Eur J Med Chem (1990) 25, 217-226
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Original paper

Isocytosine H_2-receptor histamine antagonists. III. The synthesis and biological activity of lupitidine (SK&F 93479) and related compounds

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(Received 18 May 1989; accepted 24 July 1989)

Summary — A series of 2-[2-(5-dimethylaminomethyl-2-furanylmethylthio)ethylamino]-4-pyrimidones is described containing a variety of substituents in the 5-position of the pyrimidone (isocytosine) ring. Good H_2-receptor histamine antagonist activity is retained over a range of basic and neutral, polar and non-polar substituents and the majority of compounds are selective for the H_2-receptor. Modifications have also been made to the furan ring and *N,N*-dimethylaminomethyl substituent and structure-activity observations are discussed. Compound **(16f)**, 2-[2-(5-dimethylaminomethyl-2-furanylmethylthio)ethylamino]-5-(6-methyl-3-pyridylmethyl)-4-pyrimidone (SK&F 93479, lupitidine), a compound which shows an extended duration of anti-secretory activity in animal models, was selected for clinical investigation.

Résumé — Dérivés de l'isocytosine antagonistes des récepteurs H_2 de l'histamine. III. Synthèse et activité biologique de la lupitidine (SK&F 93479) et de composés apparentés. *Une série de 2-[2-(5-diméthylaminométhyl-2-furanylméthylthio)éthylamino]-4-pyrimidones contenant une variété de substituants en position 5 du cycle pyrimidone (isocytosine) est décrite. Un bon pouvoir inhibiteur du récepteur H_2 à l'histamine est maintenu pour une sélection de substituants basiques et neutres, polaires et non polaires et la plupart des composés ont une action sélective pour le récepteur H_2.*
Le noyau furane et le substituant N,N-*diméthylaminométhyle ont également subi quelques modifications et les relations structure–activité sont discutées. Le composé **(16f)**, soit 2-[2-(5-diméthylaminométhyle-2-furanylméthylthio)éthylamino]-5-(6-méthyl-3-pyridylméthyl)4-pyrimidone (SK&F 93479, lupitidine), qui a manifesté une action anti-sécrétoire plus prolongée sur modèle animal, a été choisi pour les études cliniques.*

H_2-receptor antagonists / Mannich bases / furans / thiophenes / 2-amino-4-pyrimidones (isocytosines) / lupitidine (SK&F 93479)

Introduction

The success of cimetidine (**1**) in the treatment of peptic ulcer disease and other hypersecretory aliments has promoted a great deal of research interest in H_2 receptor histamine antagonists.

In our laboratories a number of alternative, neutral, dipolar systems were successfully introduced as replacements for the cyanoguanidine group of cimetidine including nitrodiaminoethene (*eg* **2**) and 2-amino-4-pyrimidone (*eg* **3**). We recently described further developments [1, 2] in the area of 2-amino-4-pyrimidone (isocytosine) H_2-antagonists, including the discovery of the clinically effective agent oxmetidine (**4**) and compound (**5**).

Modification of the imidazole ring terminal of cimetidine has also been extensively explored and reviewed [3] and work in this area led to the discovery [4] at Glaxo laboratories of ranitidine (**6**), in which the imidazole ring of (**2**) is replaced by the *N,N*-dimethylamino-methylfuranyl moiety. Cimetidine and ranitidine are today the most widely used agents in the therapy of hypersecretory states.

In this paper we describe the synthesis and biological activity of a series of compounds derived by replacing the imidazole ring of (**3**)–(**5**) and other

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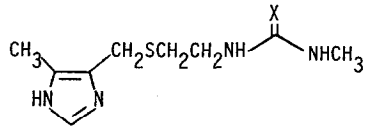
Table I. Chemical characteristics of new isocytosines in tables II and III.

