Eur J Med Chem (1996) 31, 273–280

Synthesis and potential antiallergic activity of new pteridinones and related compounds

G Ferrand[1], H Dumas[1], JC Depin[2], Y Quentin[1]

[1]*Department of Chemistry, Lipha, Centre de recherche et développement;*
[2]*Department of Pharmacology, Lipha, Centre de recherche et développement, 115, avenue Lacassagne, 69003 Lyon, France*

(Received 9 October 1995; accepted 27 November 1995)

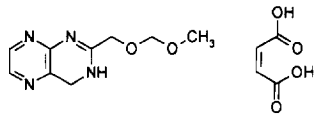
Summary — The synthesis and pharmacological profile of a series of 2-alkoxy and 2-phenoxymethyl-3*H*-pteridin-4-ones and related compounds are described. These compounds displayed antiallergic activity in the passive cutaneous anaphylaxis test in rats. In contrast to both sodium cromoglycate and nedocromil sodium, many of our compounds were active when administered orally. The most potent in this series, 2-ethoxymethyl-3*H*-pteridin-4-one **2b** (LCB 2183) was investigated in other models of allergy and inflammation. The antiallergic activity was confirmed and interesting antiinflammatory properties were observed. It was therefore selected for further development as a human therapeutic agent.

3*H*-pteridin-4-one / antiallergic activity / antiasthmatic activity / antiinflammatory activity

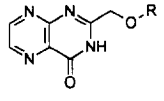
Introduction

G Ferrand et al [1] previously reported hypouricemic activity with a class of pteridines and focused on 2-(methoxymethoxymethyl)-3,4-dihydropteridine maleate **1**. Unlike the well-known reference compound, allopurinol, the dihydropteridine **1** was not an in vitro xanthine oxidase inhibitor, but the ex vivo inhibition could be proved. A metabolite of **1** was assumed to be responsible for the effectiveness of prodrug **1**. In our search for such a potential metabolite, we synthesized new 3*H*-pteridin-4-ones **2**, oxidized derivatives of 3,4-dihydropteridines.

The first compound obtained **2** (R = Me) did not possess the expected hypouricemic activity and xanthine oxidase inhibition. However, pharmacological screening discovered potential antiallergic properties. This paper describes the synthesis and the initial pharmacological studies of compounds **2** and some of their derivatives.



1

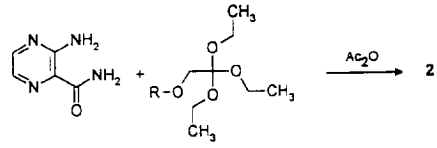


2

Chemistry

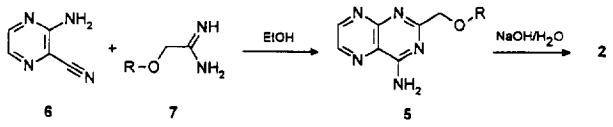
We used the method of Felder et al [2] for the synthesis of 2-methyl-3*H*-pteridin-4-one. Hence, condensation of 3-aminopyrazine-2-carboxamide **3** with orthoester **4** led to the desired structure (scheme 1, *Method A*).

Another route (scheme 2, *Method B*) consisted of preparing 4-aminopteridine **5** and hydrolyzing it with diluted aqueous alkali, as is known for 2-amino-3*H*-pteridin-4-one [3, 4]. In that route, the 4-aminopteridines **5** were obtained by condensing 3-aminopyrazine-2-carbonitrile **6** with acetamidine **7**.



3 **4**

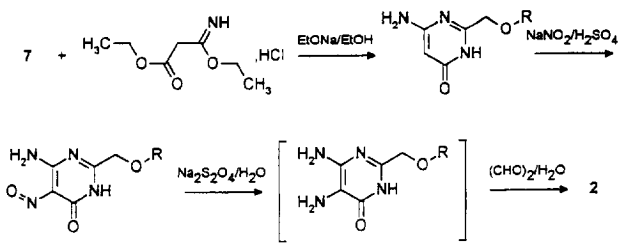
Scheme 1.



Scheme 2.

For practical purposes, an improved synthesis was investigated. Hence, starting from a pyrimidine nucleus, the pyrazine ring was added at a later stage (scheme 3, *Method C*).

The physical characteristics of compounds **2** appear in table I, where the indicated yields are those obtained in most cases in a single reaction and are not optimized. Using *Methods A* or *B*, some structural modifications of compounds **2** were then carried out (table II): different substituents were introduced in positions 3, 6 or 7 of pteridin-4-ones (compounds **8–10**); the oxygen atom of the position 2 chain was changed for a sulfur atom (compound **11**); and the pyrazine ring was changed for a pyridine or a quinoxaline ring (compounds **12–14**).



Scheme 3.

Table III shows the physical properties of the intermediate compounds, the 4-aminopteridines **5** and their derivatives.

