A READY ONE-POT PREPARATION FOR PTERIDINE AND ISOXAZOLO[3,4-d]PYRIMIDINE DERIVATIVES

José Mª Quintela,* Carlos Peinador,* María J. Moreira, Rosa Toba, and Marcos Chas

Department of Fundamental Chemistry. Faculty of Sciences. University of A Coruña. Campus A Zapateira. E-15071. A Coruña. Spain. e-mail: jqqoqf@udc.es

Abstract – Several new substituted pteridines and isoxazolo[5,4-*d*]pyrimidines are easily obtained by an efficient one-pot procedure from the reaction of *N,N*-dimethyldichloromethyleniminium chloride (phosgeniminium chloride) with the precursors β-enaminonitriles 2-amino-3-cyano-5-phenylpyrazine (**5**) and 5-amino-4-cyano-3-methylisoxazole (**1**), respectively.

The development of efficient and mild methods of heterocyclic compound synthesis represents a broad area of organic chemistry.[1] In this context, nitrogen-containing rings are among the most useful heterocycles and their utility has been widely demonstrated, as a consequence of their exciting biological properties and their role as pharmacophores of considerable historical importance.[2]

Macrophages play a crucial role in modulating the initiation and perpetuation of the inflammatory response. One means by which macrophages modulate inflammation is via their capacity to elaborate biological mediators, like nitric oxide (NO) and prostaglandins (PGs) which have numerous cardiovascular and inflammatory effects.[3] NO is formed by the enzyme NO synthase (NOS), which exists as three distinct isoforms.[4] The inducible isoform iNOS is expressed after stimulation of cells with a variety of agents including endotoxin (bacterial lipopolysaccharide, LPS) and cytokines.[4,5] The NO production during inflammatory response may become "self-destructive" owing to its oxidative properties, as occurs in chronic inflammatory diseases.[3,6]

Cyclooxigenase (COX) is the first enzyme in the pathway in which arachidonic acid is converted to PGs, prostacyclin and thromboxane A2 (TXA$_2$).[7] COX-2 isoform, whose expression is restricted under basal conditions, is upregulated by inflammatory stimuli resulting in increased PGE$_2$ production, which has been suggested to play an important role in the pathophysiology of inflammation and arthritis.[8]

The co-induction of iNOS and COX-2 in cells stimulated with bacterial endotoxins and other inflammatory stimuli has been described.[9] Since the induction of COX-2 and iNOS results in the increased synthesis of PGs and NO, the selective modulation of NO and PGE_2 overproduction by iNOS and COX-2 might represent an important therapeutical goal in different inflammatory pathologies.

Many compounds described as inhibitors of NOS are nitrogen-containing heterocycles.[10] During the last years we reported the synthesis of substituted heterocycles containing the pyrazolopyrimidine, pyridothienopyrimidine and pyridothieno-1,2,3-triazine skeletons with the aim of finding compounds with antiinflammatory and antihistaminic activity.[11] During a project directed toward the identification of new inhibitors of iNOS and COX-2 induction, we selected for study the isoxazolo[5,4-*d*]- and pyrazino[2,3-*d*]pyrimidine nucleus. In a recent paper,[12] we reported that certain isoxazolopyrimidines are potent inhibitors of nitrite and PGE_2 production in endotoxin-stimulated macrophages, which can be related to a reduced expression of iNOS and COX-2 in some cases. These products were also found active on nitrite levels by oral administration, in the mouse air pouch model of inflammation. In this paper we describe the synthesis of a series of these isoxazolo[5,4-*d*]pyrimidine and pyrazino[2,3-*d*]pyrimidine (pteridine) compounds.
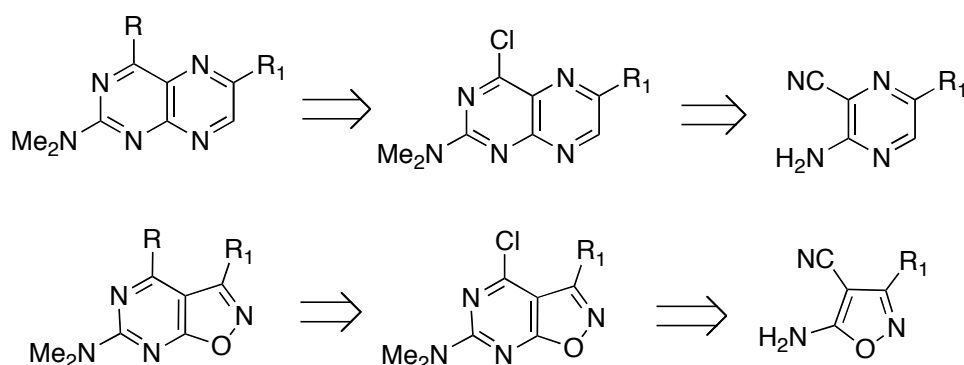
Figure 1 Retrosynthetic pathway for synthesis of the pteridines and isoxazolo[5,4-*d*]pyrimidines
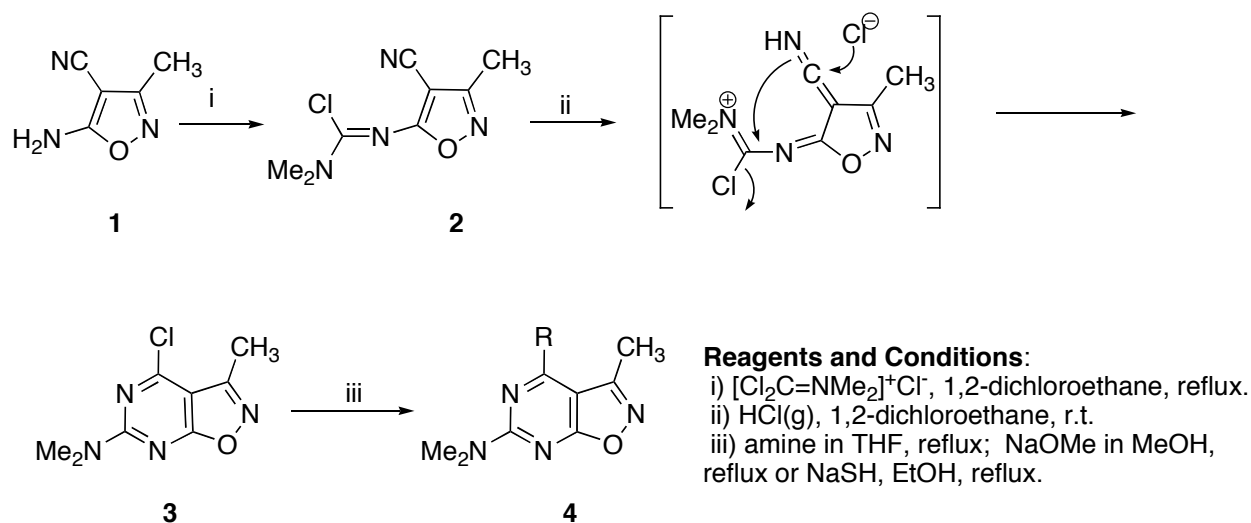
For the preparation of the heterocycles we devised suitable procedure for the introduction of a variety of substituents with a minimum change in the nature of the chemistry used. For the preparation of the title compounds (Figure 1), we used a synthetic procedure based on the use of β-enaminonitriles[13] and phosgeniminium chloride that allows the introduction of two substituents R and $N(CH_3)_2$ on the heterocycle.[14] Thus, substituent dimethylamino is introduced during the formation of the pyrimidine ring. Substituent R is introduced in the last step by means of an aromatic nucleophilic substitution of a chloro-substituent. Several nucleophiles of nitrogen, oxygen and sulfur have been shown effective in this reaction. We considered this methodology would be attractive due to its conciseness and utilization of readily synthesized or commercially available starting materials.

The *N,N*-dichloromethylenedimethyliminium salts, particularly *N,N*-dichloromethylenedimethyl iminium chloride (Viehe's salt) are very useful reagents that can function as Vilsmeier or Mannich acceptors and

lead to the insertion of a one-carbon unit bearing a dialkylamino group.[15] This makes them especially useful in one-step heterocyclization reactions such as those considered here.

