compounds (**2a-c**) were observed around 330 nm both in MeCN and in MeOH. Emission maximum of compound (**2b**) in MeOH was observed at about 30 nm longer wavelength than that in MeCN. However, in a case of this compound, its fluorescence intensity was remarkably decreased.

Table 3 UV-VIS and fluorescence spectral data of compounds (2a-c) and (9)

Compd.	UV[a]/ λ_{max} nm (ε)		Fluorescence[b]/ λ_{max} nm	
	in MeCN	in MeOH	in MeCN	in MeOH
2a	330 (7810)	330 (7320)	381	382
2b	328 (8010)	329 (7370)	385	412
2c	332 (7880)	331 (7670)	382	391
9	290(23800)	293 (23140)	474	495
	369 (8500)	373 (7570)	474	497

a) Concentration: 1.0 x 10^{-4} M

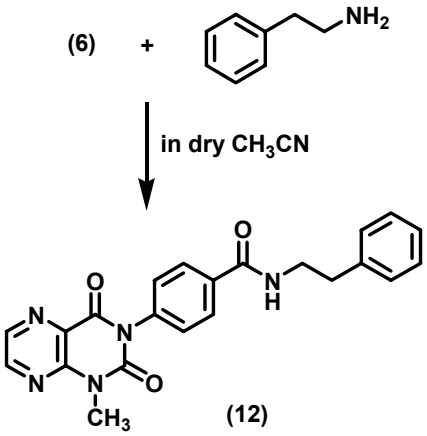
b) Concentration: 1.0 x 10^{-6} M

On the other hand, absorption maxima of compound (**9**) were observed at around 290 and 370 nm both in MeCN and in MeOH. From this result, it was suggested that compound (**9**) exists in the lactim tautomer like **D** both in MeCN and in MeOH. Emission maximum of compound (**9**) in MeOH was observed at 20 nm longer wavelength than that in MeCN. The difference in the fluorescence intensity between in MeCN and in MeOH was found to be large. Compound (**9**) showed the strongest emission band at 474 nm in MeCN when the excitation wavelength of 290 nm was applied.

Application of pteridinediones to fluorescence derivatization reagents of phenethylamine

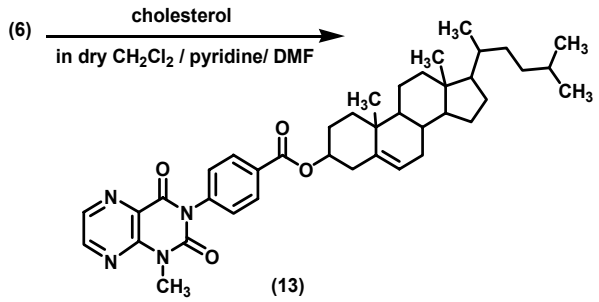
A solution of pteridinedione (**6**) and phenethylamine in dry MeCN was stirred at room temperature to give the

product (**12**) as pale yellow powders in 80% yield. From TLC monitoring, it was found that the derivatization completed within a few minutes. (Scheme 7) The signals due to the phenethyl group were observed at δ 2.88, 3.52, and 7.26-7.34 ppm in CD_3OD. The absorption band due to the N-H and C=O stretching vibrations of amide were observed at 3305 and 1636 cm^{-1}, respectively.