Pharmacology, results and discussion

Rat passive cutaneous anaphylaxis

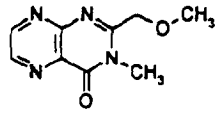
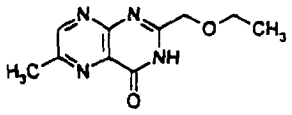
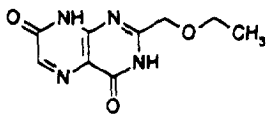
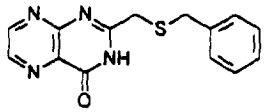
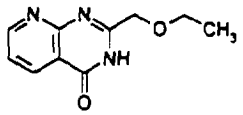
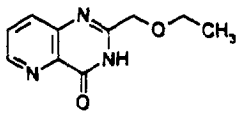
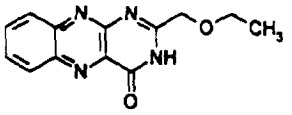
The antiallergic activity of compounds **2** and derivatives was measured in rats using the passive cutaneous anaphylaxis (PCA) test [5, 6]. The compounds were

Table I. Structures, physical properties and measured effects on PCA test of compounds **2**.

Compound	*R*	*Method*	*Yield (%)*	*Mp (°C)*	*Analysis*[a]	*PCA test ED_{50} (mg/kg) ip route [95% confidence interval]*
2a	CH_3	A	75	187–189[c]	$C_8H_8N_4O_2$	13.8 [11.3–17.0]
2b	CH_3CH_2	A	44	168–169[c]	$C_9H_{10}N_4O_2$	7.6 [5.8–10.0]
		B	65			
		C	61			
2c	$CH_3CH_2CH_2$	A	36	158–160[c]	$C_{10}H_{12}N_4O_2$	9.0 [7.0–12.0]
2d	$(CH_3)_2CH$	B	66	198–199[c]	$C_{10}H_{12}N_4O_2$	14.3 [8.9–23.0]
2e	CH_3OCH_2	B	41	161–163[c]	$C_9H_{10}N_4O_3$	18.5 [14.3–23.9]
2f	$CH_3CO_2CH_2CH_2$	B[f]	10	157–159[c]	$C_{11}H_{12}N_4O_4$	Inactive
2g	CF_3CH_2	B	50	165–167[d]	$C_9H_7F_3N_4O_2$	Inactive
2h	C_6H_5	B	70	220–221[c]	$C_{13}H_{10}N_4O_2$	38.8 [19.1–81.2]
2i	4-ClC_6H_4	B	50	267–269[e]	$C_{13}H_9ClN_4O_2$	Inactive
2j	4-$CH_3C_6H_4$	B	52	232–233[e]	$C_{14}H_{12}N_4O_2$	20.4 [16.1–25.3]
2k	4-$CH_3OC_6H_4$	B	31	233–234[e]	$C_{14}H_{12}N_4O_3$	71.4 [8.9–573]
2l	2,3-$Cl_2C_6H_3$	B	50	219–220.5[c]	$C_{13}H_8Cl_2N_4O_2$	Inactive
2m	3,4-$Cl_2C_6H_3$	B	66	285–287[e]	$C_{13}H_8Cl_2N_4O_2$	Inactive
2n	$C_{11}H_{13}O_2$[b]	B	63	224–226[e]	$C_{18}H_{18}N_4O_4$	Inactive
2o	$C_6H_5CH_2$	B	83	157–159[c]	$C_{14}H_{12}N_4O_2$	9.8 [7.4–12.9]
2p	CH_3CO	A	65	210–212[c]	$C_9H_8N_4O_3$	Inactive
Sodium cromoglycate						16.2 [9.5–27.5]
Nedocromil sodium						18.3 [15.2–22.3]

[a]Analysed elements: C, H, Cl, F, N; [b]4-acetyl-3-hydroxy-2-propylphenyl; [c]ethanol; [d]ethyl acetate; [e]ethanol/*N,N*-dimethylformamide; [f]see *Experimental protocols*.

Table II. Structures, physical properties and measured effects on PCA test of compounds **8–14**.

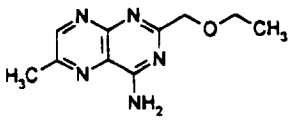
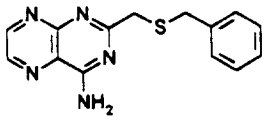
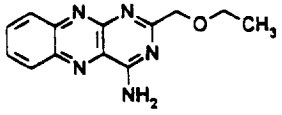
Compound	*Formula*	*Method*	*Yield (%)*	*Mp (°C)*	*Analysis*[a]	*PCA test ED_{50} (mg/kg) ip route [95% confidence interval]*
8		A	47	130.5–132.5[b]	$C_9H_{10}N_4O_2$	Inactive
9		B	66	181–183[c]	$C_{10}H_{12}N_4O_2$	Inactive
10		A*	40	255–256[d]	$C_9H_{10}N_4O_3$	20.7 [13.4–32.0]
11		B	31	224–226[e]	$C_{14}H_{12}N_4OS$	Inactive
12		A	48	144–146[f]	$C_{10}H_{11}N_3O_2$	Inactive
13		A	23	152–154[f]	$C_{10}H_{11}N_3O_2$	Inactive
14		A B	31 37	190–193[g]	$C_{13}H_{12}N_4O_2$	Inactive

[a]Analysed elements: C, H, N, S; [b]ethyl acetate; [c]acetone; [d]methanol/*N,N*-dimethylformamide; [e]ethanol/*N,N*-dimethylformamide; [f]hexane/ethyl acetate; [g]ethanol. *LCB 2183 metabolite isolated by filtration from the urine of rats and dogs treated with high oral doses of LCB 2183.

administered by the intraperitoneal (ip) route; the results are given in tables I and II.