The construction of fused pyrimidines from *ortho*-aminonitriles and phosgene iminium salts has been successfully applied to the preparation of many heterocyclic fused systems.[14] For the synthesis of isoxazolo[5,4-*d*]pyrimidines and pyrazino[2,3-*d*]pyrimidines, we used that strategy starting with *o*-aminocyanoisoxazole (**1**) and *o*-aminocyanopyrazole (**5**), respectively. The starting 5-amino-4-cyano-3-methylisoxazole (**1**)[16] and 2-amino-3-cyano-5-phenylpyrazine (**5**)[17] were readily obtained by previously described procedures.

In this way, 5-amino-4-cyano-3-methylisoxazole (**1**), was made to react with dichloromethylenedimethyliminium chloride in refluxing 1,2-dichloroethane (Scheme 1) affording the 3-chloroamidine (**2**), which was reacted with dry hydrogen chloride to undergo cyclization to fused heterocyclic compound 4-chloro-6-dimethylamino-3-methylisoxazolo[5,4-*d*]pyrimidine (**3**). Aromatic nucleophilic displacement of the chlorine atom in **3** was achieved by reaction with nucleophiles and resulted in the formation of substituted products (**4a-k**) in good yields (62-98%).

Reagents and Conditions:
i) [Cl$_2$C=NMe$_2$]$^+$Cl$^-$, 1,2-dichloroethane, reflux.
ii) HCl(g), 1,2-dichloroethane, r.t.
iii) amine in THF, reflux; NaOMe in MeOH, reflux or NaSH, EtOH, reflux.

Scheme 1 Synthesis and structure of isoxazolo[5,4-*d*]pyrimidines (3) and (4)

It is known that dichloromethylene iminium salts do not normally react with the cyano group so the formation of isoxazolo[5,4-*d*]pyrimidine (**3**) from chloroamidine (**2**) can be assumed to proceed through the corresponding chloroiminium chloride.[18] One-pot high yield synthesis (80%) of isoxazolopyrimidine (**3**) from β-enaminonitrile (**1**) was carried out by refluxing **1** and phosgene iminium chloride in 1,2-dichloroethane for 1 h followed by treatment with hydrogen chloride.

All the compounds shown in Table 1 gave satisfactory elemental analyses and spectral data (IR, MS, and ^{1}H and ^{13}C NMR) coherent with the structures proposed. The structure of compounds (**2**) and (**3**) were determined from spectral data. The mass spectra showed the expected molecular ion peak and the IR spectrum of **2** exhibited a strong absorption band at ν = 1610 cm^{-1} due to the imino group and presented

the characteristic signal at $\nu = 2220$ cm^{-1} (CN), while the decoupled ^{13}C NMR spectrum showed one signal at $\delta = 112.2$ due to the carbon atom in the one cyano group. After cyclization, the spectrum of **3** did not include those types of signals. The most salient features of the ^{1}H NMR and ^{13}C NMR spectra are given on Experimental.

Table 1 Physicochemical data for isoxazolo[5,4-*d*]pyrimidines (**3**) and (**4a-k**)

Compound[a]	R	Mp(°C)	Yield(%)	Molecular formula[b]
3	Chloro	146-148	80[c]	C$_8$H$_9$N$_4$OCl
4a	Piperidino	89-90	70[c]	C$_{13}$H$_{19}$N$_5$O
4b	3,4-Methylenedioxybenzylpiperazino	148-150	99[d]	C$_{20}$H$_{24}$N$_6$O$_3$
4c	Benzylamino	170-172	79[c]	C$_{15}$H$_{17}$N$_5$O
4d	Morpholino	105-107	75[e]	C$_{12}$H$_{17}$N$_5$O$_2$
4e	Thiomorpholino	120-122	60[e]	C$_{12}$H$_{17}$N$_5$OS
4f	4-Methylpiperidino	74-76	79[e]	C$_{14}$H$_{21}$N$_5$O
4g	4-(4-Acetylphenyl) piperazino	190-192	70[f]	C$_{20}$H$_{24}$N$_6$O$_2$
4h	Diethylamino	100-102	84[e]	C$_{12}$H$_{19}$N$_5$O
4i	*n*-Propylamino	120-122	85[e]	C$_{11}$H$_{17}$N$_5$O
4j	Methoxy	116-118	68[c]	C$_9$H$_{12}$N$_4$O$_2$
4k	Mercapto	196-198	75[h]	C$_8$H$_{10}$N$_4$OS

[a] All spectra data were consistent with the assigned structures.

[b] All compounds analyzed for C, H, N; analytical results are within ±0.4% of theoretical values.

[c] Recrystallized from ethanol.

[d] Purified by medium-pressure column chromatography using dichloromethane:ethyl acetate (1:1, v/v) as eluent.

[e] Purified by medium-pressure column chromatography using hexane:ethyl acetate (3:1, v/v) as eluent.

[f] Purified by medium-pressure column chromatography using hexane:ethyl acetate (1:1, v/v) as eluent.

[g] Purified by medium-pressure column chromatography using dichloromethane as eluent.

Some isoxazolopyrimidine derivatives are potent inhibitors of nitrite and PGE$_2$ production in endotoxin-stimulated macrophages, which can be related to a reduced expression in iNOS and COX-2 in some cases.[12] These products were also active on nitrite levels by oral administration, in the mouse air pouch model of inflammation.[12] This group of compounds may offer new drugs for the modulation of NO production in different inflammatory and NO-related pathologies.

Annelated pyrazines, such as quinoxaline and pteridine derivatives are very important nitrogen-containing heterocycles found in biological systems and widely used as pharmaceuticals.[19] Pyrazino[2,3-*d*]pyrimidines are known as "pteridines",[20] because the first examples of the ring system, as natural products, were found in pigments, like xanthopterin (yellow), in the wings of butterflies (*Lepidoptera*). Folic acid (vitamin B-9), a well-known pteridine derivative,[21] is essential to the body for the formation of new cells; it is a key vitamin in cellular function and repair. Flavines also represent

pteridine derivatives[22] and are involved in the formation of red blood cells, in respiration, antibody production, in human growth regulation and reproduction. The pteridine ring system has subsequently been found in coenzymes which use tetrahydrofolic acid, the molybdenum cofactor on the oxomolybdoenzymes, and in the anti-cancer drug methotrexate, a classical dihydrofolate reductase inhibitor clinically used in cancer chemoterapy.[23] Aminopterin and Methotrexate, synthesized as potential antimetabolites of folic acid (Figure 2), induced remissions of acute lymphoblastic leukemia.[24] The biological basis for the cytotoxicity of these two compounds was found to be their potent inhibitory activity towards dihydrofolate reductase (DHFR), an enzyme which regenerates tetrahydrofolate from dihydrofolate, and therefore maintains a cellular supply of this coenzyme which plays critical roles in a wide spectrum of cellular one-carbon transfer reactions.[25]
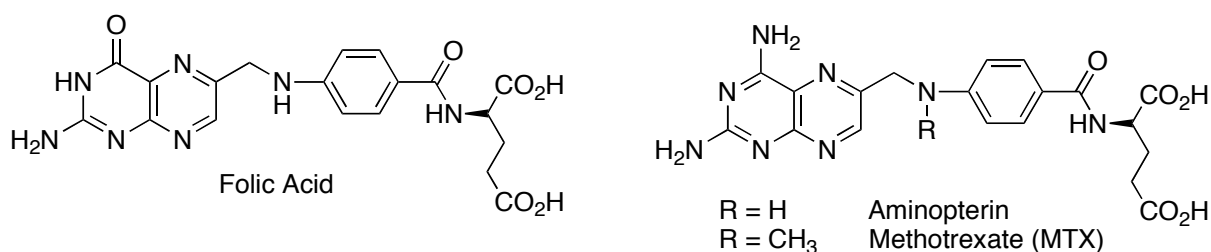
Figure 2