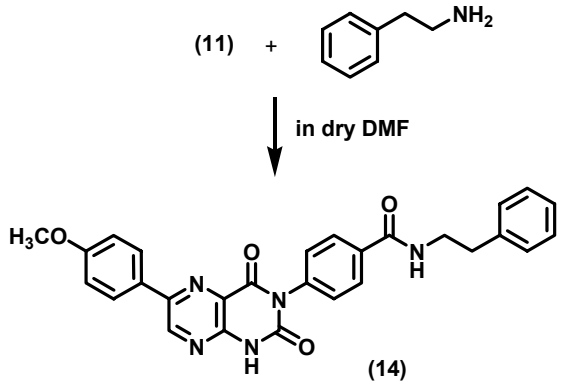
Scheme 7

To a solution of pteridinedione (**6**) in dry CH_2Cl_2, pyridine, and DMF was added cholesterol, and then the reaction mixture was stirred at room temperature to give the product (**13**) as white solids. From TLC analysis, it was found that the derivatization completed within 10 minutes. (Scheme 8) The signals assignable to protons of the cholesteryl group were observed at δ 0.70, 0.86, 0.92, 0.99-2.01, 2.47, 4.89, and 5.44 ppm in $CDCl_3$. The absorption bands due to ester and amide C=O stretching vibrations were observed at 1731 and 1695 cm^{-1}, respectively.



Scheme 8

A solution of pteridinedione (**11**) and phenethylamine in dry MeCN and DMF was stirred at room temperature to give the product (**14**) as yellow solids. (Scheme 9) The signals due to the phenethyl group were observed at δ 2.95, 3.63, and 7.26-7.33 ppm in CD_3OD. The absorption band due to the N-H and C=O stretching vibrations of amide were observed at 3425 and 1687 cm^{-1}, respectively.



Scheme 9

Measurement of UV-VIS and fluorescence spectra of the derivatized products

UV-VIS and fluorescence spectral data of the derivatized products (**12**), (**13**), and (**14**) are summarized in Table 4. Absorption and emission maxima of compounds (**12**) and (**13**) were observed at around 330 and 380 nm in MeCN, respectively. The absorption and emission maxima of compounds (**12**) and (**13**) were the almost same as compound (**2a**) regardless of the ester or amide linkage.

Table 4 UV-VIS and fluorescence spectral data of the derivatized products (12), (13), and (14) in MeCN

Compd.	UV[a]/ λ_{max} nm (ε)	Fluorescence[b] λ_{max} nm
12	329 (7266)	383
13	330 (6900)	381
14	291 (16130)	474
	369 (5192)	473

a) Concentration: 1.0 x 10^{-4} M

b) Concentration: 1.0 x 10^{-6} M

From these results, pteridinedione (**6**) is applicable to a new fluorescence derivatization reagent for amines and alcohols, although the emission maximum is fairly short wavelength. On the other hand, the absorption maxima of compound (**14**) were observed at 291 and 369 nm. The emission maximum of compound (**14**) were observed

at around 470 nm. The fluorescence intensity of compound (**14**) was apparently stronger than those of compounds (**12**) and (**13**). The Stokes' shift of compound (**14**) was remarkably larger than those of compounds (**12**) and (**13**). From these results, pteridinedione (**11**) was demonstrated to be applicable to a new fluorescence derivatization reagent for amines.

Experimental

General. Melting points were recorded on a Melting Point Apparatus SMP3 in open capillaries and are uncorrected. IR spectra were recorded on a JASCO FT/IR-470 plus Fourier Transform Infrared spectrometer. UV-VIS spectra were recorded on a JASCO V-550 UV/VIS spectrophotometer. Fluorescent spectra were recorded on a JASCO FP-777 and JASCO FP-6500 spectrofluorometers. ^{1}H-NMR spectra were recorded on JEOL JNMLA400D NMR spectrometer in $CDCl_3$, DMSO-d_6, and CD_3OD are reported in ppm (δ) downfield from internal Me_4Si. Thin-layer chromatography (TLC) was performed on silica gel 60F-254 with a 0.2 mm Layer thickness. Column chromatography was carried out with KANTO CHEMICAL silica gel 60N (spherical, neutral, 63-210 μm). Flash column chromatography was carried out with Merck silica gel 60 (40-63 μm). Recycle GPC was carried out with a JAI LC-9201 equipped with a MDL-101 by using columns packed with a JAIGEL-1H and JAIGEL-2H. High performance liquid chromatography (HPLC) was carried out with JASCO 800-PU, a 821-FP equipped with a JASCO 807-IT integrator by using a column packed with a Crestpak C18T-5. Combustion analyses were performed on a Perkin Elmer 2400 Series II CHNS/O analyzer.

