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Novel tetrahydropyrido[4,3-*d*]pyrimidines as gastric antilesion agents

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Summary — A variety of substituted tetrahydropyrido[4,3-*d*]pyrimidines was prepared and found to possess gastric antilesion against ethanol-induced lesions in rats. The more potent compounds possessed similar activity against aspirin-induced gastric lesions. A selective group of compounds was determined to be inactive as gastric antisecretory agents in rabbit isolated parietal cells. The antilesion properties of these tetrahydropyrido[4,3-*d*]pyrimidines make them a potential alternative to prostaglandin therapy for gastric antilesion therapy and may have clinical utility in peptic ulcer disease.

tetrahydropyrido[4,3-*d*]pyrimidines / gastric antilesion / peptic ulcer disease / prostaglandin

Introduction

Peptic ulcer disease is associated with an imbalance between aggressive factors acting on the gastroduodenal mucosa such as acid, pepsin, or ulcerogenic drugs, and factors such as mucus and bicarbonate secretion that defend the mucosa against aggression [1]. Successful treatment of peptic ulcer disease depends upon reestablishing this dynamic balance in the gut, thereby restoring the integrity of the compromised gastrointestinal mucosal barrier.

The most widely used strategy for increasing the rate of peptic ulcer healing has been the reduction of the aggressive stimulus of gastric acid with anti-acids or histamine H_2-receptor antagonists. Recently, there has been increased interest in the defensive factors that relate to mucosal ulceration, primarily due to information about the pathophysiological significance and pharmacological potential of prostaglandins in peptic ulcer disease [2]. Prostaglandins can prevent experimentally induced gastrointestinal mucosal damage caused by a large variety of noxious agents or procedures such as steroidal or non-steroidal anti-inflammatory drugs, bile, reserpine, serotonin, restraint or pylorous-ligation, or by necrotizing agents such as strong acid, strong base, ethanol or even boiling water [3–5].

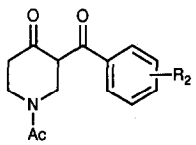
However, use of prostaglandins is also associated with a variety of potentially untoward effects including contraction of uterine smooth muscle, stimulation of intestinal secretion and motility as well as a variety of cardiovascular effects [6]. These potential side effects make the discovery of non-prostaglandin therapies for anti-ulcer therapy an attractive goal. The present study describes, as a result of broad screening, the discovery of a novel series of tetrahydropyrido[4,3-*d*]pyrimidines that exhibit selective antilesion activity in rats.

Chemistry

The preparation of a variety of substituted tetrahydropyrido[4,3-*d*]pyrimidines was envisioned as resulting from the direct condensation of an appropriately substituted amidine with the enaminoketone derivative of diketone **I**. The diketones **I** were prepared by acylation of the morpholine enamine of 1-acetyl-4-piperidone [7] and their physical properties are described in table I. Diketone **I** failed to give the desired substituted tetrahydropyrido[4,3-*d*]pyrimidine derivatives upon direct reaction with substituted amidines under a variety of conditions such as aqueous sodium bisulfite, refluxing acetic acid or sodium hydride in THF. Interestingly, when **Ib** was treated with ethanolic piperidine and a substituted amidine, hemiketal **II** was isolated (scheme 1) which could not be forced to react to form our target compounds.

This unreactivity of **I** was circumvented by converting the diketone to the morpholine or pyrrolidine enaminoketone **III** that has been used to activate similar systems for condensation [8]. Treatment of **III**

Table I. Physical properties of diketones **I**.