Methotrexate itself, although it has been in clinical use for some 40 years, is a non-selective cytotoxic agent. Its extreme toxicity coupled with its in-effectiveness against many types of human cancers has severely limited its clinical effectiveness.[25] Extensive efforts over many years have been made to modify the aminopterin/methotrexate/folic acid structure in an effort to develop less toxic cofactor antagonists which possess more selective transport and inhibitory properties and which might therefore be effective against a broader range of human cancers.[26]

On the other hand, tetrahydrobiopterin derivatives and dihydropteridine derivatives are of great interest for their dihydrofolate reductase inhibiting, mental retardation, neurotransmitter and use in treating other neurological problems.[27] In addition, methotrexate has been shown to possess immunosuppressive activity, and the establishment of a facile and regioselective construction of these fundamental pteridine heterocyclic skeleton is important.[28]

The synthesis of the pteridine system has been approached by two obvious routes: one is the fusion of the pyrazine ring onto a preformed 4,5-diaminopyrimidine, and the second, the elaboration of the pyrimidine ring on a preformed pyrazine. The first of these, the Gabriel-Isay condensation, suffers from the disadvantage that condensation of the heterocyclic 1,2-diamine with an unsymmetrical 1,2-dicarbonyl compound usually leads to a mixture of two 5/6-substituted isomers.[29] Other approaches for the pteridine nucleus from pyrimidine intermediates are the Timmis reaction, which makes use of the regioselective condensations of 6-amino-5-nitrosopyrimidines with aldehydes, ketones, esters, and nitriles under basic

catalysis,[30] and the Polonovski-Boom cyclization,[31,32] an unambiguous regioselective approach by reacting 6-chloro-5-nitro- and 6-chloro-5-arylazopyrimidines, respectively, with α-aminocarbonyl compounds followed by reduction to the 5-amino derivatives and subsequent cyclization.[33] (6R)- and (6S)-5,6,7,8-tetrahydrofolic acids were prepared by this approach.[34]

Pteridine synthesis from pyrazine precursors is usually applied in such cases where the formation of special derivatives cannot be achieved easily by the pyrimidine approach. It was to avoid this difficulty that the alternative strategy, the Taylor synthesis, now widely used, starting with a pyrazine, was developed.[35] This versatile new approach to pteridines utilizes as its key first (and unequivocal) step the cyclization of an α-oximinocarbonyl compound with an α-aminonitrile to give a 2-amino-3-cyano- (or carbalkoxy-) pyrazine 1-oxide which can then be converted to pteridines and pterins by a series of simple deoxygenation and cyclization steps.[36,37] Various antileukemic agents related to methotrexate are also synthetically available by the Taylor's method.[38]

We found that the pteridine nucleus is easily formed by the synthetic procedure based on the use of β-enaminonitriles and phosgeniminium chloride. Thus, the reaction of the 2-amino-3-cyano-5-phenylpyrazine (5) with phosgeniminium chloride gave 6 (Scheme 2). Amide halide intermediate (6) underwent cyclization to the corresponding fused compounds (7) by reaction with dry hydrogen chloride. On treatment with phosgeniminium salt in refluxing 1,2-dichloroethane for 1 h and a subsequent treatment with hydrogen chloride, 5 directly afforded the substituted fused heterocyclic compound (6) which, in turn, showed the remarkable reactivity of its chloro substituent towards nucleophilic agents. The structures of the new compounds were confirmed by elemental and spectroscopic features and also are summarized on Experimental.
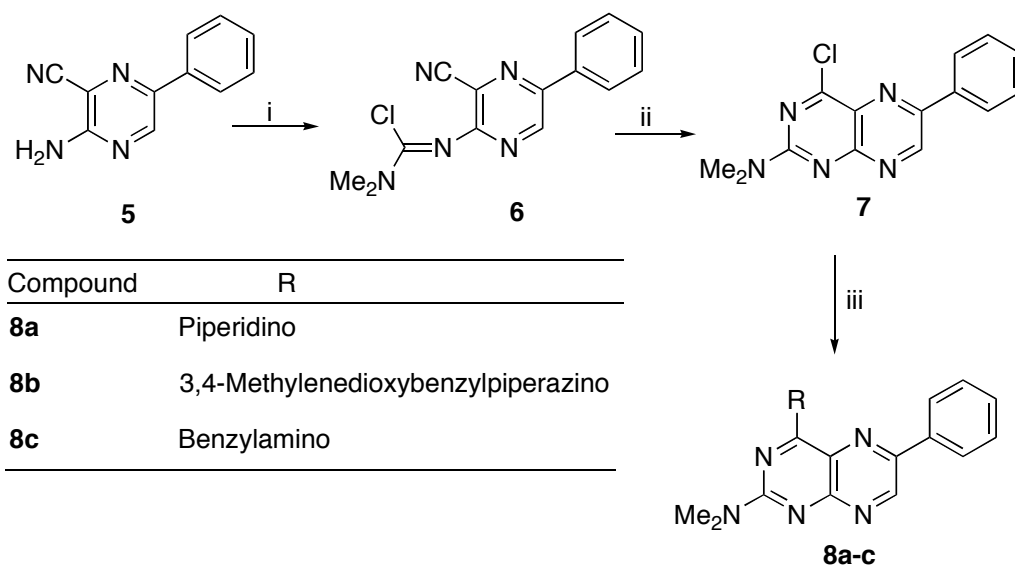
Compound	R
8a	Piperidino
8b	3,4-Methylenedioxybenzylpiperazino
8c	Benzylamino

Reagents and Conditions: i) [Cl$_2$CNMe$_2$]$^+$Cl$^-$, 1,2-dichloroethane, reflux; ii) HCl(g), 1,2-dichloroethane, rt; iii) amine in THF, reflux.

Scheme 2 Synthesis and structure of pteridines (8a-c)

Although several methods are available for the synthesis of pteridines,[39] in this simplicity, the affordability of the starting materials, good yields obtained and, straightforward product isolation, the proposed one-pot procedure compares favorably with other synthesis for the pyrazino[2,3-*d*]pyrimidine (pteridine) system. This approach has the further advantage that because the 2-amino-3-cyano-5-substituted pyrazine is presynthesized, it eventually produces, regioselectivity, 6-substituted pteridines and substitution at the 6-position is the common pattern in natural pteridines.

EXPERIMENTAL

Melting points were determined on a Bibby SMP3 apparatus and are uncorrected. All reagents used were commercial grade chemicals from freshly opened containers. IR spectra were recorded as potassium bromide disks on a Bruker vector 22FT-IR. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AC 200F instrument at room temperature. MS were obtained on a VG-QUATTRO spectrometer. The Silica gel 60F-254 used for analytical thin layer chromatography was purchased from Merck. Microanalyses for C. H, N, and S were performed by the elemental analyses general services of the University of A Coruña.

5-Chlorodimethylaminomethylenamino-4-cyano-3-methylisoxazole (**2**).

A solution of **1**[16] (1.23 g, 10 mmol) and dichloromethylene-dimethyliminium chloride (2 g, 12 mmol) in 1,2-dichloroethane (30 mL) was heated at reflux until all starting material has disappeared as checked by TLC (1 h). The solvent was removed under reduced pressure and the resulting solid was purified by flash chromatography using hexane/dichloromethane (1:1, v/v) as eluent to obtain **2** (2.01 g, 95%); mp 78-80 º C (EtOH). IR (KBr, cm^{-1}): v = 2220 (CN), 1630 cm^{-1}. MS (EI, *m/z* %) = 214 (M$^+$ + 2, 16), 212 (M$^+$, 47), 107 (100). ^{1}H NMR (CDCl$_3$): δ = 2.23 (s, 3 H, CH$_3$); 3.23 (s, 6H, NMe$_2$). ^{13}C NMR (CDCl$_3$): δ = 10.8 (CH$_3$); 40.5 (NMe$_2$); 79 (C-4); 112.2 (CN). *Anal*. Calcd for C$_8$H$_9$N$_4$OCl: C, 45.19; H, 4.27; Cl, 16.67; N, 26.35. Found: C, 45.32; H, 4.22; Cl, 16,54; N, 26.52.