Compound No	*Molecular Formula*[a]	*Solvent of crystallisation*	*mp (°C)*	*Yield*[b] *(%)*	*Route of synthesis by scheme*	*PMR δ (ppm)*
16a	$C_{14}H_{20}N_4O_2S{\cdot}2C_4H_4O_4$[c]	EtOH	113–116	76	1 (pyridine)	2.85(m, 2H), 2.87(s, 6H), 3.69(m, 2H), 3.87(s, 2H), 4.33 (s, 2H), 6.01(d, 1H), 6.34(s, 2H), 6.39(d, 1H), 6.64(d, 1H), 7.64(d, 1H) in D_2O.
16b	$C_{22}H_{26}N_4O_4S{\cdot}2HCl$	MeOH/EtOH	188–191	73	2	2.93(s, 6H), ~2.9(m, 2H), 3.62(s, 2H), 3.67(m, 2H), 3.93(s, 2H), 4.35(s, 2H), 6.00(s, 2H), 6.46(d, 1H), 6.66(d, 1H), 6.86(m, 3H), 7.56(s, 1H) in D_2O.
16c	$C_{21}H_{25}ClN_4O_2S{\cdot}2C_4H_4O_4$	MeOH/iso-PrOH	105–107	26	1 (pyridine)	2.99(s, 6H), ~3.0(m, 2H), ~3.7(m, 2H), ~3.7(s, 2H), 3.94(s, 2H), 4.41(s, 2H), 6.5(s, 2H), 6.50(d, 1H), 6.73(d, 1H), 7.33(m, 4H), 7.60(s, 1H) in D_2O.
16d	$C_{21}H_{26}N_4O_3S{\cdot}2C_4H_4O_4$[d]	MeOH/iso-PrOH	147–148	85	1 (fusion)	2.62(m) and 2.72(s) obscured by DMSO–d_5, 3.40(s, 2H), ~3.4(m, 2H), 3.81(s, 2H), 4.33(s, 2H), 6.14(s, 2H), 6.38(d, 1H), 6.60(d, 1H), 6.65(m, 2H), 7.00(m, 2H), 7.41(s, 1H) in DMSO–d_6.
16e	$C_{20}H_{25}N_5O_2S{\cdot}3HCl$[e]	MeOH	205–208	73	2	2.91(s, 6H), 2.95(t, 2H), 3.67(t, 2H), 3.92(s, 2H), 4.01(s, 2H), 4.40(s, 2H), 6.46(d, 1H), 6.72(d, 1H), 7.83(s, 1H), 8.07(d of d, 1H), 8.59(m, 1H), 8.75(m, 2H) in D_2O.
16f	$C_{21}H_{27}N_5O_2S{\cdot}3HCl$	MeOH/EtOH	212–214	88	1,2,3 (yield for 1 in pyridine)	[2.79(s), 2.9(s), ~2.9(m), 11H], 3.65(m, 2H), [3.91(s), 3.90(s), 4H], 4.39(s, 2H), 6.44(d, 1H), 6.70(d, 1H), 7.77(s, 1H), 7.85(d, 1H), [8.38(m), 8.56(m), 2H] in D_2O.
16g	$C_{20}H_{25}N_5O_2S{\cdot}3HCl{\cdot}0.5H_2O$	MeOH/EtOH	194–197	38	1 (pyridine)	[2.89(s), 2.9(m), 8H], 3.65(m, 2H), [3.90(s), 4.08(s), 4H], 4.38(s, 2H), 6.44(d, 1H), 6.69(d, 1H), [7.83(s), 7.97(m), 3H], 8.72(m, 2H) in D_2O.
16h	$C_{20}H_{25}N_5O_2S{\cdot}3HCl{\cdot}H_2O$[f]	MeOH/iso-PrOH	208–210	58	1 (pyridine)	[2.89(s), 2.88(m), 8H], 3.63(m, 2H), 3.91(s, 2H), 4.20(s, 2H), 4.38(s, 2H), 6.43(d, 1H), 6.69(d, 1H), [7.89(s), 7.95(m), 3H], [8.55(m), 8.70(m), 2H] in D_2O.
16i	$C_{24}H_{27}N_5O_2S{\cdot}3HCl{\cdot}0.4H_2O$	EtOH/iso-PrOH	228–230	25	1 (pyridine)	[2.82(s), 2.82(s), 8H], 3.68(m, 2H), 3.93(s, 2H), 4.16(s, 2H), 4.40(s, 2H), 6.46(d, 1H), 6.70(d, 1H), 7.90(s, 1H), 7.9–8.4(m, 4H), [9.06(m), 9.16(m), 2H] in D_2O.
16j	$C_{19}H_{24}N_4O_2S_2{\cdot}2HCl$	MeOH/iso-PrOH	154 (dec)	29	1 (pyridine)	[2.84(s), 2.88(m), 8H], 3.60(m, 2H), [3.88(s), 3.91(s), 4H], 4.23(s, 2H), [6.40(d), 6.57(d), 2H], 7.05(m, 2H), 7.55(m, 1H), 7.6(s, 1H) in D_2O.
16k	$C_{19}H_{24}N_4O_3S{\cdot}2HCl$	EtOH	187–189	43	2	[2.86(s), 2.9(s), 8H], 3.60(m, 2H), 3.73(s, 2H), 3.88(s, 2H), 4.28(s, 2H), 6.25–6.6(m, 4H), [7.39(s), 7.55(s), 2H] in D_2O.
16l	$C_{22}H_{29}N_5O_2S{\cdot}3HCl{\cdot}1.2H_2O$	MeOH/EtOH	221–224	36	2	2.47(s, 3H), 2.71(s, 3H), [2.90(s), 2.90(m), 8H], 3.66(m, 2H), [3.90(s), 3.91(s), 4H], 4.40(s, 2H), 6.45(d, 1H), 6.70(d, 1H), 7.77–8.40(broad, 3H) in D_2O.
16m	$C_{20}H_{25}N_5O_3S$	oil (solidified with Et_2O)	50– 55	26	2	2.11(s, 6H), [2.62(m) + DMSO–d_5], [3.77(s), ~3.5(s), 6H], 3.78(s, 2H), 6.19(d of d, 2H), 6.57(br m, 1H), 7.3(m, 2H), 7.66(s, 1H), 8.0–8.15(m, 2H) in DMSO–d_6.
16n	$C_{21}H_{27}N_5O_3S{\cdot}3HCl{\cdot}0.5H_2O$[g]	EtOH	176–179	70	2	2.61(s, 3H), [~2.9(m), 8H], [3.62(t), 3.81(s), 3.89(s), 6H], 4.38(s, 2H), 6.43(d, 1H), 6.67(d, 1H), ~7.7(m, 3H), 8.43(d, 1H) in D_2O.
16o	$C_{20}H_{25}N_5O_3S{\cdot}3HCl$	MeOH	192–194 (dec)	70	2	[2.89(s), 2.9(t), 8H], 3.46(t, 2H), [~3.9(s), 4H], 4.39(s, 2H), 6.44(d, 1H), 6.68(d, 1H), [7.68–7.74(3H)], 8.42(d, 2H) in D_2O.