Compd	R_2	*Yield %*	*Empirical formula* [a]	*mp, °C* [b]
Ia	H	75	$C_{14}H_{15}NO_3$	Oil
Ib	4-Cl	33	$C_{14}H_{14}ClNO_3$	96–98
Ic	4-F	56	$C_{14}H_{14}FNO_3$	Oil
Id	3-Cl	45	$C_{14}H_{14}ClNO_3$	102–104
Ie	3-F	42	$C_{14}H_{14}FNO_3$	Oil
If	3,4-Cl_2	34	$C_{14}H_{13}Cl_2NO_3$	100–102
Ig	4-CH_3	45	$C_{15}H_{17}NO_3$	Oil
Ih	4-MeO	56	$C_{15}H_{17}NO_4$	Oil
Ii	2-thienyl	65	$C_{12}H_{13}N_2O_3S$	108–110
Ij	4-pyridyl	42	$C_{13}H_{14}N_2O_3$	116–118
Ik	3-pyridyl	37	$C_{13}H_{14}N_2O_3$	120–121

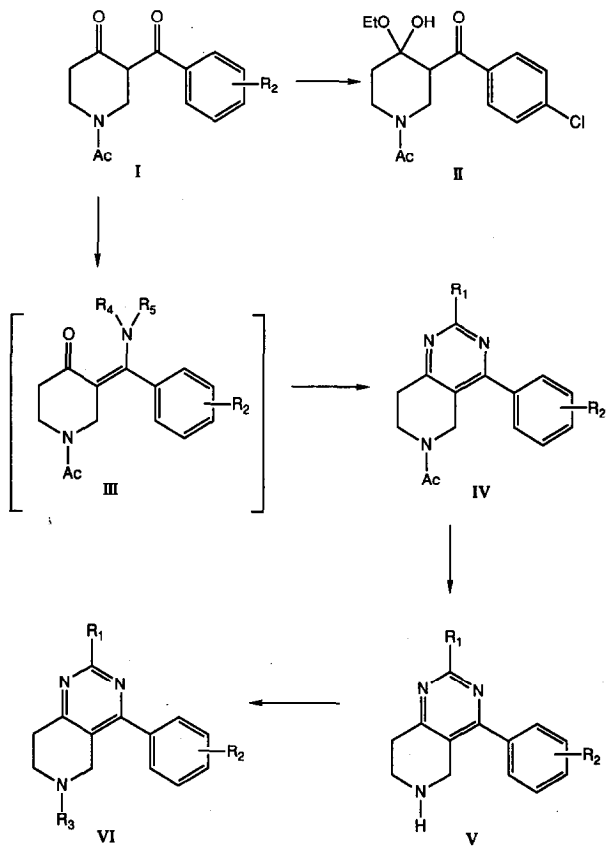
[a]All compounds obtained as solids exhibited satisfactory (± 0.4%) elemental analysis and as oils characterized by MS and ^1H NMR. [b]Recrystallized from ethyl ether/hexanes

with substituted amidines in the presence of sodium ethoxide gave the substituted-6-acetyl-tetrahydropyrido[4,3-*d*]pyrimidine derivatives **IV** in moderate to good yields.

The N-6 acetyl group was removed by treatment of **IV** with aqueous 10% hydrochloric acid at reflux to give **V**. Reaction of **V** with various alkyl halides and sodium hydride in DMF gave the N-6-substituted tetrahydropyrido[4,3-*d*]pyrimidine derivatives **VI**. The various tetrahydropyrido[4,3-*d*]pyrimidines prepared are listed in tables II–IV.

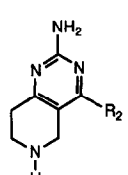
Results and discussion

Histological studies have shown that prostaglandins and other antilesion agents do not prevent the destruction of superficial epithelial gastric mucosal cells in rats even in areas of the stomach without macroscopic lesions [9, 10]. Therefore, in the present study, the term 'antilesion' has been used instead of 'cytoprotection' since this descriptive term does not specify any mechanism of action or imply that all cells are protected from damage. Test compounds were administered orally (*po*), 1 h before oral administration of 50% ethanol or 40 mg/kg aspirin. The effect of the compound in preventing mucosal lesions induced by ethanol or aspirin was determined 1 h later. Total prevention of mucosal (or intramucosal) gastric bleeding was used as measure of



Scheme 1.