4-Chloro-6-dimethylamino-3-methylisoxazolo[5,4-*d*]pyrimidine (**3**).

Method A:

A mixture of **1** (0.617 g, 5 mmol) and dichloromethylene-dimethyliminium chloride (1 g, 6 mmol) in 1,2-dichloroethane (30 mL) was heated at reflux for 1h. A stream of dry hydrogen chloride was passed through the mixture for 3 h and the reaction mixture was allowed to stand 24 h at rt. The solvent was then removed under reduced pressure and the resulting solid was recrystallized from EtOH to afford **3** (0.85 g, 80%); mp 146-148 ℃. IR (KBr, cm^{-1}) v = 2940, 1630. MS (EI, *m/z* %) = 214 (M$^+$ + 2, 2), 212 (M$^+$, 5), 57 (100). ^{1}H NMR (CDCl$_3$) δ = 2.53 (s, 3H, CH$_3$); 3.25 (d, 6H, *J* = 8.3 Hz, NMe$_2$). ^{13}C NMR (CDCl$_3$): δ = 11.2 (CH$_3$); 36.7 (NMe$_2$); 100 (C-3a); 153.8, 154.5, 161.1, 175.7 (C-3, C-4, C-6, C-7a). *Anal*.

Calcd for $C_8H_9N_4OCl$: C, 45.19; H, 4.27; Cl, 16.67; N, 26.35. Found: C, 45.26; H, 4.16; Cl, 16.78; N, 26.29.

Method B

A stream of dry hydrogen chloride was passed through a mixture of **2** (1.06 g, 5 mmol) in 1,2-dichloroethane (25 mL) for 3 h. The reaction mixture was allowed to stand overnight at rt. The solvent was then removed under reduced pressure and the resulting solid was recrystallized from EtOH to afford **3** (0.78 g, 74%).

4- Substituted 6-dimethylamino-3-methylisoxazolo[5,4-*d*]pyrimidines **(4a-i).** General Procedure:

A solution of **3** (106 mg, 0.5 mmol) and the appropriate amine (0.56 mmol) in THF (5 mL) was heated at reflux until all starting material had disappeared as checked by TLC. The solvent was then removed under reduced pressure and the resulting solid was recrystallized from EtOH or purified by flash cromatography.

6-Dimethylamino-3-methyl-4-piperidinoisoxazolo[5,4-*d*]pyrimidine **(4a)** (70%): mp 89-90 ºC (EtOH). IR (KBr, cm^{-1}) ν = 2980, 1620. MS (EI, *m/z* %) = 261 (M$^+$, 82), 232 (42), 84 (100). ^{1}H NMR (CDCl$_3$) δ = 1.64 (m, 6H, CH$_2$); 2.41 (s, 3H, CH$_3$); 3.13 (s, 6H, NMe$_2$); 3.56 (m, 4H, NCH$_2$). ^{13}C NMR (CDCl$_3$): δ = 14.7 (CH$_3$); 24.3, 25.5 (CH$_2$); 36.9 (NMe$_2$); 48.7 (NCH$_2$); 89.2 (C-3a); 152.7, 160.2, 161.2, 178.7 (C-3, C-4, C-6, C-7a). *Anal*. Calcd for $C_{13}H_{19}N_5O$: C, 59.75; H, 7.33; N, 26.80. Found: C, 59.92; H, 7.06; N, 26.98.

6-Dimethylamino-3-methyl-4-(3,4-methylenedioxybenzylpiperazino)isoxazolo[5,4-*d*]pyrimidine **(4b)** (99%): mp 148-50 ºC (EtOH). IR (KBr, cm^{-1}) ν = 2980, 1620. MS (EI, *m/z* %) = 396 (M$^+$, 8), 206 (57), 135 (100). ^{1}H NMR (CDCl$_3$) δ = 2.37 (s, 3H, CH$_3$); 2.45-250 (m, 4H, NCH$_2$); 3.11 (s, 6H, NMe$_2$); 3.39 (s, 2H, CH$_2$); 3.60-3.65 (m, 4H, NCH$_2$); 5.87 (s, 2H, OCH$_2$O); 6.68 (s, 2H, C$_6$**H**$_3$CH$_2$O$_2$); 6.81 (s, 1H, C$_6$**H**$_3$CH$_2$O$_2$). ^{13}C NMR (CDCl$_3$): δ = 14.9 (CH$_3$); 36.9 (NMe$_2$); 47.5, 52.3 (NCH$_2$); 62.3 (CH$_2$); 89.2 (C-3a); 100.6 (OCH$_2$O); 107.6, 109.0 (C-2', C-5'); 122.0 (C-6'); 131.3 (C-1'); 146.4, 147.4 (C-3', C-4'); 152.4, 160.0, 161.0 (C-3, C-4, C-6, C-7a). *Anal*. Calcd for $C_{20}H_{24}N_6O_3$: C, 60.59; H, 6.10; N, 21.20. Found: C, 60.75; H, 5.76; N, 21.34.

4-Benzylamino-6-dimethylamino-3-methylisoxazolo[5,4-*d*]pyrimidine **(4c)** (79%): mp 170-172 ºC (EtOH). IR (KBr, cm^{-1}) ν = 3380 (NH), 2880, 1640. MS (EI, *m/z* %) = 283 (M$^+$, 53), 206 (28), 91 (100). ^{1}H NMR (CDCl$_3$) δ = 2.45 (s, 3H, CH$_3$); 3.20 (s, 6H, NMe$_2$); 4.79 (d, 2H, *J* = 5.86 Hz, CH$_2$); 5.29 (br t, 1H, NH); 7.36 (m, 5H, C$_6$H$_5$). ^{13}C NMR (CDCl$_3$): δ = 12.5 (CH$_3$); 37.3 (NMe$_2$); 44.6 (CH$_2$); 88.0 (C-3a); 127.4, 127.5, 128.2, 128.6, 138.6 (C$_6$H$_5$); 152.8 157.5, 162.7, 178.0 (C-3, C-4, C-6, C-7a). *Anal*. Calcd for $C_{15}H_{17}N_5O$: C, 63.59; H, 6.05; N, 24.72. Found: C, 63.77; H, 5.96; N, 24.58.

6-Dimethylamino-3-methyl-4-morpholinoisoxazolo[5,4-*d*]pyrimidine **(4d)** (75%): mp 105-107 ºC (EtOH). IR (KBr, cm^{-1}) ν = 2980, 1600, 1540. MS (EI, *m/z* %) = 263 (M$^+$, 100), 205 (82), 107 (68), 86 (69), 69

(72). ^{1}H NMR (CDCl$_3$) δ = 2.48 (s, 3H, CH$_3$); 3.21 (s, 6H, NMe$_2$); 3.67-3.72 (m, 4H, NCH$_2$); 3.80-3.85 (m, 4H, OCH$_2$). ^{13}C NMR (CDCl$_3$): δ = 15.1 (CH$_3$); 37.2 (NMe$_2$); 48.3 (NCH$_2$); 66.5 (OCH$_2$); 89.7 (C-3a); 152.5 160.6, 161.4, 178.9 (C-3, C-4, C-6, C-7a). *Anal*. Calcd for C$_{12}$H$_{17}$N$_5$O$_2$: C, 54.74; H, 6.51; N, 26.60. Found: C, 54.56; H, 6.70; N, 26.47.

6-Dimethylamino-3-methyl-4-thiomorpholinoisoxazolo[5,4-*d*]pyrimidine (**4e**) (60%): mp 120-122 ºC (EtOH). IR (KBr, cm^{-1}) ν = 2980, 1640, 1580. MS (EI, *m/z* %) = 279 (M$^+$, 21), 206 (100), 107 (49), 69 (85). ^{1}H NMR (CDCl$_3$) δ = 2.43 (s, 3H, CH$_3$); 2.72-2.76 (m, 4H, SCH$_2$); 3.16 (s, 6H, NMe$_2$); 3.89-3.95 (m, 4H, NCH$_2$). ^{13}C NMR (CDCl$_3$): δ = 14.9 (CH$_3$); 27.0 (SCH$_2$); 37.2 (NMe$_2$); 50.4 (NCH$_2$); 89.6 (C-3a); 152.5 160.5, 161.3, 178.9 (C-3, C-4, C-6, C-7a). *Anal*. Calcd for C$_{12}$H$_{17}$N$_5$OS: C, 51.59; H, 6.13; N, 25.07; S, 11.48. Found: C, 51.70; H, 6.02; N, 24.93; S, 11.67.