The best compounds were 2-alkoxymethyl-3*H*-pteridin-4-ones **2a–e**, 2-phenoxymethyl-3*H*-pteridin-4-ones **2h,j** and 2-benzyloxymethyl-3*H*-pteridin-4-one **2o**. All the other trials with the modification of the chain in position 2 of pteridin-4-ones induced a loss of activity. The same was true for the modification or for other substitutions of the pteridin-4-ones, with the exception of hydroxylation in position 7 (compound **10**). The products showing the best results were also tested orally (table IV). The best compound **2b** (code number LCB 2183) is about twice as potent as sodium cromoglycate in the PCA test by ip admi-

Table III. Structures and physical properties of intermediate compounds **5** and derivatives.

Compound	*R*	*Yield (%)*	*Mp (°C)*	*Analysis*[a]
5b	CH_3CH_2	84	152–154[c]	$C_9H_{11}N_5O$
5d	$(CH_3)_2CH$	28	139–141[d]	$C_{10}H_{13}N_5O$
5e	CH_3OCH_2	71	129–131[c]	$C_9H_{11}N_5O_2$
5f	$HOCH_2CH_2$	70	149–151[c]	$C_9H_{11}N_5O_2$
5g	CF_3CH_2	42	145–147[d]	$C_9H_8F_3N_5O$
5h	C_6H_5	85	202–204[c]	$C_{13}H_{11}N_5O$
5i	4-Cl-C_6H_4	84	246–248[e]	$C_{13}H_{10}ClN_5O$
5j	4-CH_3-C_6H_4	17	225–227[f]	$C_{14}H_{13}N_5O$
5k	4-CH_3O-C_6H_4	24	238–240[g]	$C_{14}H_{13}N_5O_2$
5l	2,3-Cl_2-C_6H_3	84	216–218[c]	$C_{13}H_9Cl_2N_5O$
5m	3,4-Cl_2-C_6H_3	70	261–263[g]	$C_{13}H_9Cl_2N_5O$
5n	$C_{11}H_{13}O_2$[b]	30	179–181[g]	$C_{18}H_{19}N_5O_3$
5o	C_6H_5-CH_2	58	131–133[d]	$C_{14}H_{13}N_5O$
		34	166–168[h]	$C_{10}H_{13}N_5O$
		100	Amorphous solid, not purified	
		81	200 (decomposition)[i]	$C_{13}H_{13}N_5O$

[a]Analysed elements: C, H, Cl, F, N, S; [b]4-acetyl-3-hydroxy-2-propylphenyl; [c]ethanol; [d]ethyl acetate; [e]*N,N*-dimethylformamide; [f]acetone/ethanol; [g]ethanol/*N,N*-dimethylformamide; [h]acetone/diisopropyl ether; [i]anhydrous ethanol.

nistration. Moreover, it is active by the oral route, which is not the case for sodium cromoglycate and nedocromil sodium. Further tests were performed with LCB 2183 (see below).

Anaphylactic bronchoconstriction in the anaesthetized guinea pig

An immediate hypersensitivity reaction was provoked by antigen challenge in guinea pigs which had been passively sensitized using rabbit anti-egg albumin serum [7]. The bronchoconstriction that followed the intravenous injection of antigen, measured by the method of Konzett and Rössler [8], was inhibited by

Table IV. Inhibition of PCA by the oral route.

Compound	*ED_{50} (mg/kg) po route [95% confidence interval]*
2a	34.6 [22.8–52.6]
2b	8.5 [5.8–12.4]
2c	≫ 50 mg/kg
2e	≫ 50 mg/kg
2o	≫ 50 mg/kg
Sodium cromoglycate	Inactive
Nedocromil sodium	Inactive

LCB 2183 given orally, 10 min before the antigen. Activity was proportional to the dose with a calculated ED_{50} of 15.7 (9.9–25.0) mg/kg (fig 1).

Bronchial inflammation

Another experiment was carried out in conscious guinea pigs. A local bronchial inflammation was produced in animals actively sensitized with egg albumin aerosol. Antigen challenge by inhalation caused an infiltration of cells into the airways, predominantly eosinophilic in nature [9]. LCB 2183 was given as a preventive treatment by the ip route 5 min before challenged test. This experiment showed that LCB 2183, at a dose of 50 mg/kg, inhibited the inflammatory reaction in the bronchi of guinea pigs. By comparison sodium cromoglycate was inactive at the same dose (table V).

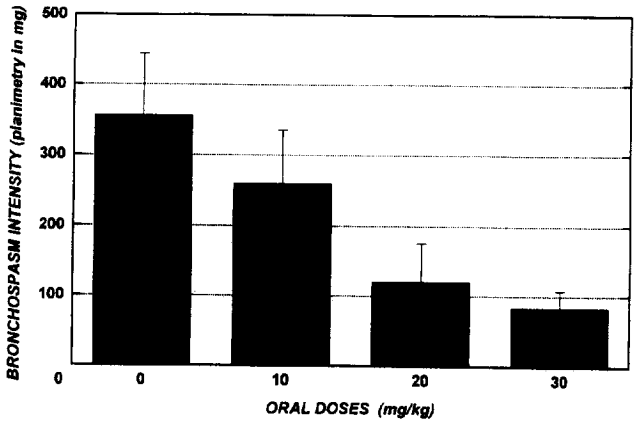


Fig 1. Anaphylactic bronchospasm inhibition by oral doses of LCB 2183.