Table I. *(Continued).*

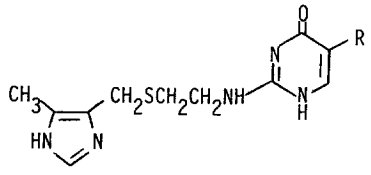
Compound No	*Molecular Formula* [a]	*Solvent of crystallisation*	*mp (°C)*	*Yield* [b] *(%)*	*Route of synthesis by scheme*	*PMR δ (ppm)*
17a	$C_{18}H_{20}N_4O_2S$	iso-PrOH/H_2O	166–168	83	2	[2.40(s), 2.62(t) + DMSO–d_5], [3.4(s), 3.50(s), 4H], 3.80(s, 2H), 6.29(d, 1H), 6.39(d, 1H), 6.5(broad t, 1H), 7.12(d, 1H), [7.57–7.82(3H)], 8.33(d, 1H) in DMSO–d_6.
17b	$C_{23}H_{29}N_5O_2S$	EtOAc	118–120	31	3	1.63(m, 4H), [2.40–2.6, 9H + DMSO–d5], [3.39–3.50, 6H], 3.77(s, 2H), 6.16 (ABq, 2H), 6.51(br t, 1H), 7.11(d, 1H), [7.51(d of d), 7.56(s), 2H], 7.56(d, 1H) in DMSO–d_6.
17c	$C_{24}H_{31}N_5O_2S$	EtOAc	136–137	30	3	~1.4(m, 6H), [2.31(t), 2.39(s), 2.64(t) + DMSO–d_5], [3.4(m), 3.37(s), 3.48(s), 6H], 3.86(s, 2H), [6.1–6.4(m), 3H], 7.09(d, 1H), [7.47(q), 7.50(s), 2H], 8.3(d, 1H) in DMSO–d_6.
17d	$C_{23}H_{29}N_5O_3S$	EtOAc	129–130	41	2	[2.34(t), 2.40(s), 2.62(t) + DMSO–d_5], [3.42(s), 3.4–3.5(m), 3.78(s), 12H], 6.2(s, 2H), 6.48(br s, 1H), [7.13–8.33(m), 4H], 10.95(br s, 1H) in DMSO–d_6.
17e	$C_{24}H_{32}N_6O_2S{\cdot}4HCl{\cdot}0.5H_2O$	MeOH/EtOH	224–226	12	3	[2.81(s), 2.91(t), 5H], 3.08(s, 3H), ~3.80(m, 14H), 4.58(s, 2H), 6.48(d, 1H), 6.77(d, 1H), [7.78(s), 7.84(d), 2H], [8.41(d of d), 8.59(d), 2H] in D_2O.
17f	$C_{22}H_{26}F_3N_5O_2S$	EtOAc/Et_2O	115–118	18	2	[2.44(s), 2.50(s), 2.69(m), 8H], 3.03(q, 2H), [3.47(m), 3.61(s), 3.68(s), 3.68(s), 8H], 6.10(m, 2H), 6.58(br, 1H), 7.05(d, 1H), [7.47(m), 7.51(s), 2H], 8.39(m, 1H) in $CDCl_3$.
17g	$C_{22}H_{29}N_5O_2S$	EtOAc	116–118	50	2	2.26(s, 6H), 2.49(s, 3H), ~2.7(m, 6H), [3.40(m), 3.61(s), 3.64(s), 6H], [5.88(d), 5.99(d), 2H], 6.50(br m, 1H), 7.03(d, 1H), [7.41(s), 7.44(d of d), 2H], [8.20(br res), 8.38(d), 2H] in $CDCl_3$.
17h	$C_{19}H_{22}N_4O_3S{\cdot}0.7H_2O$	EtOH/H_2O	80– 82	32	2	[2.4(s), 2.61(t) + DMSO–d_5], 3.47(s, 2H), 3.76(s, 2H), 4.32(d, 2H), 5.19(t, 1H), 6.15–6.18(ABq, 2H), 6.48(br t, 1H), 7.12(d, 1H), [7.50(q), 7.55(s), 2H], 8.32(s, 1H), 10.9(br s, 1H) in DMSO–d_6.
17i	$C_{21}H_{24}N_4O_4S{\cdot}$	iso-PrOH/H_2O	101–104	26	2	1.28(t, 3H), [2.4(s), 2.68(t) + DMSO–d_5], [3.40–3.48, 4H, includes H_2O], 3.90(s, 2H), 4.28(q, 2H), 6.53(d, 2H), [7.12(d), 7.21(d), 2H], [7.51(q), 7.57(s), 2H], 8.33(br s, 1H), 10.96(br s, 1H) in DMSO–d_6.
18a	$C_{21}H_{27}N_5OS_2$	EtOAc	147–149	18	2	2.14(s, 6H), [2.39(s), 2.65(t), + DMSO–d_5], [3.40(m), 3.50(s+s) obscured by HOD], 5.92(s, 2H), [6.40(br), 6.70(m), 4H], 7.02(d, 1H), [7.42(m), 7.44(s), 2H], 8.25(m, 1H) in DMSO–d_6.
18b	$C_{21}H_{27}N_5OS_2{\cdot}3HCl$	MeOH	234–238	28	2	[2.78(s), 2.89(s), 2.91(m), 11H], 3.60(m, 2H), 3.90(s, 2H), 4.15(s, 2H), 4.39(s, 2H), [7.10(d), 7.41(d), 2H], [7.74(s), 7.84(d), 2H], [8.38(d of d), 8.57(d), 2H] in D_2O.