3-(*p*-Ethoxycarbonyl)phenyl-2,4(1*H*,3*H*)-pteridine-dione (1)

A mixture of methyl 3-amino-2-pyrazinecarboxylate (529 mg, 3.5 mmol) and ethyl *p*-isocyanatobenzoate (950 mg, 5 mmol) in dry pyridine (100 mL) was refluxed at 135 ºC for 9 h. After evaporation of the solvent, ether was added to the residue for washing. The collected crude product was recrystallized from MeOH to give the pure product (**1**) as yellow crystals. Yield: 480 mg (45%); mp: 280-285 ºC; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 1.34 (3H, t, J = 7.1 Hz, CH_3), 4.36 (2H, q, J = 7.1 Hz, CH_2), 7.52 (2H, d, J = 8.3 Hz, 3',5'-*N*-Ph), 8.09 (2H, d, J = 8.3 Hz, 2',6'-*N*-Ph), 8.59 (1H, d, J = 2.4 Hz, C_6-H), and 8.72 ppm (1H, d, J = 2.4 Hz, C_7-H); IR (KBr): 3426 (ν_{N-H}), 1717 ($\nu_{C=O}$), and 1677 cm^{-1} ($\nu_{C=O}$). EA Calcd for $C_{15}H_{12}N_4O_4$: C, 57.69; H, 3.80; N, 17.94%. Found: C, 57.47; H, 4.09; N, 17.97%

3-(*p*-Ethoxycarbonyl)phenyl-1-methyl-2,4(1*H*,3*H*)-pteridinedione (2a)

A solution of compound (**1**) (489 mg, 1.6 mmol) in dry DMF (50 mL) was added dropwise to a solution of NaH (73 mg of 60 weight% dispersion in paraffin oil, 1.8 mmol) in dry DMF (10 mL), and then the mixture was stirred at room temperature for 1 h. CH_3I (426 mg, 3.0 mmol) in dry DMF (5 mL) was added dropwise to the mixture, and the reaction mixture was stirred at 80 ºC for 4.5 h. After addition of EtOH (0.5 mL), the solvent was evaporated off under reduced pressure, and then the organic materials were extracted with $CHCl_3$ (100 mL x 2). The organic layer was washed with H_2O (100 mL x 4), saturated NaCl aqueous solution (50 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography on silica gel with $CHCl_3$: acetone : EtOH (100 : 10 : 1) mixture to give the pure product (**2a**) as pale yellow powders. Yield: 493 mg (96%); mp: 238-239 ºC; ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 1.41 (3H, t, J = 7.2 Hz, CH_3), 3.76 (3H, s, 2-*N*-CH_3), 4.42 (2H, q, J = 7.2 Hz, CH_2), 7.39 (2H, d, J = 8.8 Hz, 3',5'-*N*-Ph), 8.23 (2H, d, J = 8.8 Hz, 2',6'-*N*-Ph), 8.65 (1H, d, J = 2.2 Hz, C_6-H), and 8.71 ppm (1H, d, J = 2.2 Hz, C_7-H); IR (KBr): 1726 ($\nu_{C=O}$), 1691($\nu_{C=O}$), and 1282 cm^{-1} (ν_{C-O}). EA Calcd for $C_{16}H_{14}N_4O_4$•0.5H_2O: C, 57.31; H, 4.51; N, 16.71%. Found: C, 57.22; H, 4.26; N, 16.50%

The following products (**2b** and **2c**) were prepared acording to the similar manner to compound **(2a)**.