Conclusion

We have shown the potential antiallergic activity of a series of 2-alkoxy- and 2-phenoxymethyl-3*H*-pteridin-4-ones, particularly for compound **2b** (LCB 2183) which inhibits orally PCA in rats and the anaphylactic bronchoconstriction in guinea pigs. LCB 2183 also showed a potential antiinflammatory activity by inhibition of the bronchial inflammation in guinea pigs. This pharmacological property confirmed the inhibition of the oxazolone-induced contact hypersensitivity inflammatory response in mice by LCB 2183, which was reported by Murray et al [10]. These results have motivated us to develop this compound for the clinical treatment of allergic and inflammatory diseases.

Experimental protocols

Chemistry

The non-corrected melting points were measured on a Gallenkamp apparatus. Analyses (C, H, Cl, F, N, S) were performed by the Service central d'analyse du CNRS and gave satisfactory results within ±0.40% of the theoretical values. Infrared spectra were recorded on a Beckman Acculab 2 apparatus and ^{1}H-NMR spectra on Bruker WP 60 CW apparatus; the internal standard was tetramethylsilane; the results are consistent with the proposed structures.

Method A

Triethyl orthoacetates ***4***
Triethyl orthomethoxyacetate and triethyl orthoethoxyacetate were prepared according to literature procedures [11, 12].

Triethyl orthopropoxyacetate ***4***
Hydrogen chloride was bubbled for 1 h though a solution of 29.7 g (0.30 mol) of propoxyacetonitrile [13] and 13.8 g (0.30 mol) of absolute ethanol in 500 mL of diethyl ether cooled to 0°C. The reaction mixture was left for 2 days at 0 °C and was then taken up with 300 mL diethyl ether. The precipi-

Table V. Effect of LCB 2183 and sodium cromoglycate on cellular infiltration of the airways induced in conscious guinea pig by inhalation of egg albumin.

Compound	*Number of animals*	*Dose mg/kg, ip*	*Neutrophil and eosinophil total cells, mean ± SE*	*Significance t or U test vs challenged controls*
Unchallenged controls	20	0	715 ± 64	
Challenged controls	18	0	3058 ± 570	[a]
LCB 2183	10	50	986 ± 74	$P < 0.05$
Sodium cromoglycate	10	50	2193 ± 593	NS[b]

[a] $P < 0.01$ challenged controls/unchallenged controls; [b] non-significant.

tate formed, composed of ethyl 2-propoxyacetimidate hydrochloride, was isolated by filtration. It was washed with diethyl ether, dried under reduced pressure and used without further purification. Yield: 30.2 g (55%). This product (30.2 g; 0.17 mol) was dissolved in 160 mL absolute ethanol. The solution was left for 2 days at room temperature; it was then concentrated under reduced pressure. The residue obtained was taken up with diethyl ether and filtered. The ethereal solution was dried over potassium carbonate, filtered and concentrated under reduced pressure. The liquid obtained was used in the next step without further purification. Yield: 29.3 g (80%); ^{1}H-NMR ($CDCl_3$): δ = 0.9 (3H, t), 1.3 (9H, t), 1.3–2.0 (2H, m), 3.4 (2H, t), 3.5 (2H, s), 3.6 (6H, q); $C_{11}H_{24}O_4$.

Triethyl orthoacetoxyacetate **4**

Hydrogen chloride was bubbled to saturation through a solution of 30.0 g (0.303 mol) acetoxyacetonitrile [14] and 15.4 g (0.334 mol) absolute ethanol in 300 mL diethyl ether cooled to 0 °C. The reaction mixture was then left for 5 h at 0 °C. The precipitate, composed of ethyl 2-acetoxyacetimidate hydrochloride was isolated by filtration. It was washed with diethyl ether, dried under reduced pressure and used without further purification. Yield: 52.2 g (95%); ^{1}H-NMR (DMSO-d_6 + CF_3COOD): δ = 1.1 (3H, t), 1.7 (3H, s), 3.4 (2H, q), 3.8 (2H, s). A mixture of 34.0 g (0.187 mol) of this hydrochloride and 340 mL absolute ethanol was left for 3 days at room temperature. The precipitate formed was removed by filtration; the filtrate was concentrated under reduced pressure. The residue obtained was taken up with 800 mL diethyl ether and cooled to about –10 °C. A new precipitate was formed and removed by filtration. The filtrate was concentrated under reduced pressure and the liquid obtained was used in the next step without further purification. Yield: 34.5 g (84%); ^{1}H-NMR ($CDCl_3$): δ = 1.0 (9H, t), 1.9 (3H, s), 3.4 (6H, q), 4.1 (2H, s); $C_{10}H_{20}O_5$.