[a]All micro-analytical data for C, H, N, S and Cl^- to within ±0.4% of theory except where noted. [b]Yield in final stage of the synthesis. [c]$C_4H_4O_4$ refers to the maleate salt. [d]N (theory 8.7%, found 8.2%). [e]Cl^- (theory 20.9%, found 20.4%). [f]Cl^- (theory 20.2%, found 19.4%). [g]H (theory 5.7%, found 5.2%).

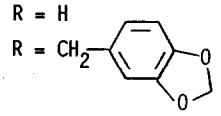


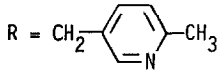
(1), X = N.CN (cimetidine)

(2), X = $CHNO_2$ (SK&F 92456)

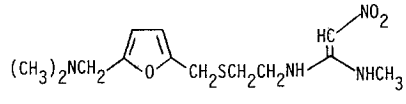


(3), R = H

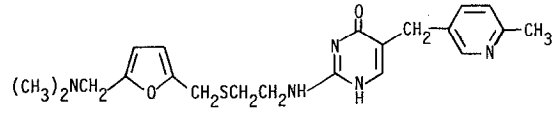
(4), R =  (oxmetidine)

(5), R =  (SK&F 93241)

selected isocytosines [1, 2], with the *N,N*-dimethylaminomethylfuranyl moiety of **(6)**. In addition we describe isocytosines of this type where the *N,N*-dimethylaminomethyl substituent on the furan ring is modified to include alternative dialkylaminomethyl (Mannich) and other groups and some compounds in which the furan ring is replaced by a thiophene ring.



(6), ranitidine



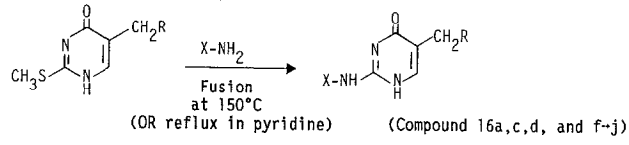
(16f), lupitidine (SK&F 93479)

From this work compound **(16f)** (SK&F 93479, lupitidine) was selected for further evaluation and was shown to be a specific and potent H_2-receptor histamine antagonist and a very effective anti-secretory agent with an extended duration of action relative to cimetidine and ranitidine in animal models and in man.

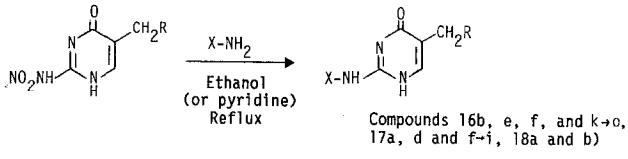
Chemistry

The preparation of the required isocytosines was achieved using one of three routes (schemes 1–3). The first two routes have been described in detail previously [1, 2] and involve nucleophilic displacement with a primary amine of either 2-methylthio (scheme 1) or 2-nitroamino (scheme 2) groups from the appropriate pyrimidone. In the third route (scheme 3) a dialkylaminomethyl substituent was introduced into the furan ring at the 2-position of compound **(17a)**, using standard Mannich-reaction conditions. A further development of this reaction using *bis*-dimethylaminomethane (BDAM) as the Mannich reagent has been outlined [5] for the preparation of **(16f)**. Physical properties and analytical data of all the isocytosines prepared are given in table I.