3-(*p*-Ethoxycarbonyl)phenyl-1-benzyl-2,4(1*H*,3*H*)-pteridinedione (2b) as white crystals; yield: 258 mg (64%); mp: 215-216 ºC; ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 1.41 (3H, t, J = 7.1 Hz, CH_3), 4.41 (2H, q, J = 7.1 Hz, CH_2), 5.56 (2H, s, 1-*N*-$\underline{CH_2}$Ph), 7.31-7.35 (3H, m, 1-*N*-$CH_2\underline{Ph}$), 7.39 (2H, d, J = 8.8 Hz, 3',5'-*N*-Ph), 7.53-7.56 (2H, m, 1-*N*-$CH_2\underline{Ph}$), 8.22 (2H, d, J = 8.8 Hz, 2',6'-*N*-Ph), 8.65 (1H, d, J = 2.2 Hz, C_6-H), and 8.73 ppm (1H, d, J = 2.2 Hz, C_7-H); IR (KBr): 1726 ($\nu_{C=O}$), 1687 ($\nu_{C=O}$), 1273 (ν_{C-O}), 747 and 702 cm^{-1} (δ_{C-H}). EA Calcd for $C_{22}H_{18}N_4O_4$: C, 65.66; H, 4.51; N, 13.92%**.** Found: C, 65.66; H, 4.49; N,

13.88%

3-(*p*-Ethoxycarbonyl)phenyl-1-propyl-2,4(1*H*,3*H*)-pteridinedione (2c) as white powders; yield: 264 mg (74%); mp: 184-186 ºC; ^{1}H-NMR (δ, DMSO-d_6 at 120 ºC, 400 MHz): 0.95 (3H, t, J = 7.5 Hz, 1-*N*-$CH_2CH_2\underline{CH_3}$), 1.37 (3H, t, J = 7.1, Hz, CH_3), 1.77 (2H, sext, J = 7.3 Hz, 1-*N*-$CH_2\underline{CH_2}CH_3$), 4.23 (2H, t, J = 7.3 Hz, 1-*N*-$\underline{CH_2}CH_2CH_3$), 4.39 (2H, q, J = 7.1 Hz, CH_2), 7.50 (2H, d, J = 8.5 Hz, 3',5'-*N*-Ph), 8.08 (2H, d, J = 8.5 Hz, 2',6'-*N*-Ph), 8.62 (1H, d, J = 2.2 Hz, C_6-H), and 8.78 ppm (1H, d, J = 2.2 Hz, C_7-H); IR (KBr): 1728 ($\nu_{C=O}$), 1692 ($\nu_{C=O}$) and 1273 cm^{-1} (ν_{C-O}). EA Calcd for $C_{18}H_{18}N_4O_4$: C, 61.01; H, 5.12; N, 15.81%. Found: C, 60.94; H, 4.91; N, 15.70%

3-(*p*-Carboxy)phenyl-1-methyl-2,4(1*H*,3*H*)-pteridine-dione (5)

[Method A]: To a solution of 3-(*p*-ethoxycarbonyl)-phenyl-1-methyl-2,4(1*H*,3*H*)-pteridinedione (100 mg, 0.31 mmol) in MeOH:DMF (1:1) mixture (50 mL) was added 1M NaOH (0.6 mL, 0.6 mmol), and the mixture was stirred at room temperature for 2.5 h. An additional 1 M NaOH (0.6 mL, 0.6 mmol) was added to the mixture and the reaction mixture was stirred at room temperature for another 3 h. After evaporation of the solvent, to the residue was added H_2O (50 mL) and $CHCl_3$ (50 mL). The aqueous layer was adjusted about pH 2 with 10% citric acid and extracted with $CHCl_3$ (200 mL x 2), which was washed with H_2O (100 mL) and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was recrystallized from AcOEt to give the pure product (**3**) as yellow powders. On the other hand, the $CHCl_3$ layer was evaporated off under reduced pressure and the residue was purified by column chromatography on silica gel with $CHCl_3$:acetone:EtOH (100:10:1) mixture to give the product (**4**) as yellow powders. However, compound (**5**) could not be obtained.

3-(Methylamino)pyrazine-2-carboxylic acid (3): Yield: 23 mg (48%); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 3.10 (3H, d, J = 4.9 Hz, CH_3), 7.74 (1H, d, J = 2.2 Hz, C_5-H), 7.90 (1H, br, NH), and 8.36 ppm (1H, d, J = 2.2 Hz, C_6-H); IR (KBr): 3365 (ν_{N-H}), 2845 (broad, ν_{O-H}), and 1679 cm^{-1} ($\nu_{C=O}$). EA Calcd for $C_6H_7N_3O_2$: C, 47.06; H, 4.61; N, 27.44%. Found: C, 47.25; H, 4.57; N, 27.19%.