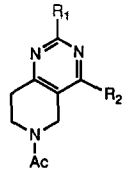
Table III. Substituted tetrahydropyrido[4,3-*d*]pyrimidines **V**.



Compd	R_2	*Empirical formula* [a]	*mp, °C*	*Yield (%)*
Va	4-ClPh	$C_{13}H_{13}ClN_4 \cdot 2HCl$	283–285	91
Vb	4-FPh	$C_{13}H_{13}FN_4 \cdot 2HCl$	287 (dec)	75
Vc	Ph	$C_{15}H_{18}N_4 \cdot H_2O$	132–134	85

[a]All compounds exhibited satisfactory (± 0.4%) elemental analysis except where noted

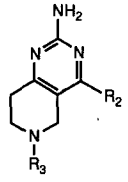
Table II. Various *N*-acetyl-tetrahydropyrido[4,3-*d*]pyrimidines **IV**.



Compd	R_1	R_2	*Empirical formula* [a]	*mp (°C)*	*Yield (%)*	*Method* [b]
IVa	CH_3	4-ClPh	$C_{16}H_{16}ClN_3O$	139–141	76	A
IVb	Ph	4-ClPh	$C_{21}H_{18}ClN_3O$	182–184	40	A
IVc	Ph	4-FPh	$C_{21}H_{18}FN_3O{\cdot}H_2O$	265 (dec)	36	B
IVd	PhNH	4-FPh	$C_{21}H_{19}FN_4O{\cdot}H_2O$	243 (dec)	45	B
IVe	EtNH	4-FPh	$C_{17}H_{19}FN_4O$	136–138	86	B
IVf	NH_2	4-ClPh	$C_{15}H_{15}ClN_4O$	218–220	82	A
IVg	NH_2	4-FPh	$C_{15}H_{15}FN_4O{\cdot}H_2O$	205–207	39	B
IVh	NH_2	3-FPh	$C_{15}H_{15}FN_4O$	185–187	45	B
IVi	NH_2	3-ClPh	$C_{15}H_{15}ClN_4O$	178–180	49	A
IVj	NH_2	Ph	$C_{15}H_{16}N_4O{\cdot}H_2O$	294 (dec)	76	B
IVk	NH_2	3,4-Cl_2Ph	$C_{15}H_{14}Cl_2N_4O$	232–234	52	A
IVl	NH_2	4-CH_3Ph	$C_{16}H_{18}N_4O{\cdot}H_2O$	199–200	35	B
IVm	NH_2	4-CH_3OPh	$C_{16}H_{18}N_4O_2{\cdot}HCl{\cdot}H_2O$	164–166	41	B
IVn	NH_2	2-thienyl	$C_{13}H_{14}N_4OS$	235–237	56	B
IVo	NH_2	4-pyridyl	$C_{14}H_{15}N_5O$	227–229	44	B
IVp	NH_2	3-pyridyl	$C_{14}H_{15}N_5O$	188–190	51	B

[a]All compounds exhibited satisfactory (± 0.4%) elemental analysis except where noted. [b]Methods as described in *Experimental protocols*

Table IV. Various N-substituted-tetrahydropyrido[4,3-*d*]pyrimidines **VI**.



Compd	R_2	R_3	*Empirical formula* [a]	*mp (°C)*	*Yield (%)*
VIa	4-ClPh	CH_3	$C_{14}H_{15}ClN_4$	172–175	11
VIb	4-ClPh	Et	$C_{15}H_{17}ClN_4{\cdot}2HCl$	295 (dec)	79
VIc	4-FPh	Et	$C_{15}H_{17}FN_4{\cdot}2HCl$	242–243	48
VId	4-ClPh	Propyl	$C_{16}H_{19}ClN_4{\cdot}2HCl$	244–246	51
VIe	4-FPh	Propyl	$C_{16}H_{19}FN_4{\cdot}2HCl$	239–241	55
VIf	4-ClPh	Allyl	$C_{16}H_{17}ClN_4{\cdot}2HCl$	248–250	39
VIg	4-FPh	Allyl	$C_{16}H_{17}FN_4{\cdot}2HCl$	241–243	59
VIh	4-ClPh	CH_2Ph	$C_{20}H_{19}ClN_4$	138–141	47