2-Ethoxymethyl-3H-pteridin-4-one **2b**

A mixture of 8.3 g (0.060 mol) 3-aminopyrazine-2-carboxamide **3** [15], 49.5 g (0.24 mol) triethyl orthoethoxyacetate and 53 mL acetic anhydride was refluxed under a nitrogen atmosphere for 3 h. With the passing of time, the reflux temperature dropped from 124 to 96 °C, remaining constant at the latter temperature. After cooling, the precipitate formed was isolated by filtration. It was washed with diethyl ether and recrystallized from ethanol. Yield: 5.5 g (44%); mp = 168–169 °C; IR: ν (CO) = 1690 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ = 1.3 (3H, t), 3.7 (2H, q), 4.6 (2H, s), 8.8 (1H, d), 8.9 (1H, d), 10.3 (1H, peak exchangeable with CF_3COOD); anal $C_9H_{10}N_4O_2$ (C, H, N).

Method B

Acetamidines **7**

Compounds **7**, 2-phenoxyacetamidine, 2-(4-chlorophenoxy)-acetamidine and 2-(4-methylphenoxy)acetamidine [17, 18] and 2-benzyloxyacetamidine and 2-(benzylthio)acetamidine [19, 20] were prepared according to literature procedures.

2-Ethoxyacetamidine **7**. To a solution of 39 g (2.29 mol) ammonia in 1.225 mL absolute ethanol maintained at 10 °C was added 53.5 g (0.457 mol) methyl 2-ethoxyacetimidate. The solution obtained was left for 6 days at room temperature and was then concentrated under reduced pressure. It was used in the next step without further purification. Yield: 46.7 g (quantitative); $C_4H_{10}N_2O$.

The following acetamidines **7** were obtained in the same manner.

2-Isopropoxyacetamidine **7**. Starting with 14.7 g (0.12 mol) methyl 2-isopropoxyacetimidate [16] and 9.50 g (0.56 mol) ammonia in 300 mL absolute ethanol. Reaction time: 2 days. Yield: 13.0 g (quantitative), $C_5H_{12}N_2O$.

2-(Methoxymethoxy)acetamidine **7**. Starting with 20.0 g (0.15 mol) methyl 2-(methoxymethoxy)acetimidate [1] and 13.0 g (0.75 mol) ammonia in 400 mL absolute ethanol. Reaction time: 2 days. Yield: 18.0 g (quantitative); ^{1}H-NMR ($CDCl_3$ + D_2O): δ = 3.4 (3H, s), 4.0 (2H, s), 4.6 (2H, s); $C_4H_{10}N_2O_2$.

2-(2,2,2-Trifluoroethoxy)acetamidine **7**. Starting with 15.6 g (0.091 mol) methyl 2-(2,2,2-trifluoroethoxy)acetimidate [1] and 7.7 g (0.455 mol) ammonia in 225 mL absolute ethanol. Reaction time: 24 h. Yield: 14.2 g (quantitative); $C_4H_7F_3N_2O$.

2-(3,4-Dichlorophenoxy)acetamidine **7**. Starting with 28.2 g (0.12 mol) methyl 2-(3,4-dichlorophenoxy)acetimidate and 10.2 g (0.60 mol) ammonia in 480 mL absolute ethanol. Reaction time: 3 h. Yield: 26.3 g (quantitative); $C_8H_8Cl_2N_2O$.

2-(Hydroxyethoxy)acetamidine **7**. To a solution of 22.6 g (1.33 mol) ammonia in 1080 mL absolute ethanol maintained at 10 °C was added 39.2 g (0.266 mol) ethyl 2-(2-hydroxyethoxy)acetimidate [1]. The reaction mixture was left for 2 days at room temperature and the excess ammonia was then driven off with a stream of nitrogen. The solution obtained contained 2-(hydroxyethoxy)acetamidine and was used in the next step without further purification; $C_4H_{10}N_2O_2$.

The following acetamidines **7** were obtained in the same manner.

2-(4-Methoxyphenoxy)acetamidine **7**. Starting with 23.9 g (0.122 mol) methyl 2-(4-methoxyphenoxy)acetimidate and 10.4 g (0.61 mol) ammonia in 480 mL absolute ethanol; $C_9H_{12}N_2O_2$.

2-(4-Acetyl-3-hydroxy-2-propylphenoxy)acetamidine **7**. Starting with 25.0 g (0.0895 mol) ethyl 2-(4-acetyl-3-hydroxy-2-propylphenoxy)acetimidate and 7.6 g (0.45 mol) ammonia in 300 mL absolute ethanol; $C_{13}H_{18}N_2O_3$.

2-(2,3-Dichlorophenoxy)acetamidine **7**. To a solution of 3.5 g (0.21 mol) ammonia in 100 mL absolute ethanol, cooled to 10 °C were added rapidly 11.8 g (0.041 mol) ethyl 2-(2,3-dichlorophenoxy)acetimidate hydrochloride [1]. The reaction mixture was left for 3 days at room temperature. After removal of a few suspended particles by filtration, the reaction mixture was concentrated to dryness under reduced pressure. The solid residue obtained, which was 2-(2,3-dichlorophenoxy)-acetamidine hydrochloride, was purified by washing with diethyl ether and recrystallization from isopropanol. Yield: 8.0 g (75%); mp = 204.5–206.5 °C; ^{1}H-NMR (DMSO-d_6 + D_2O): δ = 5.1 (2H, s), 7.1–7.7 (3H, m); anal $C_8H_9Cl_3N_2O$ (C, H, Cl, N). This product (8.0 g; 0.031 mol) was suspended in sodium hydroxide solution and extracted with chloroform. The organic extract was concentrated to dryness under reduced pressure. The solid residue was washed with hexane and dried; it was used in the next step without further purification. Yield: 6.7 g (98%); mp = 104–108 °C; $C_8H_8Cl_2N_2O$.