2-[(*p*-Ethoxycarbonyl)phenyl]amido-3-(methylamino)pyrazine (4): Yield: 11 mg (12 %); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 1.39 (3H, t, J = 7.1 Hz, CH_3), 3.80 (3H, s, *N*-CH_3), 4.36 (2H, q, J = 7.1 Hz, CH_2), 7.54-7.99 (4H, m, -NH-$\underline{Ph}$), 7.99 (1H, d, J = 2.2 Hz, C_5-H), and 8.00 ppm (1H, d, J = 2.2 Hz, C_6-H); IR (KBr): 3316 (ν_{N-H}), 1736 ($\nu_{C=O}$), 1695 ($\nu_{C=O}$), and 770 cm^{-1} (δ_{C-H}).

[Method B]: To a solution of compound (**2a**) (103 mg, 0.3 mmol) in MeOH (360 mL) was added 1M NaOH (10 mL, 10 mmol), and the mixture was stirred at room temperature for 4 h. After evaporation of the solvent, to the residue was added H_2O (50 mL) and $CHCl_3$ (50 mL). The aqueous layer was adjusted about pH 2 with 1 M HCl, extracted with $CHCl_3$ (200 mL x 2), and then the $CHCl_3$ layer was dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was recrystallized from AcOEt to give the pure product (**5**) as pale yellow crystals. Yield: 53 mg (58%); ^{1}H-NMR (δ, CD_3OD, 400 MHz): 3.72 (3H, s, 2-*N*-CH_3), 7.47 (2H, d, J = 8.5 Hz, 3',5'-*N*-Ph), 8.18 (2H, d, J = 8.5 Hz, 2',6'-*N*-Ph), 8.59 (1H, d, J = 2.4 Hz, C_6-H), and 8.81 ppm (1H, d, J = 2.4 Hz, C_7-H); IR (KBr): 3077 (ν_{O-H}), 1718 ($\nu_{C=O}$), 1663 ($\nu_{C=O}$), and 779 cm^{-1} (δ_{C-H}). EA Calcd for $C_{14}H_{10}N_4O_4$: C, 56.38; H, 3.38; N, 18.78%. Found: C, 56.25; H, 3.14; N, 18.55%.

3-(*p*-Chlorocarbonyl)phenyl-1-methyl-2,4(1*H*,3*H*)-pteridinedione (6)

A solution of compound (**5**) (30 mg, 0.1 mmol) in $SOCl_2$ (3 mL, 0.3 mmol) was refluxed for 8 h. The solvent was evaporated under reduced pressure to give the product (**6**) as pale yellow powders; IR (KBr): 1770 ($\nu_{C=O, COCl}$), 1727 ($\nu_{C=O}$), and 1683 cm^{-1} ($\nu_{C=O}$)

Methyl 3-amino-6-bromopyrazine-2-carboxylate (7)

To a solution of commercially available methyl 3-amino-2-pyrazinecarboxylate (1.53 g, 10 mmol) and Bu_4NBr_3 (6.79 g, 14 mmol) in dry $CHCl_3$ (25 mL) was added dry pyridine (2 mL), and then the mixture was refluxed for 15 h. To the reaction mixture was added H_2O (100 mL), and then the aqueous layer was extracted with $CHCl_3$ (100 mL x 4). The combined organic layers were washed with 10% $NaHSO_3$ (30 mL), saturated NaCl aqueous solution (30 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography on silica gel with $CHCl_3$:acetone:EtOH (100:10:1) mixture to give the pure product (**7**) as yellow powders. Yield: 1.14 g (49 %); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 3.98 (3H, s, CH_3) and 8.29 ppm (1H, s, C_5-H); IR (KBr): 3416 and 3363 (ν_{N-H}),