6-Dimethylamino-3-methyl-4-methylpiperidinoisoxazolo[5,4-*d*]pyrimidine (**4f**) (79%): mp 74-76 ºC (EtOH). IR (KBr, cm^{-1}) ν = 2940, 1580, 1440. MS (EI, *m/z* %) = 275 (M$^+$, 73), 232 (32), 98 (100). ^{1}H NMR (CDCl$_3$) δ = 0.95 (d, 3H, *J* = 6.35 Hz, CH$_3$); 1.19-1.38 (m, 2H, CH$_2$); 1.63-1.75 (m, 3H, CH, CH$_2$); 2.43 (s, 3H, CH$_3$); 2.89-3.03 (m, 2H, CH$_2$); 3.15 (s, 6H, NMe$_2$); 4.17-4.25 (m, 2H, CH$_2$). ^{13}C NMR (CDCl$_3$): δ = 14.9 (CH$_3$); 21.8 (CH$_2$); 30.2 (CH); 31.0 (CH$_2$); 37.1 (NMe$_2$); 48.2 (NCH$_2$); 89.4 (C-3a); 152.9 160.3, 161.4, 178.9 (C-3, C-4, C-6, C-7a). *Anal*. Calcd for C$_{14}$H$_{21}$N$_5$O: C, 61.07; H, 7.69; N, 25.43. Found: C, 60.92; H, 7.82; N, 25.50.

4-(*N*-4'-Acetylphenylpiperazino)-6-dimethylamino-3-methylisoxazolo[5,4-*d*]pyrimidine (**4g**) (70%): mp 190-192 ºC (EtOH). IR (KBr, cm^{-1}) ν = 2860, 1700 (CO), 1580, 1400. MS (EI, *m/z* %) = 380 (M$^+$, 6), 206 (100), 69 (22). ^{1}H NMR (CDCl$_3$) δ = 2.53 (s, 3H, CH$_3$CO); 2.54 (s, 3H, CH$_3$); 3.23 (s, 6H, NMe$_2$); 3.54-3.57 (m, 4H, NCH$_2$); 3.86-3.91 (m, 4H, NCH$_2$); 6.90, 7.92 (AA'XX' system, 4H, *J* = 8.8 Hz, C$_6$H$_4$). ^{13}C NMR (CDCl$_3$): δ = 15.1 (CH$_3$); 26.1 (CH$_3$); 37.2 (NMe$_2$); 46.7 (NCH$_2$); 47.2 (NCH$_2$); 89.6 (C-3a); 113.2, 127.9, 130.4 (C$_6$H$_4$); 152.6, 153,6, 161.3, 178.8 (C-3, C-4, C-6, C-7a). *Anal*. Calcd for C$_{20}$H$_{24}$N$_6$O$_2$: C, 63.14; H, 6.36; N, 22.09. Found: C, 63.31; H, 6.24; N, 21.97.

4-Diethylamino-6-dimethylamino-3-methylisoxazolo[5,4-*d*]pyrimidine (**4h**) (84%): mp 100-102 ºC (EtOH). IR (KBr, cm^{-1}) ν = 2960, 1580, 1400. MS (EI, *m/z* %) = 249 (M$^+$, 55), 220 (63), 206 (75), 72 (100). ^{1}H NMR (CDCl$_3$) δ = 1.27 (t, 6H, *J* = 7.1 Hz, 2CH$_3$); 2.54 (s, 3H, CH$_3$); 3.20 (s, 6H, NMe$_2$); 3.66 (q, 4H, *J* = 7.1 Hz, 2CH$_2$). ^{13}C NMR (CDCl$_3$): δ = 13.3 (CH$_3$); 15.6 (CH$_3$); 37.0 (NMe$_2$); 43.5 (NCH$_2$); 87.9 (C-3a); 152.5, 157.1, 158.8, (C-3, C-4, C-6, C-7a). *Anal*. Calcd for C$_{12}$H$_{19}$N$_5$O: C, 57.81; H, 7.68; N, 28.09. Found: C, 58.02; H, 7.50; N, 28.28.

6-Dimethylamino-3-methyl-4-*n*-propylaminoisoxazolo[5,4-*d*]pyrimidine (**4i**) (85%): mp 120-122 ºC (EtOH). IR (KBr, cm^{-1}) ν = 3380 (NH); 2980, 1640. MS (EI, *m/z* %) = 235 (M$^+$, 46), 178 (47), 107 (62), 69 (100). ^{1}H NMR (CDCl$_3$) δ = 0.87 (t, 3H, *J* = 7.3 Hz, CH$_3$); 1.58 (m, 2H, CH$_2$); 2.36 (s, 3H, CH$_3$); 3.09 (s, 6H, NMe$_2$); 3.36-3.46 (m, 2H, CH$_2$); 5.22 (t, 1H, *J* = 5.6 Hz, NH). ^{13}C NMR (CDCl$_3$): δ = 11.4 (CH$_3$);

12.3 (CH_3); 22.6 (CH_2); 37.1 (NMe_2); 42.5 (NCH_2); 87.8 (C-3a); 153.1, 157.7, 162.7, 177.8 (C-3, C-4, C-6, C-7a). *Anal*. Calcd for $C_{11}H_{17}N_5O$: C, 56.15; H, 7.28; N, 29.77. Found: C, 55.98; H, 7.44; N, 29.66.

6-Dimethylamino-4-methoxy-3-methylisoxazolo[5,4-*d*]pyrimidine (4j)

A solution of **3** (106 mg. 0.5 mmol) and sodium methoxide (33mg, 0.7 mmol) in MeOH (5 mL) was heated at reflux until all starting material has disappeared as checked by TLC (1.5 h). The solvent was then removed at reduced pressure and the resulting solid was recrystallized from ethanol to afford **4j** (67mg, 68%): mp 116-118 °C. IR (KBr, cm^{-1}) ν = 2940, 1630. MS (EI, *m/z* %) = 208 (M$^+$, 100), 179 (78). ^{1}H NMR (CDCl$_3$) δ = 2.45 (s, 3H, CH$_3$); 3.24 (s, 6H, NMe$_2$); 4.04 (s, 3H, OCH$_3$). ^{13}C NMR (CDCl$_3$): δ = 11.9 (CH$_3$); 37.4 (NMe$_2$); 53.7 (OCH$_3$); 90.0 (C-3a); 154.3, 162.5, 165.0 (C-3, C-4, C-6, C-7a). *Anal*. Calcd for $C_9H_{12}N_4O_2$: C, 51.92; H, 5.81; N, 26.91. Found: C, 52.12; H, 5.66; N, 27.04.

6-Dimethylamino-3-methylisoxazol[5,4-*d*]pyrimidine-4(5*H*)-thione (4k)

NaSHxH$_2$O (0.20 g) was added in portions to a solution of **3** (106 mg, 0.5 mmol) in EtOH (10 mL). The reaction mixture was stirred at rt until all starting material has disappeared as checked by TLC. The solvent was removed at reduced pressure and water (10 mL) was added. The solution was neutralized with 2N HCl and the precipitate was filtered off and purified by MPLC using dichloromethane as eluent to obtain **4k** (75 mg, 75%): mp 196-198 °C. IR (KBr, cm^{-1}) ν = 3180 (SH), 1580, 1300. MS (EI, *m/z* %) = 210 (M$^+$, 100), 195 (34), 166 (33), 69 (61). ^{1}H NMR (CDCl$_3$) δ = 2.62 (s, 3H, CH$_3$); 3.28 (s, 6H, NMe$_2$). ^{13}C NMR (CDCl$_3$): δ = 12.4 (CH$_3$); 37.9 (NMe$_2$); 105.2 (C-3a); 135.5, 159.3, 179.7 (C-3, C-4, C-6, C-7a). *Anal*. Calcd for $C_8H_{10}N_4OS$: C, 45.70; H, 4.79; N, 26.65; S, 15.25. Found: C, 45.58; H, 4.94; N, 26.83; S, 15.13.