Acetimidates

Most of the alkyl acetimidates used to obtain the acetamidines **7** are known and were prepared from the corresponding acetonitriles in basic medium in accordance with Schaefer and Peters conditions [16]. They were used untreated without purification. The characteristics of new methyl acetimidates are as follows.

Methyl 2-ethoxyacetimidate. Yield: 59%; bp_{15} = 38–40 °C; ^{1}H-NMR ($CDCl_3$): δ = 1.2 (3H, t), 3.5 (2H, q), 3.7 (3H, s), 7.6 (1H, peak exchangeable with D_2O); $C_5H_{11}NO_2$.

Methyl 2-(4-methoxyphenoxy)acetimidate. Yield: 73%; mp = 95–96 °C (diisopropyl ether); $C_{10}H_{13}NO_3$.

Methyl 2-(3,4-dichlorophenoxy)acetimidate. Yield: 80%; mp = 55–56 °C (diisopropyl ether); $C_9H_9Cl_2NO_2$.

Ethyl 2-(4-acetyl-3-hydroxy-2-propylphenoxy)acetimidate. Ethyl 2-(4-acetyl-3-hydroxy-2-propylphenoxy)acetimidate hydrochloride was obtained from (4-acetyl-3-hydroxy-2-propylphenoxy)acetonitrile [21] in acidic medium in accordance with Pinner's conditions [22]. Yield: 97%; mp = 157–159 °C; ^{1}H-NMR (DMSO-d_6): δ = 0.9 (3H, t), 1.1–1.9 (2H, m), 1.4 (3H, t), 2.4–2.9 (2H, m), 2.6 (3H, s), 4.6 (2H, q), 5.3 (2H, s), 6.7 (1H, d), 7.8 (1H, d), 10.8 (2H, peak exchangeable with CF_3COOD), 12.8 (1H, s exchangeable with CF_3COOD); $C_{15}H_{22}ClNO_4$.

To a suspension of 40.0 g (0.127 mol) of this chlorhydrate in 1 L of chloroform was added 10.2 g (0.121 mol) sodium hydrogen carbonate and this mixture was stirred for 1 h. The solid was removed by filtration and the filtrate was concentrated to dryness under reduced pressure. The residue obtained was extracted with a mixture of 100 mL hexane and 100 mL diisopropyl ether under reflux. This extract, concentrated under reduced pressure, was recrystallized from hexane to give 25.0 g (70%) ethyl 2-(4-acetyl-3-hydroxy-2-propylphenoxy)acetimidate; mp = 50–52 °C; ^{1}H-NMR ($CDCl_3$ + D_2O): δ = 1.0 (3H, t), 1.1–1.9 (2H, m), 1.4 (3H, t), 2.6 (3H, s), 2.7 (2H, t), 4.3 (2H, q), 4.7 (2H, s), 6.3 (1H, d), 7.6 (1H, d), 12.7 (1H, s partially exchanged); $C_{15}H_{21}NO_4$.

4-Amino-2-(ethoxymethyl)pteridine ***5b***

A mixture of 36.0 g (0.30 mol) 3-aminopyrazine-2-carbonitrile **6** [23] and 46.7 g 2-ethoxyacetamidine in 700 mL absolute ethanol was refluxed for 2 h 30 min under a nitrogen atmosphere. After cooling, the precipitate obtained was isolated by filtration. It was purified by recrystallization from ethanol. Yield: 52.0 g (84%); mp = 152–154 °C; ^{1}H-NMR (DMSO-d_6): δ = 1.1 (3H, t), 3.5 (2H, q), 4.4 (2H, s), 8.2 (2H, peak exchangeable with CF_3COOD), 8.7 (1H, d), 9.0 (1H, d); anal $C_9H_{11}N_5O$ (C, H, N).

2-Ethoxymethyl-3H-pteridin-4-one ***2b***

A solution of 30.0 g (0.146 mol) of 4-amino-2-(ethoxymethyl)-pteridine in 800 mL of 5% aqueous sodium hydroxide was brought slowly to 75 °C and maintained at this temperature for 2 h. After cooling, the solution obtained was acidified with acetic acid to pH 6 and was then extracted with chloroform. The organic extracts were dried over sodium sulphate and concentrated to dryness under reduced pressure. The solid residue was recrystallized from ethanol in the presence of activated carbon. Yield: 19.5 g (65%). The product was identical to that obtained by *Method A*.

2-[(2-Acetoxyethoxy)methyl]-3H-pteridin-4-one ***2f***

A solution of 10.0 g (0.0452 mol) of 4-amino-2-[(2-hydroxyethoxy)methyl]pteridine **5f** in 350 mL of 5% aqueous sodium hydroxide was brought gradually to 85 °C and maintained at this temperature for 1 h 30 min. After cooling, the solution obtained was acidified with acetic acid to pH 5.5, and then concentrated to dryness under reduced pressure. The residue was taken up in 500 mL isopropanol. The insoluble inorganic portion was removed by filtration. The isopropanolic solution was concentrated to dryness under reduced pressure. The solid residue was treated with 250 mL acetic anhydride and this mixture was refluxed for 1 h. After cooling, the inorganic salts were removed by filtration; the filtrate was concentrated to dryness under reduced pressure. The residue was purified by four successive recrystallizations from ethanol in the presence of activated carbon. Yield: 1.2 g (10%); mp = 157–159 °C; IR: ν (CO) = 1690 and 1720 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 2.0 (3H, s), 3.7–4.0 (2H, m), 4.1–4.4 (2H, m), 4.5 (2H, s), 8.8 (1H, d), 9.0 (1H, d), 12.6 (1H, peak exchangeable with CF_3COOD); anal $C_{11}H_{12}N_4O_4$ (C, H, N).