1701 ($\nu_{C=O}$), and 516 cm^{-1} (ν_{C-Br}). EA Calcd for $C_6H_6BrN_3O_2$: C, 31.06; H, 2.61; N, 18.11%. Found: C, 31.19; H, 2.37; N, 17.82%

3-(*p*-Ethoxycarbonyl)phenyl-6-bromo-2,4(1*H*,3*H*)-pteridinedione (8)

A solution of compound (**7**) (1.05 g, 4.5 mmol) and ethyl *p*-isocyanatobenzoate (1.77 g, 9.2 mmol) in dry pyridine (60 mL) was refluxed for 11 h. After evaporation of the solvent, ether (100 mL) was added to the residue for washing. The collected crude product was recrystallized from MeOH to give the product (**8**) as brown crystals. Yield: 782 mg (58 %); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 1.41 (3H, t, *J* = 7.1 Hz, CH_3), 4.42 (2H, q, *J* = 7.1 Hz, CH_2), 7.38 (2H, d, *J* = 8.1 Hz, 3',5'-*N*-Ph), 8.23 (2H, d, *J* = 8.1 Hz, 2',6'-*N*-Ph), and 8.70 ppm (1H, s, C_7-H); IR (KBr): 1739 ($\nu_{C=O}$), 1693 ($\nu_{C=O}$), 1292 (ν_{C-O}), 869 (δ_{C-H}), and 517 cm^{-1} (ν_{C-Br}).

3-(*p*-Ethoxycarbonyl)phenyl-6-(*p*-methoxy)phenyl-2,4(1*H*,3*H*)-pteridinedione (9)

[Method I]: A solution of compound (**8**) (196 mg, 0.5 mmol) in dioxane (20 mL) was degassed. To the mixture was added bis(triphenylphosphone) palladium(II) dichloride (19 mg, 0.026 mmol, 5.3 mol%), triphenylphosphine (15 mg, 0.05 mmol), *p*-methoxy- phenylboronic acid (77 mg, 0.5 mmol), and 2.0 M Na_2CO_3 (0.6 mL), and then the reaction mixture was refluxed for 10 h under argon atmosphere. After cooling, the precipitate was filtered off. After evaporation of the filtrate, H_2O (30 mL) was added to the residue, the aqueous layer was extracted with $CHCl_3$ (40 mL x 5), and the organic layer was dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography on silica gel with $CHCl_3$:acetone:EtOH (100:20:4) mixture to give the product (**9**) as yellow solids. Yield: 40 mg (20 %)**;** ^{1}H-NMR (δ, $CDCl_3$ 400 MHz) : 1.41 (3H, t, *J* = 7.1 Hz, CH_3), 3.89 (3H, s, OCH_3), 4.42 (2H, q, *J* = 7.1 Hz, CH_2), 7.04 (2H, d, *J* = 8.8 Hz, 2",6"-Ph), 7.42 (2H, d, *J* = 8.3 Hz, 3',5'-*N*-Ph), 8.08 (2H, d, *J* = 8.8 Hz, 3",5"-Ph), 8.24 (2H, d, *J* = 8.3 Hz, 2',6'-*N*-Ph), 8.64 (1H, br, NH), and 9.03 ppm (1H, s, C_7-H); IR (KBr) : 3518 (ν_{N-H}), 1735 ($\nu_{C=O}$), 1690 ($\nu_{C=O}$), 1275 (ν_{C-O})and 840 cm^{-1} (δ_{C-H}).[Method II]: To a solution of compound (**8**) (78 mg, 0.2 mmol) in benzene (18 mL) was added bis- (triphenylphosphone)palladium(II) dichloride (8.0 mg, 0.01 mmol, 5 mol%), triphenyl phosphine (7.2 mg, 0.03 mmol), *p*-methoxyphenylboronic acid (34 mg, 0.2 mmol), and 2.0 M Na_2CO_3 (0.22 mL), and the reaction mixture was refluxed for 15 h under argon atmosphere. The same work-up as method I, but compound (**9**) could not be obtained.