[a]All compounds exhibited satisfactory (± 0.4%) elemental analysis except where noted

gastric antilesion activity. Use of these criteria for gastric antilesion activity rather than attempting to quantify a reduction in lesion area may be a better indication of whether a drug is preventing breakage of the gastric mucosal barrier. This ability may also correspond more closely to the goal of clinical therapy, *ie* prevention of gastroduodenal lesions. The use of 50% ethanol as the gastric irritant, as opposed to the more noxious level of 100% ethanol, permits sensitive, dose-related observations of this type of antilesion activity. Previous studies have shown that 50% ethanol will cause mucosal bleeding in 100% of control rats [11].

Derivatives in this study were found to possess gastric antilesion activity against ethanol and aspirin-induced lesions in rats. These results are summarized in table V.

The variation of substituents at the 2-position, while maintaining an acetyl group on the piperidine nitrogen and either a 4-chlorophenyl or 4-fluorophenyl group in the 4-position of the tetrahydropyrido[4,3-*d*]pyrimidine ring system was first studied (table II). The presence of a small basic group at the 2-position appears necessary for good activity against ethanol-induced lesions in rats. Methyl (**IVa**) or anilino (**IVd**) substitution does not show significant activity. A phenyl group (**IVb, c**) eliminates activity against ethanol-induced lesions. An ethyl substituent on the 2-amino group (**IVe**), as well as an unsubstituted amine group at the 2-position (**IVf, g**) exhibits complete inhibition against ethanol-induced lesions at the screening dose of 10 mg/kg. Evaluation of **IVe–g** for their ability to inhibit aspirin-induced lesions shows **IVe** to be much less active against aspirin-induced compared to ethanol-induced lesions in rats. Compounds **IVf** and **IVg** exhibit the best antilesion activity against both ethanol- and aspirin-induced lesions and are equipotent in both assays.

Table V. Antilesion activity against ethanol- and aspirin-induced lesions.

Example	*EtOH-induced lesions* [a]		*Aspirin-induced lesions* [b]	
	% Inhibition at 10 mg/kg po	*ED_{50} (mg/kg po) (95% conf limits)*	*% Inhibition at 10 mg/kg po*	*ED_{50} (mg/kg po) (95% conf limits)*
VIa	20	–	–	–
IVb	0	–	–	–
IVc	0	–	–	–
IVd	10	–	–	–
IVe	100	5.0 (3.0–7.8)	80[d]	–
IVf	100	7.7 (3.1–13.5)	100	9.1 (4.8–13.3)
IVg	100	4.6 (2.8–7.5)	100	8.7 (4.2–13.4)
IVh	100	2.5 (1.7–5.2)	35	–
IVi	100	3.2 (2.0–5.1)	30[c]	–
IVj	100	2.3 (2.2–7.4)	100	9.5 (4.0–18.4)
IVk	100	–	20[d]	–
IVl	0	–	–	–
IVm	0	–	–	–
IVn	20	–	–	–
IVo	100	–	30[d]	–
IVp	90	–	15[d]	–
Va	40	–	–	–
Vb	100	3.2 (1.0–4.8)	20[d]	–
Vc	80	–	20	–
VIa	100	7.2 (4.8–11.0)	60[c]	–
VIb	100	3.2 (1.3–5.3)	100	4.5 (2.2–7.4)
VIc	100	3.7 (0.6–6.5)	80[d]	–
VId	100	2.7 (1.6–5.4)	70[d]	–
VIe	100	2.4 (1.1–3.8)	60[d]	–
VIf	100	7.9 (4.6–14.0)	100	4.4 (3.1–6.6)
VIg	100	5.0 (2.0–12.8)	75[d]	–
VIh	20[c]	–	–	–
Rioprostil	100	1.9 (1.7–2.2) μg/kg	100	1.2 (0.1–2.2) μg/kg