Method C

6-Amino-2-ethoxymethyl-3H-pyrimidin-4-one

A stream of ammonia was bubbled through a solution of 75.7 g (0.577 mol) ethyl 2-ethoxyacetimidate in 650 mL absolute ethanol maintained at 0 °C so as to dissolve 39.1 g (2.30 mol) of the gas. The solution obtained was left stirring at room temperature for 2 days. The excess ammonia was then driven off with a stream of nitrogen. The reaction mixture was treated with 75.0 g (0.383 mol) ethyl (1-ethoxyformimidoyl)acetate hydrochloride [24] and then with 27.3 g (0.401 mol) sodium ethoxide at about 0 °C. After 15 min at 0 °C, the suspended solid was removed by filtration; the filtrate was stirred for 24 h at room temperature and was then refluxed for 2 h. This solution was concentrated by the removal of 425 mL ethanol by means of a Dean–Stark apparatus; it was then cooled to about –20 °C. The precipitate formed was isolated by filtration and washed with diethyl ether. The solid obtained was dried and used in the next step without further purification. Yield: 39.5 g (61%); mp = 224–226 °C. An analytical sample was obtained by recrystallization from a mixture of isopropanol and diisopropyl ether; mp = 228 °C; IR: ν (CO) = 1610 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.1 (3H, t), 3.5 (2H, q), 4.1 (2H, s), 4.9 (1H, s), 6.4 (2H, s exchangeable with CF_3COOD), 11.2 (1H, s exchangeable with CF_3COOD); anal $C_7H_{11}N_3O_2$ (C, H, N).

6-Amino-2-ethoxymethyl-5-nitroso-3H-pyrimidin-4-one

To a solution of 39.5 g (0.233 mol) 6-amino-2-ethoxymethyl-3*H*-pyrimidin-4-one in 336 mL (0.336 mol) 1 N sodium hydroxide, was added 17.7 g (0.256 mol) sodium nitrite. A solution of 17.9 mL (0.320 mol) 96% sulphuric acid diluted in 179 mL water was then added dropwise at between 0 and 5 °C. The blue precipitate formed was isolated by filtration immediately after completion of the addition. The solid obtained was washed with cold water and then with diethyl ether. After drying, it was used in the next step without further purification. Yield: 46.2 g (quantitative); mp = 163–165 °C. An analytical sample was obtained by recrystallization from methanol; mp = 166–167 °C; IR: ν (CO) = 1660 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.15 (3H, t), 3.55 (2H, q), 4.25 (2H, s), 9.15 (1H, peak exchangeable with CF_3COOD); anal $C_7H_{10}N_4O_3$ (C, H, N).

2-Ethoxymethyl-3H-pteridin-4-one ***2b***

A solution of 19.1 g (0.11 mol) of sodium dithionite in 88 mL water was added dropwise to a suspension, maintained at 20 °C, of 10.0 g (0.050 mol) 6-amino-2-ethoxymethyl-5-nitroso-3*H*-pyrimidin-4-one in 50 mL water. After stirring for 30 min at room temperature, the reaction mixture, still maintained at 20 °C, was treated with 47.9 g (0.33 mol) of a 40% aqueous solution of glyoxal. Stirring was continued for 20 h at room temperature. The solution obtained was then extracted with methylene chloride. The organic extracts were dried over sodium sulphate and concentrated to dryness under

reduced pressure. The solid residue was recrystallized from methanol in the presence of activated carbon. Yield: 6.5 g (63%). The product is identical to that obtained by *Methods A* and *B*.

Pharmacology

Rat PCA

Homologous anti-egg albumin (anti-EA) serum was obtained from female Sprague–Dawley rats (180–200 g) receiving simultaneously 10 mg/kg im EA, distributed between the muscles of the four legs, and Bordetella Pertussis (2×10^{10} organisms) ip. Rats were bled 10 days later and serum isolated from citrated blood and kept at –20 °C.

The skin of female Sprague–Dawley rats (180–200 g) was then sensitized with four intradermal injections (0.05 mL each) of the homologous anti-EA serum diluted 15-fold in order to produce a skin reaction of about 10 mm diameter. On the day following the sensitization, the animals received 1 mL/kg of a 2.5% saline solution of Evans blue and 1 mL/kg of a 2.5% saline EA solution simultaneously by intravenous injection.

For the ip route, the test compounds were dissolved in apyrogenic physiological saline and injected (5 mL/kg) 5 min before the antigen. For the oral route, test compounds were suspended in 0.5% methylcellulose and given (10 mL/kg) 10 min before the antigen. Each compound was given at three increasing doses. Four rats were used for each dose.