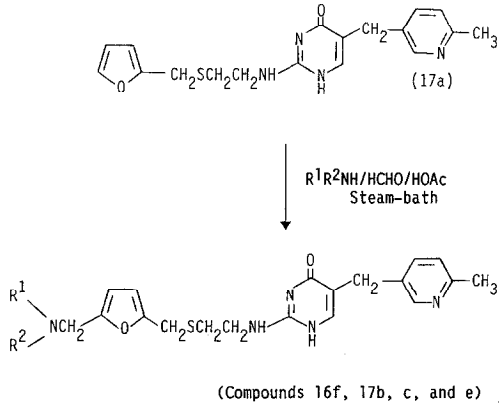
The majority of the amines used in schemes 1 and 2 are known compounds (see *Experimental protocols*).



Scheme 1. Identities of R and amine ($X\text{-}NH_2$) as shown in table II.



Scheme 2. Identities of R and amine ($X\text{-}NH_2$) as shown in table II.



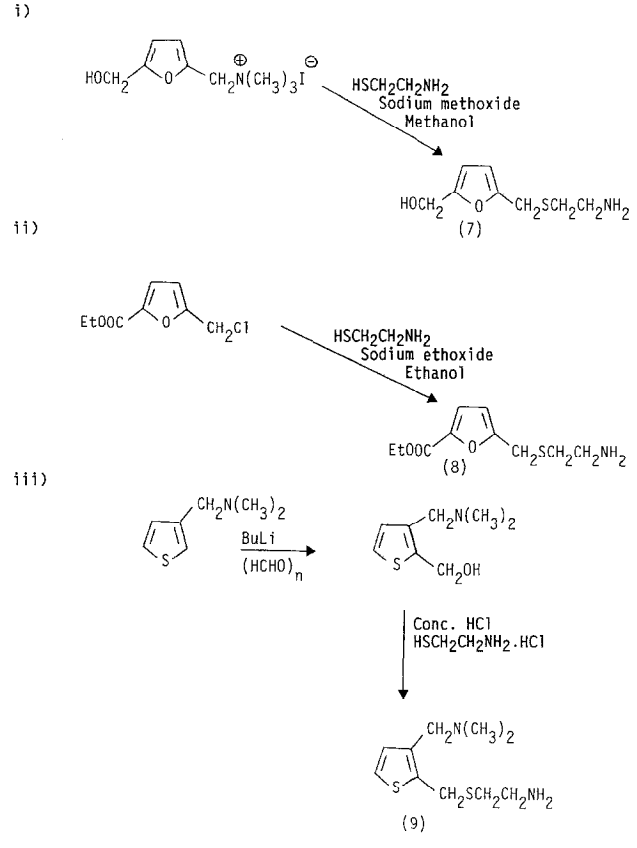
Scheme 3. Identities of R^1 and R^2 as shown in tables II and III as part of the Mannich groups.

The preparations of novel amines are outlined in scheme 4 and described in the *Experimental protocols*. Reaction of cysteamine under basic conditions with 5-hydroxymethylfurfuryltrimethylammonium iodide (scheme 4(i)) and ethyl (5-chloro-methyl)-2-furoate (scheme 4(ii)) led to amines (**7**) and (**8**) respectively. The 2,3-disubstituted thiophene amine (**9**) was prepared by reaction of cysteamine under acidic conditions with 3-dimethylaminomethyl-2-hydroxymethylthiophene, which, in turn, was prepared from 3-dimethyl-aminomethylthiophene using butyl lithium and para-formaldehyde (scheme 4(iii)). The position of hydroxymethylation in the latter reaction was proved by NMR spectroscopy.