[a]Inhibition against 50% EtOH-induced intramucosal lesions with test compound dose of 10 mg/kg. [b]Inhibition against 40 mg/kg aspirin-induced intramucosal lesions with test compound dose of 10 mg/kg. [c]Test compound dose of 20 mg/kg. [d]Test compound dose of 40 mg/kg

Substituent effects were then studied at the 4-position of the tetrahydropyrido[4,3-*d*]pyrimidine ring system while maintaining the desired unsubstituted amino group in the 2-position (**IVh–p**). A fluoro- or chlorophenyl group whether substituted in the *para* (**IVf, g**) or *meta* (**IVh, i**) position, or disubstituted (**IVk**) produce good activity against ethanol-induced lesions. The unsubstituted phenyl system (**IVj**) is also equipotent to the halo-substituted phenyls against ethanol-induced lesions. However, electron-donating groups such as methyl or methoxy on the phenyl ring (**IVl, m**) reduce activity. In addition, substituting an electron-rich heterocycle such as thiophene (**IVn**) in the 4-position of the pyrimidine ring diminishes antilesion activity. In contrast, an electron-deficient heterocycle such as pyridine (**IVo, p**) maintains interesting levels of antilesion activity. Evaluation of the more potent compounds for their ability to inhibit aspirin-induced lesions shows **IVf, g** and **IVj** to be equipotent against aspirin- and ethanol-induced lesions in rats.

Substituent effects on the tetrahydropyridine nitrogen (N-6) were examined next (**Va–c, VIa–h**). Removal of the acetyl group to give the secondary amine (**Va–c**) maintains good activity against ethanol-induced lesions while alkyl substitution with methyl, ethyl, propyl and allyl groups (**VIa–g**) enhance the potency against ethanol-induced lesions. However, when the steric bulk at the 6-position is increased such as with a benzyl group (**VIh**), antilesion activity is diminished. While these compounds show equally interesting activity against ethanol-induced lesions, evaluation of the 6-alkyl substituted compounds for inhibition of aspirin-induced lesions shows a distinction between compounds that have a 4-fluoro and 4-chloro-substituted phenyl group on the tetrahydropyrido[4,3-*d*]pyrimidine ring system. The 4-chlorophenyl compounds (**VIb, VIf**) were more potent than the 4-fluorophenyl compounds (**VIc, VIg**) against aspirin-induced lesions in rats. The more potent compounds of the study are **VIb** and **VIf** that have an oral ED_{50} of 3.2 and 7.9 mg/kg against ethanol-induced lesions and an oral ED_{50} of 4.5 and 4.4 mg/kg against aspirin-induced lesions, respectively. The prostaglandin derivative rioprostil was used as a standard in this assay and shown to have an oral ED_{50} of 1.9 (1.7–2.2) and 1.2 (0.1–2.2) μg/kg against ethanol- and aspirin-induced lesions [12].

To evaluate the gastric antilesion specificity of these compounds, a selected group of the more potent tetrahydropyrido[4,3-*d*]pyrimidines was evaluated for gastric antisecretory activity in an *in vitro* antisecretory activity in rabbit isolated parietal cell assay (table VI). Unlike the prostaglandins, these compounds possess no *in vitro* antisecretory activity in rabbit parietal cells stimulated with histamine up to a screening dose of 100 μM. Rioprostil and cimetidine, a potent gastric antisecretory histamine H_2-receptor antagonist, were used as standards in this assay and shown to have an IC_{50} value of 3.8 (0.4–5.4) μM and 0.64 (0.47–0.81) μM, respectively [12]. These data suggest that the tetrahydropyrido[4,3-*d*]-pyrimidines are selective gastric antilesion agents with no antisecretory activity.