Thirty minutes after the injection of the EA/dye mixture, the animals were sacrificed and the intensity of the allergic response determined by measuring the area of the skin coloration. The protection provided by the test products was expressed as an ED_{50} (dose decreasing by 50% the skin area occupied by the dye).

Anaphylactic bronchoconstriction in the anaesthetized guinea pig

Heterologous anti-EA serum was obtained from New-Zealand rabbits (2.8 to 3 kg) receiving an emulsion (2 mL) prepared with an equivolume of Freund's complete adjuvant and 1% EA solution, eight times at twice a week, and bled one week after the last antigen stimulation. The antiserum was separated and kept at –20 °C.

Male Dunkin–Hartley guinea pigs (350–400 g) were sensitized passively by intravenous injection of 0.5 mL anti-EA rabbit serum at a previously determined active concentration. The dilution was in the range of 1 in 16. Twenty-four hours later, animals were anaesthetized with urethane and the trachea cannulated. Guinea pigs were then mechanically ventilated by a constant volume respiratory pump delivering 54 strokes/min of 1 mL of air per 100 g body weight. Pulmonary inflation pressure, an index of intrathoracic airway calibre, was measured by means of a Statham P23 X L pressure transducer connected to a pen-recorder.

After a 5 min stabilization period, the animals were challenged iv with 0.5 ml of 2% EA, which induced an anaphylactic bronchoconstriction.

Each test dose suspended in 0.5% methylcellulose was administered orally (5 mL/kg) 10 min before the antigen challenge. Bronchoconstriction was recorded for 20 min after antigen challenge. The effect was measured by weighing the bronchoconstriction area (planimetry in mg)

Four guinea pigs were used for each dose of compounds and seven for the controls.

Bronchial inflammation

Male Dunkin–Hartley guinea pigs (450–500 g) were sensitized by inhalation of EA (1%) for 3 min on days 0 and 7. On day 14, they were challenged by inhalation of EA (2%) for 5 min under cover of mepyramine maleate (10 mg/kg) injected intraperitoneally 30 min beforehand. A bronchoalveolar lavage was performed with 2×5 mL volumes of sterile saline. Total nucleated cell counts were performed in a Coulter counter. Results are expressed as the total number of cells recovered from each animal following bronchoalveolar lavage. Ten guinea pigs were used for each group.

The test compounds were dissolved in apyrogenic physiological saline and injected (5 mL/kg) by the ip route 5 min before the challenge test.

Statistical analysis

Statistical significance between means was determined by Student's *t*-test after comparison of variances (*F*-test), *P* values of less than 0.05 being considered as significant. ED_{50} values were evaluated by regression analysis after logarithmic transformation of the doses.

Acknowledgments

We thank D Bardet, A Battesti, C Didone and R Verchère for their technical collaboration on the syntheses and N Amouyal, MP Fesquet and C Gicquel for help with the pharmacological tests.

References

1 Ferrand G, Dumas H, Decerprit J (1992) *Eur J Med Chem* 27, 309–320
2 Felder EC, Pitrè D, Boveri S (1972) *J Med Chem* 15, 210–211
3 Taylor EC, Abdulla RF, Tanaka K, Jacobi PA (1975) *J Org Chem* 40, 2341–2347
4 Taylor EC, Portnoy RC, Hochstetler DC, Kobayashi T (1975) *J Org Chem* 40, 2347–2351
5 Goose J, Blair AMJN (1969) *Immunology* 16, 749–760
6 Ovary Z, Bier G (1952) *Proc Soc Exp Biol Med* 81, 584–586
7 Fukuda T, Saito T, Tajima S, Schimoara K, Ito K (1984) *Arzneim Forsch/Drug Res* 34, 805–810
8 Konzett H, Rössler R (1940) *Arch Exp Path Pharmakol* 195, 71–74
9 Hutson PA, Holgate ST, Church MK (1988) *Br J Pharmacol* 94, 6–8
10 Murray N, Zoerkler N, Brown T, Bonhomme Y (1994) *Int J Immunopharm* 16, 675–683
11 Stiller ET (1947) *US Patent* 2 422 598; *Chem Abstr* 41, 5904a
12 McElvain SM, Walters PM (1942) *J Am Chem Soc* 64, 1963–1965
13 Gauthier D (1906) *CR Acad Sci* 143, 831–832
14 Henry L (1905) *Recl Trav Chim Pays-Bas* 24, 169
15 Ellingson RC, Henry RL, McDonald FG (1945) *J Am Chem Soc* 67, 1711–1713
16 Schaefer FC, Peters GA (1961) *J Org Chem* 26, 412–418
17 Djerassi C, Scholz CR (1947) *J Am Chem Soc* 69, 1688–1692
18 Djerassi C, Scholz CR (1951) *US Patent* 2 517 468; *Chem Abstr* 45, 661f
19 Haggerty WJ Jr, Rost WJ (1969) *J Pharm Sci* 58, 50–55
20 McManus JM (1968) *J Heterocycl Chem* 5, 137–139
21 Marshall WS, Goodson T, Cullinan GJ et al (1987) *J Med Chem* 682–689
22 Pinner A, Klein F (1877) *Ber Dtsch Chem Ges* 10, 1889
23 Albert A, Ohta K (1970) *J Chem Soc C* 1540–1547
24 Ursprung JJ (1968) *US Patent* 3 337 579; *Chem Abstr* 68, 68986k