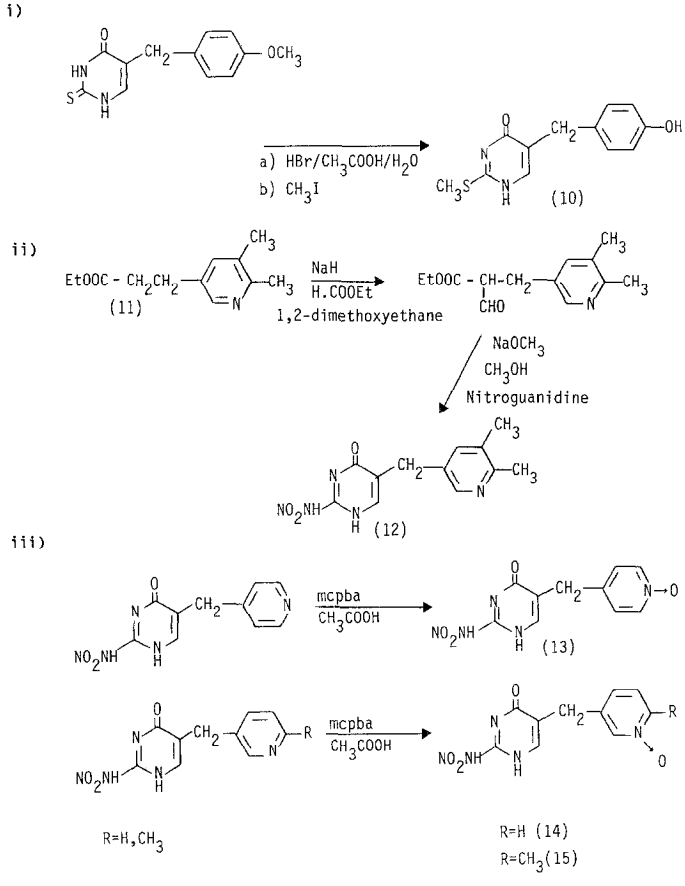
The majority of the isocytosine precursors used in schemes 1 and 2 have previously been described [1, 2]. The preparations of novel compounds of this type are outlined in scheme 5 and described in the *Experimental protocols*. 5-(4-Hydroxybenzyl)-2-methylthiopyrimidin-4-one (**10**) was prepared from 5-(4-methoxybenzyl)-2-thiouracyl [1] by demethylation with conc.HBr/acetic acid, followed by *S*-methylation with methyl iodide (scheme 5(i)). The two transformations were carried out in the same flask, without purification of intermediate, since some *S*-methylated product (**10**) was observed after first stage demethylation, presumably arising from reaction of thione with methyl bromide by-product. 5-(5,6-Dimethyl-3-pyridyl-methyl)-2-nitroaminopyrimidin-4-one (**12**) was prepared from ethyl 3-(5,6-dimethyl-3-pyridyl)propionate (**11**) by formylation with ethyl formate followed by cyclisation with nitroguanidine (scheme 5(ii)). The preparation of the propionate ester (**11**) from simple pyridine precursors has been outlined previously [6]. The pyridine-*N*-oxide intermediates (**13–15**) were prepared by selective oxidation, with *meta*-chloroperbenzoic acid (mcpba) in acetic acid of the appropriate pyridine analogues (scheme 5(iii)).

Pharmacology

Compounds were assayed *in vitro* for H_2-receptor histamine antagonist activity by measurement of the



Scheme 4. Preparation of new amines used in schemes 1 and 2.

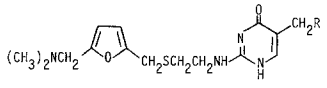


Scheme 5. Preparation of new isocytosine precursors used in schemes 1 and 2.

antagonism of histamine-stimulated tachycardia in the guinea pig isolated right atrial preparation [7] and activities were expressed as pA_2 values when possible. Some of the compounds were slow to reach equilibrium and were difficult to wash out of the tissue.

Anti-secretory activities of the compounds were obtained by measurement of the inhibition of histamine-stimulated gastric acid secretion in the lumen-perfused stomach of the anaesthetised rat after rapid intravenous injection.

Table II. Biological activity of isocytosines containing the side-chain of **(6)**.



Compound No	R	H_2-Antagonist pA_2(slope)[a] (G.P. Atrium)	ED_{50}(rat)[b] (μmol/Kg)	H_1-Antagonist pA_2(slope)[c] (G.P. Ileum)
1)	(Cimetidine)	6.1(0.82)(8')	1.4	-
4)	(Oxmetidine)	*6.9(0.65)(30')	0.09	5.44(1.11)(2')
5)	(SK&F 93241)	7.05(1.15)(30')	0.09	5.49(0.88)(2')
16a	(CH_2R=H)	6.02(1.1)	0.25	-
16b	C_6H_3,3,4-OCH_2O-	7.04(0.81)(8')	0.12	4.99(0.97)
16c	C_6H_4,4-Cl	7.49(0.91)	0.67	-
16d	C_6H_4,4-OH	*8.08(0.63)	0.037	-
16e	3-Pyridyl	7.77(1.02)	0.06	4.65(0.8)
16f	6-Methyl-3-pyridyl	7.78(0.88)	0.14	*4.24(1.5)
16g	4-Pyridyl	7.37(0.83)	0.11	-
16h	2-Pyridyl	*7.90(0.58)	0.08	-
16i	3-Quinolinyl	7.17(1.08)	0.14	5.18(0.74)
16j	2-Thienyl	8.18(0.82)	0.2	5.12(1.17)
16k	2-Furyl	*8.07(0.65)	0.27	*4.77(1.4)
16l	5,6-Dimethyl-3-pyridyl	*8.61(0.55)	0.01	*4.60(1.2)
16m	3-Pyridyl-N-oxide	*7.87(0.74)	0.008	3.5
16n	6-Methyl-3-pyridyl-N-oxide	*7.47(1.39)	0.01	-
16o	4-Pyridyl-N-oxide	7.59(1.09)	0.04	-

[a]Antagonism of histamine-stimulated tachycardia in guinea pig right atrium *in vitro*. Unless otherwise indicated, an equilibration time of 60 min was used in the determination of pA_2 values. [b]Antagonism of histamine-stimulated gastric acid secretion in the lumen-perfused stomach of the anaesthetised rat. Compounds were given by rapid intravenous injection during a near maximal plateau of acid secretion. The ED_{50} is the dose required to produce 50% inhibition and was estimated from the linear regression of log $[I / 100\text{-}I]$ on log dose, where I is the percentage inhibition. [c]Antagonism of histamine-induced contractions of the isolated guinea pig ileum *in vitro*. Equilibration was achieved within 8 min unless stated otherwise and pA_2 values determined. For a) and c), pA_2 values marked * must only be considered approximate figures, since the slope of the Schild plot was significantly different from unity.