[Method III]: To a solution of compound (**8**) (157 mg, 0.4 mmol) in dry DMF (6 mL) was added bis(triphenyl-phosphone)palladium(II) dichloride (15 mg, 0.02 mmol, 5 mol%), triphenyl phosphine (11 mg, 0.04 mmol), *p*-methoxyphenylboronic acid (62 mg, 0.4 mmol), and K_3PO_4 (196 mg, 0.9 mmol). The reaction mixture was stirred at 80 ºC for 5 h, at 90 ºC for 15 h, at 100 ºC for 8 h, at 110 ºC for 5 h, at 120 ºC for 12 h, and at 130 ºC for 1 day under argon atmosphere in which the temperature of oil bath was gradually elevated in order to complete the reaction. The same work-up as Method I afforded the pure product (**9**) in 30 % yield.

[Method IV]: A solution of compound (**8**) (201 mg, 0.5 mmol), bis(triphenylphosphone)palladium(II) dichloride (18 mg, 0.026 mmol, 5 mol%), triphenylphosphine (14 mg, 0.05 mmol), *p*-methoxyphenylboronic acid (85 mg, 0.56 mmol), and K_2CO_3 (2.5 mg, 0.02 mmol) in dry DMF (6 mL) was stirred at 120 ºC for 7 days under argon atmosphere. The same work-up as Method I afforded the pure product (**9**) in 25 % yield.

3-(*p*-Carboxy)phenyl-6-(*p*-methoxy)phenyl-2,4-(1*H*,3*H*)-pteridinedione (10)

To a solution of compound (**9**) (51 mg, 0.12 mmol) in MeOH (100 mL) was added 1 M NaOH (5 mL, 5 mmol), and the mixture was stirred at room temperature for 5 days. An additional 4 M NaOH (3 mL, 12 mmol) was added to the mixture, and then the reaction mixture was stirred at room temperature for 3 days. After evaporation of the solvent, to the residue was added H_2O (10 mL) and $CHCl_3$ (10 mL). The aqueous layer was adjusted about pH 2 with 1 M HCl, extracted with $CHCl_3$ (50 mL x 4). At this stage, the yellow solids in the aqueous phase were collected by suction filtration, and they were found to be the desired product (**10**). Yield: 11.5 mg (25 %); ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 3.83 (3H, s, OCH_3), 7.12 (2H, d, *J* = 9.0 Hz, 2",6"-Ph), 7.50 (2H, d, *J* = 8.8 Hz, 3',5'-*N*-Ph), 8.08 (2H, d, *J* = 9.0 Hz, 3",5"-Ph), 8.13 (2H, d, *J* = 8.8 Hz, 2',6'-*N*-Ph), 9.30 (1H, s, C_7-H), and 12.4 ppm (1H, s, OH); IR (KBr): 3416 (ν_{N-H}), 3060 (broad,

ν_{O-H}), 1692 ($\nu_{C=O}$), and 833 cm^{-1} (δ_{C-H}).

3-(*p*-Chlorocarbonyl)phenyl-6-(*p*-methoxy)phenyl-2,4(1*H*,3*H*)-pteridinedione (11)

A solution of compound (**10**) (11.5 mg, 0.03 mmol) in $SOCl_2$ (1 mL) was refluxed for 4 h. The solvent was evaporated off under reduced pressure to give the product (**11**) as orange powders; IR (KBr): 3425 (ν_{N-H}), 1796 ($\nu_{C=O,\ COCl}$), 1744 ($\nu_{C=O}$), and 839 cm^{-1} (δ_{C-H}).