Table VI. Effects on histamine-induced ^{14}C-aminopyrine uptake in rabbit isolated parietal cells.

Compound	*Inhibition of ^{14}C-AP uptake (%)* [a]
IVa	9
IVe	19
IVg	11
IVk	15
Vc	16
VIa	21
VIb	15
VIc	11
Cimetidine	IC_{50} = 0.64 (0.47–0.81) μM
Rioprostil	IC_{50} = 3.8 (0.4–5.4) μM

[a]Inhibition of ^{14}C-AP uptake values at 100 μM concentrations as determined in the rabbit isolated parietal cell preparation (n = 3)

This series of novel tetrahydropyrido[4,3-*d*]pyrimidines represents a new structural class of non-prostaglandin agents possessing gastric antilesion activity. The more potent compounds in this series against both ethanol- and aspirin-induced lesions are **VIb** and **VIf**. These compounds are not as potent as prostaglandins; however, unlike the prostaglandins these compounds are not gastric antisecretory agents. This combination of properties make them a potential alternative for antiulcer therapy.

Experimental protocols

Chemistry

Melting point determinations were done on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infra-red (IR) spectra were obtained on a Perkin-Elmer IR8 and are reported in wave-numbers (cm^{-1}). Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a General Electric QE300 (300 MHz) spectrometer. Chemical shifts are reported in parts per million (δ) downfield relative to tetramethylsilane as standard. Mass spectra (MS) were obtained on a Finningan-MAT model 8230. Combustion analyses are ± 0.4% of theory unless otherwise noted. Compounds in the tables are prepared according to the general procedures described. Physical properties of the compounds are summarized in tables I–IV.

General procedure for the preparation of N-acetyl-3-benzoyl-4-piperidones ***Ia–k***

Compounds **Ia–k** (table I) were synthesized by the representative procedure illustrated for **Ib**.

N-Acetyl-3-(4-chlorobenzoyl)-4-piperidone ***Ib***

A solution of *N*-acetyl-piperidone (50 g, 355 mmol) and morpholine (34 ml, 390 mmol) in benzene (400 ml) in the presence of catalytic amount of *p*-toluene sulfonic acid (100 mg) was refluxed under Dean-Stark conditions for 12 h. The excess morpholine and benzene was removed by vacuum distillation. The crude morpholine enamine was taken up in methylene chloride (150 ml) and cooled to 0°C. Triethylamine (50 ml, 355 mmol) was added followed by dropwise addition of 4-chlorobenzoyl chloride (16.5 ml, 130 mmol) and allowed to warm to room temperature overnight. The resulting mixture was treated with 5% hydrochloric acid (100 ml) and stirred at room temperature for 30 min. The organic layer was separated, washed with water, dried (sodium sulfate) and evaporated. Purification by flash chromatography (silica gel, methylene chloride as eluent) gave 32 g (33%) of *N*-acetyl-3-(4-chlorobenzoyl)-4-piperidone **Ib** as a yellow solid, mp 96–98°C (ethyl ether/hexane); IR (KBr): 1735, 1690, 1610, 1585 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.83 (d, J = 8 Hz, 2H), 7.77 (d, J = 8 Hz, 2H), 4.49 (m, 2H), 4.00–3.71 (m, 3H), 2.51 (m, 2H), 2.11 (s, 3H).

N-Acetyl-3-(4-chlorobenzoyl)-4-ethoxy-4-hydroxy-piperidine ***II***

A solution of *N*-acetyl-3-(4-chlorobenzoyl)-4-piperidone (2.4 g, 8.6 mmol), acetamidine hydrochloride (0.81 g, 8.6 mmol) in ethanol (40 ml) was stirred at room temperature for 1 h. The ethanol was removed *in vacuo* and the resulting oil was purified by flash chromatography (silica gel; 20% acetone, methylene chloride) to give 1.5 g (54%) of the title compound as an oil, MS m/z 326 (MH^+); IR (KBr): 3150, 1730, 1690, 1570 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.91 (m, 2H), 7.46 (m, 2H), 4.13 (q, 2H), 3.69 (m, 4H), 3.29 (m, 2H), 2.61 (t, 2H), 2.13 (s, 3H), 1.23 (q, 2H).