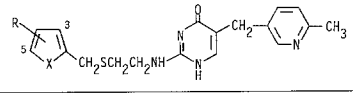
Selected compounds were assayed *in vitro* for H_1-receptor histamine antagonist activity by measurement of the inhibition of histamine-induced contractions of the guinea pig isolated ileum [7] and activities were expressed as pA_2 values.

Results and Discussion

The *in vitro* and *in vivo* H_2-receptor antagonist activities of the compounds under discussion are shown in tables II and III, together with the *in vitro* H_1-receptor antagonist activity of selected compounds. We measured the latter activity because in an earlier series of isocytosines [2] some compounds, particularly those with a heterocyclic ring in the 5-substituent of the isocytosine, had shown substantial H_1-receptor antagonist activity in addition to being potent H_2-receptor antagonists. Relevant activities of cimetidine **(1)**, oxmetidine **(4)** and SK&F 93241 **(5)** are also shown in table II for comparative purposes. Activities in this section refer to the H_2-receptor unless stated otherwise.

The novel compounds in table II all contain the *N*, *N*-dimethylaminomethylfuranyl group present in ranitidine **(6)**. The 5-unsubstituted isocytosine **(16a)** has comparable activity to cimetidine and is about ten

Table III. Biological activity of 5-(6-methyl-3-pyridylmethyl) isocytosines with modified side-chains.



Compound No	R	X	H_2-Antagonist pA_2(slope)[a] (G.P. Atrium)	ED_{50}(rat)[b] (μmol/Kg)	H_1-Antagonist pA_2(slope)[c] (G.P. Ileum)
17a	H	O	6.22(0.89)	6.8	6.03(1.31)
17b	5-CH_2N⟨ring⟩	O	7.49(0.89)	0.27	-
17c	5-CH_2N⟨ring⟩	O	*7.61(0.61)	0.34	-
17d	5-CH_2N⟨ring⟩O	O	5.66(1.11)	5.5	4.6(0.9)
17e	5-CH_2N⟨ring⟩N-CH_3	O	4.64(1.0)	54	-
17f	5-CH_2N(CH_2CF_3)(CH_3)	O	*5.93(0.6)	4.8	-
17g	5-$CH_2CH_2N(CH_3)_2$	O	*7.30(0.53)	0.51	-
17h	5-CH_2OH	O	5.86(1.02)	9.4	-
17i	5-$COOCH_2CH_3$	O	*5.80(0.55)	54	-
18a	5-$CH_2N(CH_3)_2$	S	7.38(0.94)	0.17	-
18b	3-$CH_2N(CH_3)_2$	S	5.38(1.0)	1.5	-

[a], [b] and [c]see footnotes to table II.

times as active as the analogous compound in the original isocytosine series [1] which contained the imidazole ring of **(1)**. This suggests that the 2-[[5-(dimethylamino)-methyl-2-furanyl]methylthio]-ethylamino side-chain imparts high H_2-receptor affinity to these molecules. The 3,4-methylenedioxybenzyl substituted derivative **(16b)** on the other hand has very similar activity to oxmetidine **(4)**. Noteworthy is the very high activity of **(16d)** which contains a polar hydroxy group in the phenyl ring of the 5-benzyl substituent. Compounds with a heterocyclic substituent **(16e–16k)** are all very potent, with activities at a somewhat higher level, particularly *in vitro*, than the analogous compounds in the imidazole series [2]. Thus **(16f)** is more active *in vitro* than **(5)**. Compound **(16l)**, which contains a novel isocytosine substituent, appears to be very potent but produced variable responses both *in vitro* and *in vivo*. Also containing isocytosine moieties not previously described, are the very potent *N*-oxides **(16m–16o)** and again, like **(16d)**, these compounds contain polar substituents in the 5-position of the isocytosine. (This finding of high activity in these polar molecules subsequently led to the discovery of donetidine (SK&F 93574), a potent, selective H_2-antagonist [8] and this compound will be the focus of a future medicinal chemistry publication.)

In view of the high acute toxicity seen in some of the earlier compounds [1, 2] selected analogues were similarly investigated in this series. In general the acute toxicity (LD_{50} (mouse), iv administration) in these compounds seems to be lower than in the earlier series and the order of toxicity is the same within a range of isocytosine 5-substituents. (*eg* **(16f)**, LD_{50} = 200 μmol / kg; **(16e)**, LD_{50} = 90 μmol / kg (TF Walker, unpublished results, measured as described in [1] and [2])). The Mannich group in this series of compounds is more basic (*eg* measured p*K*a of **(16f)** = 8.5 [9]) than the imidazole ring of cimetidine (p*K*a = 6.8 [10]).