3-(*p*-Phenethylamide)phenyl-1-methyl-2,4(1*H*,3*H*)-pteridinedione (12)

To a solution of compound (**6**) (10 mg, 0.03 mmol) in dry acetonitrile (30 mL) was added phenethylamine (37.6 mg, 0.3 mmol), and the reaction mixture was stirred for 24 h at room temperature. After filtration of the solvent, the filtrate was evaporated off under reduced pressure to give the solid (13 mg) as pale yellow powders. The solid was washed with H_2O and filtrated to give the derivatized product (**12**) as pale yellow powders. Yield: 10 mg (80%); ^{1}H-NMR (δ, CD_3OD, 400 MHz): 2.88 (2H, t, J = 7.3 Hz, -CONHCH$_2$<u>CH$_2$</u>-Ph), 3.52 (2H, q, J = 6.4 Hz, -CONH<u>CH$_2$</u>CH$_2$-Ph), 3.57 (3H, s, 1-*N*-CH_3), 7.26-7.34 (5H, m, -CONH- CH$_2$CH$_2$-<u>Ph</u>), 7.42 (2H, d, J = 8.5 Hz, 3',5'-*N*-Ph), 7.92 (2H, d, J = 8.5 Hz, 2',6'-*N*-Ph), 8.66 (1H, d, J = 2.2 Hz, C_6-H), and 8.85 ppm (1H, d, J = 2.2 Hz, C_7-H); IR (KBr): 3305 (ν_{N-H}), 1732 ($\nu_{C=O}$), 1683 ($\nu_{C=O}$), 1636 ($\nu_{C=O}$), and 835 cm^{-1} (δ_{C-H}).

3-(*p*-Cholesteryloxycarbonyl)phenyl-1-methyl-2,4(1*H*,3*H*)-pteridinedione (13)

A solution of compound **(6)** (30 mg, 0.1 mmol) and cholesterol (30 mg, 0.08 mmol) in dry CH_2Cl_2 (0.5 mL), dry pyridine (1.5 mL), and dry DMF (1 mL) was stirred for 24 h at room temperature. After evaporation of the solvent, the residue was purified by column chromatography on silica gel with AcOEt:hexane (1:2) mixture to give the derivatized product (**13**) as white solids. Yield: 8 mg (15%); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 0.70, 0.86, 0.92, 0.99-2.01, 2.47, 4.89, and 5.44 (43H, cholesteryl), 3.76 (3H, s, 1-*N*-CH_3), 7.39 (2H, d, J = 8.5 Hz, 3',5'-*N*-Ph), 8.23 (2H, d, J = 8.5 Hz, 2',6'-*N*-Ph), 8.66 (1H, d, J = 2.2 Hz, C_6-H), and 8.72 ppm (1H, d, J = 2.2 Hz, C_7-H); IR (KBr): 1731 ($\nu_{C=O}$), 1695 ($\nu_{C=O}$), and 812 cm^{-1} (δ_{C-H}).

3-(*p*-Phenethylamide)phenyl-6-(*p*-methoxy)phenyl-2,4(1*H*,3*H*)-pteridinedione (14)

To a solution of compound (**11**) (11.5 mg, 0.03 mmol) in dry MeCN (10 mL) and dry DMF (6 mL) was added phenethylamine (2 drops), and then the reaction mixture was stirred for 9 h at room temperature. After evaporation of the solvent, the residue was washed with H_2O to give the derivatization product (**14**) as yellow solids.; ^{1}H-NMR (δ, CD_3OD, 400 MHz): 2.95 (2H, t, J = 7.3 Hz, -CONHCH$_2$<u>CH$_2$</u>-Ph), 3.63 (2H, q, J = 7.3 Hz, -ONH<u>CH$_2$</u>CH$_2$-Ph), 3.80 (3H, s, OCH_3), 7.08 (2H, d, J = 9.0 Hz, 2",6"-Ph), 7.26-7.33 (5H, m, -CONH-CH$_2$CH$_2$-<u>Ph</u>), 7.48 (2H, d, J = 8.8 Hz, 3',5'-*N*-Ph), 7.93 (4H, d, J = 8.8 Hz, 2',6'-*N*-Ph), 8.14 (2H, d, J = 9.0, 3",5"-Ph), and 9.19 ppm (1H, s, C_7-H) ; IR (KBr): 3425 (ν_{N-H}), 1735 ($\nu_{C=O}$), 1687 ($\nu_{C=O}$), 1609 ($\nu_{C=C}$), and 836 cm^{-1} (δ_{C-H}).

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