Procedure A

6-Acetyl-4-(4-chlorophenyl)-2-methyl-5,6,7,8-tetrahydropyrido[4,3-d]-pyrimidine ***IVa***

A solution of *N*-acetyl-3-(4-chlorobenzoyl)piperidone (30.7 g, 100 mmol) and morpholine (10 ml, 100 mmol) in benzene (400 ml) was heated at reflux under Dean-Stark conditions for 5 h. The solution was cooled to room temperature and the resulting precipitate was collected by filtration, washed once with ethyl ether and dried *in vacuo* to give 20.3 g (58%) of the corresponding morpholine-enamino ketone as a yellow solid, mp 163–166°C.

To a solution of sodium (1.3 g, 56.5 mmol) in ethanol (200 ml) was added the morpholine-enamino ketone (10 g, 28.7 mmol) and acetamidine hydrochloride (R_1 = CH_3, 5.4 g, 57 mmol). The mixture was heated to reflux for 3 h, then cooled to room temperature overnight. The resulting precipitate was collected by filtration, washed with water and dried *in vacuo* to give **IVa** (6.5 g, 76%) as a white solid: mp 139–141°C; MS m/z 302 (MH^+); IR (KBr): 1685, 1590 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.65 (m, 4H), 4.58 (s, 2H), 3.61 (t, J = 2.1 Hz, 2H), 2.83 (t, J = 2.1 Hz, 2H), 2.63 (s, 3H), 2.10 (s, 3H). Anal $C_{16}H_{16}ClN_3O$ (C, H, N).

Procedure B

2-Amino-6-acetyl-4-(4-fluorophenyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine ***IVg***

A solution of *N*-acetyl-3-(4-fluorobenzoyl)piperidone (77.8 g, 290 mmol) and pyrrolidine (28 ml, 330 mmol) in toluene (500 ml) was stirred at room temperature affording a solid after 2–3 h. The resulting precipitate was collected by filtration, recrystallized from methylene chloride-ethyl ether and dried *in vacuo* to give 42.3 g (44%) of the corresponding pyrrolidine-enamino ketone as a yellow solid, mp 163–165°C.

To a solution of sodium (1.4 g, 60.9 mmol) in ethanol (200 ml) was added the pyrrolidine-enamino ketone (10 g, 30.1 mmol) and guanidine hydrochloride (R_1 = NH_2, 5.5 g, 57 mmol). The mixture was heated to reflux for 3 h, then cooled to room temperature overnight. The resulting precipitate was collected by filtration, washed with water and dried *in vacuo* to give **IVg** as a white solid: mp 205–207°C; MS m/z 287 (MH^+); IR (KBr): 3365, 3330, 3186, 1641, 1631 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.61 (m, 2H), 7.36 (m, 2H), 6.56 (s, 2H), 4.44 (s, 2H), 3.74 (t, J = 2.1 Hz, 2H), 2.81 (t, J = 2.1 Hz, 2H), 2.04 (s, 3H). Anal $C_{15}H_{15}FN_4O{\cdot}H_2O$ (C, H, N).

General procedure for the preparation of 2-amino-4-phenyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidines ***Va–c***

Compounds **Va–c** (table III) were synthesized by the representative procedure illustrated for **Va**.