3-Cyano-2-chlorodimethylaminomethylenamino-5-phenylpyrazine (6).

A solution of 2-amino-3-cyano-5-phenylpyrazine **5** (0.60 g, 3 mmol) and phosgeniminium chloride (0.56 g, 3.5 mmol) in 1,2-dichloroethane (15 mL) was heated at reflux until all starting material has disappeared as checked by TLC (1.5 h). The solvent was removed under reduced pressure and the resulting solid was purified by flash chromatography using dichloromethane as eluent to obtain **6** (0.80 g, 92%); mp 116-118 ° C. IR (KBr, cm^{-1}): ν = 2220 (CN), 1640 cm^{-1}. MS (EI, *m/z* %) = 287 (M$^+$ + 2, 9), 285 (M$^+$, 27), 76 (100). ^{1}H NMR (CDCl$_3$): δ = 3.25 (s, 6H, NMe$_2$); 7.41-7.95 (m, 5H, C$_6$H$_5$); 8.87 (s, 1H, H-6). ^{13}C NMR (CDCl$_3$): δ = 40.2 (NMe$_2$); 115.6 (CN); 123.2, 126.1, 128.7, 134.4 (C$_6$H$_5$); 142.5, 146.6, 156.1, (C-2, C-5, C-6). *Anal*. Calcd for $C_{14}H_{12}N_5Cl$: C, 58.85; H, 4.23; Cl, 12.41; N, 24.51. Found: C, 58.72; H, 4.02; Cl, 12.61; N, 24.52.

4- Chloro-2-dimethylamino-6-phenylpteridine (7)

Method A

A mixture of **5** (0.60 g, 3 mmol) and phosgeniminium chloride (0.56 g, 3.5 mmol) in 1,2-dichloroethane (15 mL) was heated at reflux until all starting material has disappeared as checked by TLC (1.5h). A

stream of dry hydrogen chloride was passed through the mixture for 2 h and the reaction mixture was allowed to stand 72 h at rt. The solvent was then removed under reduced pressure and the resulting solid was purified by flash chromatography using dichloromethane as eluent to afford **7** (0.65 g, 75%); mp 278-280 ºC (EtOH). IR (KBr, cm^{-1}) v = 2920, 1600. MS (EI, m/z %) = 287 (M$^+$ + 2, 34), 285 (M$^+$, 94), 256 (100). ^{1}H NMR (CDCl$_3$) δ = 3.35 (s, 6H, NMe$_2$); 7.45-7.52 (m, 3H, C$_6$H$_5$): 8.10-8.13 (m, 2H, C$_6$H$_5$); 9.31 (s, 1H, H-7). ^{13}C NMR (CDCl$_3$): δ = 37.7 (NMe$_2$); 126.8, 129.1, 130.0, 135.5 (C$_6$H$_5$); 150.1 (C-7); 148,2, 155.6, 159.2, 164.2 (C-2, C-4, C-4a, C-6, C-8a). *Anal*. Calcd for C$_{14}$H$_{12}$ClN$_5$: C, 58.85; H, 4.23; Cl, 12.41; N, 24.51. Found: C, 58.96; H, 4.16; Cl, 12.60; N, 24.29.

Method B

A stream of dry hydrogen chloride was passed through a mixture of **6** (0.285 g, 1 mmol) in 1,2-dichloroethane (15 mL) for 2 h. The reaction mixture was allowed to stand 72 h at rt. The solvent was then removed under reduced pressure and the resulting solid was purified by flash chromatography using dichloromethane /AcOEt (95:5 v/v) as eluent to yield **7** (0.237 g, 82%).

4- Substituted 2-dimethylamino-6-phenylpteridines **(8a-c).** General Procedure:

A solution of **7** (143 mg, 0.5 mmol) and the appropriate amine (0.7 mmol) in THF (5 mL) was heated at reflux until all starting material had disappeared as checked by TLC. The solvent was then removed under reduced pressure and the resulting solid was purified by flash cromatography using CH$_2$Cl$_2$/AcOEt (4:1, v/v) as eluent.

2-Dimethylamino-6-phenyl-4-piperidinopteridine **(8a)** (85%): mp 288-290 ºC (EtOH). IR (KBr, cm^{-1}) v = 2980, 1580. MS (EI, m/z %) = 334 (M$^+$, 100), 319 (67), 128 (75). ^{1}H NMR (CDCl$_3$) δ = 1.75 (m, 6H, CH$_2$); 3.25 (s, 6H, NMe$_2$); 4.29 (m, 4H, NCH$_2$); 7.32-7.48, 7.90-7.96 (m, 5H, C$_6$H$_5$); 9.07 (s, 1H, H-7). ^{13}C NMR (CDCl$_3$): δ = 24.7, 26.2 (CH$_2$); 37.1 (NMe$_2$); 48.9 (NCH$_2$); 125.7, 128.6, 128.8, 136.6 (C$_6$H$_5$); 145.9 (C-7); 142.4, 156.7, 159.4, 160.2 (C-2, C-4, C-4a, C-6, C-8a). *Anal*. Calcd for C$_{19}$H$_{22}$N$_6$: C, 68.24; H, 6.63; N, 25.13. Found: C, 68.40; H, 6.54; N, 25.06.

2-Dimethylamino-6-phenyl-4-(3,4-methylenedioxybenzylpiperazino)pteridine **(8b)** (73%): mp 270-272 ºC (EtOH). IR (KBr, cm^{-1}) v = 2980, 1580. MS (EI, m/z %) = 469 (M$^+$, 100), 319 (67), 128 (75). ^{1}H NMR (CDCl$_3$) δ = 2.47−2.64 (m, 4H, NCH$_2$); 3.26 (s, 6H, NMe$_2$); 3.46 (s, 2H, CH$_2$); 4.08-4.45 (m, 4H, NCH$_2$); 5.96 (s, 2H, OCH$_2$O); 6.75-6.89 (m, 3H, C$_6$**H**$_3$CH$_2$O$_2$); 7.41-7.93 (m, 5H, C$_6$H$_5$); 9.09 (s, 1H, H-7). ^{13}C NMR (CDCl$_3$): δ = 37.2 (NMe$_2$); 47.3 (NCH$_2$); 53.0 (NCH$_2$), 62.5 (CH$_2$); 100.8 (OCH$_2$O); 107.7, 109,3, (C-2', C-5'); 122.1 (C-6'); 123.9, 125.8, 128.9, 136.4 (C$_6$H$_5$); 131.5 (C-1'); 146.3 (C-7); 146.6, 147.6 (C-3', C-4'); 142.7, 156.7, 159.3, 160.0 (C-2, C-4, C-4a, C-6, C-8a). *Anal*. Calcd for C$_{26}$H$_{27}$N$_7$O$_2$: C, 66.51; H, 5.80; N, 20.88. Found: C, 66.33; H, 5.92; N, 21.00.

4- Benzylamino-2-dimethylamino-6-phenylpteridine **(8c)** (90%): mp 160-162 ºC (EtOH). IR (KBr, cm^{-1}) v = 3340 (NH), 2980, 1640. MS (EI, m/z %) = 356 (M$^+$, 25), 91 (100). ^{1}H NMR (CDCl$_3$) δ = 3.27 (s, 6H,

NMe$_2$); 4.80 (d, 2H, J = 6.3 Hz, CH$_2$); 5.42 (br t, 1H, NH); 7.26-7.95 (m, 10H, C$_6$H$_5$); 9.10 (s, 1H, H-7). ^{13}C NMR (CDCl$_3$): δ = 37.0 (NMe$_2$); 44.3 (CH$_2$); 121.3 (C-4a); 127.1, 127.4, 128.3, 128.6, 135,8, 138.1 (C$_6$H$_5$); 145.7 (C-7); 144.0, 154.7, 159,4, 160.6 (C-2, C-4, C-6, C-8a). *Anal*. Calcd for C$_{21}$H$_{20}$N$_6$: C, 70.77; H, 5.66; N, 23.58. Found: C, 70.65; H, 5.78; N, 23.57.