2-Amino-4-(4-chlorophenyl)-5,6,7,8-tetrahydropyrido[4,3-d]-pyrimidine ***Va***

A mixture of **IVf** (5.0 g, 16.5 mmol) in aqueous 10% hydrochloric acid (60 ml) was heated to reflux for 12 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The resulting solid was triturated with methanol and collected by filtration to give **Va** as a white solid (3.9 g, 91%): mp 283–285°C; MS m/z 261 (MH^+); IR (KBr) 3326, 2760, 2433, 1668 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.83 (broad s, 2H), 7.63 (m, 4H), 4.08 (m, 2H), 3.41 (m, 2H), 3.05 (t, J = 2.1 Hz, 2H). Anal $C_{13}H_{13}ClN_4{\cdot}2HCl$ (C, H, N).

General procedure for the preparation of N-substituted-2-amino-4-phenyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidines ***VIa–h***

Compounds **VIa–h** (table IV) were synthesized by the representative procedure illustrated for **VIb**.

2-Amino-4-(4-chlorophenyl)-6-ethyl-5,6,7,8-tetrahydropyrido-[4,3-d]pyrimidine ***VIb***

To a suspension of sodium hydride (0.74 g, 18.5 mmol), washed three times with pentane, in dimethylformamide (40 ml) was added **Va** (2.4 g, 9.3 mmol). The mixture was stirred at room temperature for 2 h and iodoethane (0.8 ml, 9.3 mmol) was added. The mixture was stirred at room temperature overnight, quenched with water and the resulting oil was chromatographed (silica gel; acetone). The hydrochloride salt was prepared with concentrated hydrochloric acid in methanol to give **VIb** as a white solid, (2.1 g, 79% yield): mp 295 (dec) °C; MS m/z 289 (MH^+); IR (KBr) 3224, 2641, 2311, 1623 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.63 (m, 6H), 4.32–4.11 (m, 2H), 3.70–3.08 (m, 6H), 1.25 (t, J = 2.4 Hz, 3H). Anal $C_{15}H_{17}ClN_4{\cdot}2HCl$ (C, H, N).

Biology

Gastric antilesion assay

Male, Charles River rats weighing between 140–220 g were fasted overnight, but allowed water *ad libitum*. They were, however, deprived of water during the experiment. The rats were weighed and pretreated orally with the test compound

suspended in 0.5% carboxymethyl-cellulose (CMC) (15 cps) in a dose volume of 1 ml/kg. 1 h later, 1 ml of 50% ethanol or 40 mg/kg, *po*, of aspirin also suspended in CMC was administered orally to each animal. After an additional hour the rats were asphyxiated with CO_2, the stomachs removed, opened along the greater curvature and layed out on a flat surface. The presence of mucosal bleeding was noted and, after wiping off the mucosa, the presence of submucosal bleeding sites were also noted. The incidence of lesions in the mucosa and the intramucosa were statistically analyzed by the Chi-squares method using Yates correction [13]. ED_{50} values were estimated using probit analysis [14].

Rabbit isolated parietal cell assay

Inhibition of acid secretion was measured indirectly *in vitro* using a rabbit isolated parietal cell preparation [15]. Parietal cells were isolated from the fundic mucosa of rabbit stomachs by a four-stage collagenase digestion process. The supernatant fraction from the last two stages contained the individual parietal cells. The cell suspension was centrifuged and reconstituted in a modified Hanks' buffer to contain 2–3 x 10^6 cells/ml. The cells were then tested for their ability to accumulate amino[^{14}C]pyridine (^{14}C-AP). Parietal cells were incubated with 0.12 μCi of ^{14}C-AP, 10^{-6} M histamine, 10^{-5} M isobutyl-methyl-xanthine, and test compound in 0.025 ml of Hanks' buffer (dimethyl sulfoxide final concentration = 0.25%) with a final incubation volume of 1.0 ml. The flasks were incubated at 37°C, aliquots taken and cell pellets were collected by centrifugation. Pellets were solubilized with Protosol (New England Nuclear), and radioactivity was determined using liquid scintillation spectrometry. Data are expressed as the concentration of drug required to inhibit the histamine response. The data reported are the mean of three experiments with confidence limits ± 15%.

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