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REFERENCES

1. (a) J. G. Keay and J. E. Toomey, in "Progress in Heterocyclic Chemistry", ed. by H. Suschitzky and E. F. V. Scriven, Pergamon Press, Oxford, UK, 1993, Vol. 5, p. 202. (b) M. A. Ciufolini, in "Advances in Heterocyclic Natural Product Synthesis", ed. by W. H. Pearson, JAI Press Inc., London, 1996, Vol. 3. (c) W. H. Streng, *Drug Discovery Today*, 1997, **2**, 415. (d) N. Matzanke, R. J. Gregg, and S. M. Weinreb, *Org. Prep. Proced. Int.*, 1998, **30**, 1. (e) M. J. Schneider, in "Alkaloids. Chemical and Biological Perspectives", ed. by S. W. Pelletier, Pergamon Press, Oxford, UK, 1996, Vol. 10, p. 155.

2. (a) F. Palacios, C. Alonso, P. Amezua, and G. Rubiales, *J. Org. Chem.,* 2002, **67**, 1941, and references cited therein. (b) G. Jones, in "Comprehensive Heterocyclic Chemistry II", ed. by A. R. Katritzky, C. W. Rees, E. F. V. Scriven, and A. McKillop, Pergamon Press, Oxford, 1996, Vol. 5. (c) G. Jones, in "Comprehensive Heterocyclic Chemistry", A. R. Katritzky, C. W. Rees, A. J. Boulton, and A. McKillop,   Pergamon Press, Oxford, 1984, Vol. 2. (d) M. Ihara and K. Fukumoto, *Nat. Prod. Rep.*, 1997, **14**, 413. (e) K. Neuschütz, J. Velker, and R. Neier, *Synthesis*, 1998, 227. (f) F. Palacios, M. J. Gil, E. Martínez de Marigorta, and M. Rodríguez, *Tetrahedron Lett.,* 1999, **40**, 2411. (g) F. Palacios, A. M. Ochoa de Retana, and J. Oyarzabal, *Tetrahedron Lett.,* 1996, **37**, 4577. (h) A. R. Renslo and R. L. Danheiser, *J. Org. Chem.,* 1998, **63**, 7840. (i) A. R. Katritzky, R. Mazurkiewicz, C. V. Stevens, and M. F. Gordeev, *J. Org. Chem.,* 1994, **59**, 2740. (j) L. S. Hegedus, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 1113. (k) T. Oikawa, N. Kanomata, and M. Tada, *J. Org. Chem.,* 1993, **58**, 2046.

3. E. Móilanen and H. Vapaatalo, *Ann. Med.,* 1995, **27**, 359.

4. S. Moncada and A. Higgs, *Engl. J. Med.*, 1993, **329**, 2002.

5. (a) S. T. Reddy and H. R. Herschman, *J. Biol. Chem.*, 1994, **269**, 15473. (b) J. MacMicking, Q. W. Xie, and C. Nathan, *Annu. Rev. Immunol.*, 1997, **15**, 323.

6. H. Kaur and B. Halliwell, *FEBS Lett.*, 1994, **350**, 9.

7.   J. Vane and R. Botting, *FASEB J.*, 1987, **1**, 89.

8.   (a) R. Y. Kang, J. Freire Moar, E. Sigal, and C. Q. Chu, *Br. J. Rheumatol.*, 1996, **35**, 711. (b) J. R. Vane, J. A. Mitchell, I. Appleton, A. Tomlison, D. Bishop-Bailey, J. Croxtall, and D. A. Willoughby, *Proc. Natl. Acad. Sci. U.S.A.*, 1994, **91**, 2046.

9.   A. Habib, C. Bernard, M. Lebret, C. Créminon, B. Esposito, A. Tedgui, and J. Maclouf, *J. Immunol.*, 1997, **158**, 3845.

10.   J. M. Fukuto and G. Chaudhuri, *Annu. Rev. Pharmacol. Toxicol.*, 1995, **35**, 165.

11.   (a) J. M. Quintela, C. Peinador, L. M. González, R. Riguera, I. Rioja, M. C. Terencio, A. Ubeda, and M. J. Alcaraz, *J. Med. Chem.*, 1999, **42**, 4720. (b) J. M. Quintela, C. Peinador, L. González, I. Devesa, M. L. Ferrándiz, M. J. Alcaraz, and R. Riguera, *Bioorg. Med. Chem.*, 2003, **11**, 863. (c) J. M. Quintela, C. Peinador, C. Veiga, L. González, L. M. Botana, A. Alfonso, and R. Riguera, *Bioorg. Med. Chem.*, 1998, **6**, 1911. (d) J. M. Quintela, C. Peinador, M. J. Moreira, A. Alfonso, L. M. Botana, and R. Riguera, *Eur. J. Med. Chem.*, 2001, **36**, 321. (e) I. Rioja, A. Ubeda, M. C. Terencio, I. Guillén, R. Riguera, J. M. Quintela, C. Peinador, L. M. González, and M. J. Alcaraz, *Eur. J. Pharmacol.*, 2000, **397**, 207. (f) J. M. Quintela, C. Peinador, L. M. Botana, M. Estévez, and R. Riguera, *Bioorg. Med. Chem.*, 1997, **5**, 1543. (g) I. Rioja, M. Terencio, A. Ubeda, R. Riguera, J. M. Quintela, and M. J. Alcaraz, *Naunyn-Schiedeberg's Arch. Pharmacol.*, 2002, **365**, 357.

12.   A. Vidal, M. L. Ferrándiz, A. Ubeda, I. Guillén, R. Riguera, J. M. Quintela, C. Peinador, M. J. Moreira, and M. J. Alcaraz, *Life Science*, 2000, **66**, PL 125.

13.   (a) E. C. Taylor and A. McKillop, in "The Chemistry of Cyclic Enaminonitriles and o-Aminonitriles", Interscience, New York, 1970. (b) H. Wamhoff, *Adv. Heterocycl. Chem.*, 1985, **38**, 357 (c) A. W. Erian, *Chem. Rev.*, 1993, **93**, 1991.

14.   For a review see: J. M. Quintela, C. Peinador, M. J. Moreira, and R. Alvarez-Sarandés, *Recent Res. Dev. Org. Chem.*, 1998, **2**, 409 and references cited therein.

15.   (a) H. G. Viehe and Z. Janousek, *Angew. Chem., Int. Ed. Engl.*, 1973, **12**, 806 (b) Z. Janousek and H. G. Viehe, in "Advances in Organic Chemistry, Part 1", ed. by H. Böhme and H. G. Viehe, John Wiley, New York, 1976, Vol 9, p. 343 and references cited therein. (c) B. Kokel, *J. Heterocycl. Chem.*, 1994, **31**, 845. (d) N. Guillot, H. G. Viehe, B. Tinant, and J. P. Declerq, *Tetrahedron*, 1990, **46**, 3897.

16.   B. Dumaître and N. Dodic, *J. Med. Chem.*, 1996, **39**, 1635.

17.   (a) E. C. Taylor and T. Kobayashi, *J. Org. Chem.*, 1973, **38**, 2817. (b) A. R. Haight, A. E. Bailey, W. S. Baker, M. H. Cain, R. R. Copp, J. A. DeMattei, K. L. Ford, R. F. Henry, M. C. Hsu, R. F. Keyes, S. A. King, M. A. McLaughlin, L. M. Melcher, W. R. Nadler, P. A. Oliver, S. I. Parekh, H. H. Patel, L. S. Seif, M. A. Staeger, G. S. Wayne, S. J. Wittenberger, and W. Zhang, *Org. Process Res. Dev.*,

2004, **8**, 897.

18. B. Kokel, G. Menichi, and M. Hobar-Habart, *Tetrahedron Lett*., 1984, 1557.

19. (a) A. A. Rabow, R. H. Shoemaker, E. A. Sausville, and D. G. Covell, *J. Med. Chem*., 2002, **45**, 818. (b) R. C. Reynolds, C. A. Johnson, J. R. Piper, and F. M. Sirotnak, *Eur. J. Med. Chem*., 2001, **36**, 237. (c) M. J. Sheardown, E. O. Nielsen, A. J. Hansen, P. Jacobsen, and T. Honoré, *Science*, 1990, **247**, 571. (d) U. Sehlstedt, P. Aich, J. Bergman, H. Vallberg, B. Norden, and A. Graslund, *J. Mol. Biol*., 1998, **278**, 31. (e) L. G. Fröhlich, P. Kotsonis, H. Traub, S. Taghavi-Moghadam, N. Al-Masoudi, H. Hofmann, H. Strobel, H. Matter, W. Pfleiderer, and H. H. H. W. Schmidt, *J. Med. Chem.,* 1999, **42**, 4108.

20. (a) "Pteridine Chemistry", ed. by W. Pfleiderer and E. C. Taylor, Pergamon Press, London, 1964. (b) W. Pfleiderer, *J. Heterocycl. Chem*., 1992, **29**, 583.

21. (a) R. Birnbacher, A. M. Messerschmidt, and A. P. Pollak, *Curr. Opin. Urol.,* 2002, **12**, 461. (b) H. McNulty, G. Cuskelly, and M. Ward, *Am. J. Clin. Nutr.,* 2000, **71**, 1308S. (c) M. L. Cravo, A. G. Pinto, and P. Chaves, J. A. Cruz, P. Lage, C. N. Leitao, and F. C. Mira, *Clin. Nutr.,* 1998, **17**, 45. (d) B. P. Hart, W. H. Haile, N. J. Licato, W. E. Bolanowska, J. J. McGuire, and J. K. Coward, *J. Med. Chem.,* 1996, **39**, 56.

22. (a) H. C. Benohr, R. Dreher, J. Neunhoffer, and H. D. Waller, *Verh. Dtsch. Ges. Inn. Med.,* 1973, **79**, 464. (b) H. Starlinger, *Hoppe-Seylers Z. Physiol. Chem*., 1977, **358**, 491.

23. J. Bellmunt, J. Cos, R. Cleries, M. Perez, A. Ribas, N. Eres, J. E. Murio, C. Margarit, and J. Baselga, *Cancer Investigation*, 2002, **5&6**, 673.

24. (a) S. Farber, L. K. Diamond, R. D. Mercer, R. F. Sylvester, and J. A. Wolff, *N. Engl. J. Med*., 1948, **238**, 787. (b) F. M. Huennekens, *Biochemistry*, 1963, **2**, 151. (c) R. Catane, V.H. Bono, Jr., A. C. Louie, and F. M. Muggia, *Cancer Treat. Rep*., 1978, **62**, 178. (d) F. M. Huennekens, J. R. Bertino, R. Silver, and B. W. Gabrio, *Exp. Cell. Res. Suppl*., 1963, **9**, 441.

25. "Folate Antagonists as Therapeutic Agents", Vol. 1 and 2, ed. by F. M. Sirotnak, J. J. Burchall, W. B. Ensminger, and J. A. Montgomery, Academic Press, New York, 1984.

26. (a) E. C. Taylor, D. C. Palmer, T. J. George, S. R. Fletcher, C. P. Tseng, P. J. Harrington, and G. P. Beardsley, *J. Org. Chem*., 1983, **48**, 4852. (b) D. W. Fry and R. C. Jackson, *Cancer and Metathasis Revs*., 1987, **5**, 251. (c) A. H. Calvert, D. L. Alison, S. J. Harland, B. A. Robinson, A. L. Jackman, T. R. Jones, D. R. Newell, Z. H. Siddiik, E. Wilshaw, T. J. McElvain, I. E. Smith, and K. R. Harrap, *J. Clin. Oncol*., 1986, **4**, 1245. (d) B. M. J. Cantwell, M. Earnshaw, and A. L. Harris, *Cancer Treat. Rep*., 1986, **70**, 1335.

27. (a) E. R. Werner, H. J. Habisch, A. C. Gorren, L. Schmidt, G. Canevari, G. Werner-Felmayer, and B. Mayer, *J. Biochem*., 2000, **348**, 579. (b) I. J. Butter, E. O'-Flynn, W. E. J. Seifert, and R. R. Howell,

Pediatr., 1981, **98**, 729. (c) T. Imamura, Y. Okano, H. Shintaku, Y. Hase, and G. Isshiki, *J. Hum. Genet.*, 1999, **44**, 163. (d) K. Tada, N. Narisawa, N. Arai, Y. Ogasawara, and S. Ishizawa, *Tohoku J. Exp. Med.*, 1980, **132**, 123.

28. E. C. Taylor and J. E. Dowling, *J. Org. Chem.*, 1997, **62**, 1599, and references cited therein.

29. P. Waring and W. L. F. Armarego, *Aust. J. Chem.*, 1985, **38**, 629.

30. F. Yoneda, R. Koga, S. Nishigaki, and S. Fukazawa, *J. Heterocycl. Chem.*, 1982, **19**, 949, and references cited therein.

31. P. Polonovski and H. Jerome, *C. R. Hebd. Seances Acad. Sci.*, 1950, **230**, 392.

32. W. R. Boom, W. G. M. Jones, and G. R. Ramage, *J. Chem. Soc.*, 1951, 96.

33. W. Pfleiderer, *Comp. Hterocycl. Chem. 1ˢᵗ edn.*, 1984, **3**, 315.

34. S. W. Bailey, R. Y. Chandrasekaran, and J. E. Ayling, *J. Org. Chem.*, 1992, **57**, 4470.

35. E. C. Taylor, K. L. Perlman, I. P. Sword, M. Séquin-Frey, and P. A. Jacobi, *J. Am. Chem. Soc.*, 1973, **95**, 6405.

36. E. C. Taylor and D. J. Dumas, *J. Org. Chem.*, 1980, **45**, 2485.

37. For reviews of this approach to pteridine synthesis, see (a) E. C. Taylor, in "Chemistry and Biology of Pteridines", ed. by W. Pfleiderer, Walter de Gruyter, Berlin, 1976, pp. 543-573. (b) E. C. Taylor, R. N.   Henrie II, and D. J. Dumas, in "Chemistry and Biology of Pteridines", ed. by L. Kisliuk and G. M. Brown, Elsevier/North-Holland, New York, 1979, pp. 71-75.

38. (a) E. C. Taylor, P. J. Harrington, S. R. Fletcher, G. P. Bearsdley, and R. J. Moran, *J. Med. Chem.*, 1985, **28**, 914. (b) E. C. Taylor and P. S. Ray, *J. Org. Chem.*, 1988, **53**, 35. (c) E. C. Taylor and R. Döetzer, *J. Org. Chem.*, 1991, **56**, 1816.

39. (a) W. Pfleiderer, in "Bicyclic 6-6-Systems: Pteridines: Comprehensive Heterocyclic Chemistry", ed. by A. R. Katritzky, C. W. Rees, and E. F. V. Scriven, Elsevier Science, London, 1996, Vol. 7, pp. 679-736 (b) A. Z. M. S. Chowdhury, Y. Shibata, M. Morita, K. Kaya, and K. Hiratani, *J. Heterocycl. Chem.*, 2001, **37**, 1173 (c) U. Urleb, R. Neidlein, and W. Kramer, *J. Heterocycl. Chem.*, 1990, **27**, 433 (d) D. J. Brown, in "The Chemistry of Heterocyclic Compounds, Vol. 24, Fused Pyrimidines, Part III. Pteridines", ed. by E. C.Taylor, John Wiley and Sons, New York, 1988, p. 143.