Using the 5-(6-methyl-3-pyridylmethyl)isocytosine moiety as a model system, modifications were made to the *N,N*-dimethylaminomethyl substituent in the furan ring and the activities of this series of compounds are shown in table III. Compound **(17a)**, which lacks the basic Mannich substituent at the 5-position of the furan ring, proved to be an important synthetic intermediate (scheme 3) and itself retained some activity. Compounds **(17b–17f)** have modified Mannich groups in the furan ring and only **(17b–17c)**, in which the p*K*a's of these groups will be similar to those in the first series (table II), retain H_2-antagonist activity approaching that of **(16f)**. Lowering the p*K*a of the Mannich group **(17d** and **17f)** reduces activity substantially and adding a second site of protonation distant from the original basic nitrogen **(17e)** essentially removes activity. When the carbon chain is extended **(17g)** activity is retained but non-basic substituents **(17h** and **17i)** are not favoured. These data suggest that the *N,N*-dimethylaminomethyl substituent of **(16f)** is optimised and this is supported by results in the ranitidine series [11].

Table III also shows two compounds in which the furan ring is replaced by a thiophene ring. Compound **(18a)** retains good activity but is slightly less active than the analogous furan **(16f)**. Moving the Mannich group from the 5 to the 3-position of the thiophene ring **(18b)** is not well tolerated.

Selected compounds were tested for antagonism at the histamine H_1-receptor and only **(17a)**, with no basic substituent in the furan ring, did not show high selectivity for the histamine H_2-receptor.

Compound **(16f)** (SK&F 93479, lupitidine) had a good combination of high H_2-receptor antagonist activity and low acute toxicity and was further evaluated in the acutely fistulated anaesthetized cat and in the Heidenhain-pouch dog. The compound was shown [9] to be about 16 times as active as cimetidine in the cat (iv) and the dog (iv and po) and, in addition, it produced a prolonged inhibition of gastric acid secretion, relative to cimetidine or ranitidine, independent of species or route of administration. Lupitidine also proved to be a highly effective inhibitor of nocturnal gastric acid secretion in man [12, 13]. Trials were suspended however, following the discovery of mucosal changes in rats dosed chronically at 1000 mg / kg / day [14]. Similar findings have been found in other long-acting H_2-receptor antagonists and the irreversible proton-pump inhibitor omeprazole [15, 16] and are thought to be a consequence of prolonged inhibition of gastric acid secretion leading to achlorhydria and hypergastrinaemia [17].

Experimental protocols

All new compounds quoted in table I and in this section were fully characterised spectroscopically. NMR spectra were recorded on a JEOL PFT 100P spectrometer using $(CH_3)_4Si$ for reference. Infrared spectra were recorded on a Perkin–Elmer 577 or a Perkin–Elmer 580B spectrophotometer and samples were presented as a Nujol Mull or KBr disc.

Thin-layer chromatograms were run on pre-coated silica-gel 60 F_{254} plates with ethyl acetate:methanol:0.88 NH_4OH, 5:1:1 as mobile phase. Micro-analytical data are within ± 0.4% of theoretical values unless quoted otherwise.

Melting-points were recorded in open capillaries on an electrothermal apparatus and are uncorrected.

Starting materials used in schemes 1 and 2

The preparation of the majority of the 2-methylthio (scheme 1) and 2-nitroamino (scheme 2) pyrimidone precursors has been described previously [1, 2]. The preparation of novel compounds of this type is described in this section.

Many of the side-chain primary amines used in the schemes have been described and were prepared according to literature procedures as indicated below:
2-[(2-Furanyl)methylthio]ethanamine [18].
2-[[5-(Dimethylamino)methyl-2-furanyl]methylthio]ethanamine [19].
2-[[5-(Dimethylamino)ethyl-2-furanyl]methylthio]ethanamine [19].
2-[[5-(4-Morpholinyl)methyl-2-furanyl]methylthio]ethanamine [19].
2-[[5-(Dimethylamino)methyl-2-thienyl]methylthio]-ethanamine [20].
2-[[5-(*N*-2,2,2-Trifluoroethyl-*N*-methylamino)methyl-2-furanyl]methylthio]ethanamine [21].
The preparation of novel amines is described in this section.

General procedures for the preparation of isocytosines [6] (table I)

Scheme 1
An intimate mixture of substituted 2-methylthio-4-pyrimidone (1 equivalent) and primary amine base (1 equivalent) was heated, with stirring, in an oil bath at 145–150°C for 4–5 h. Effluent gases were passed into aqueous sodium hypochlorite. After cooling the reaction mixture was triturated with water to give the required isocytosine as the free base.

Alternatively, the reaction was carried out by heating at reflux temperature in dry pyridine (200 ml pyridine / 0.1 mol) for 24 h. After cooling the pyridine was evaporated at reduced pressure and the residue triturated with water as before.

The product bases were converted to suitable crystalline salts by either a) dissolution in ethanolic hydrogen chloride, evaporation and crystallisation; or b) dissolution in methanol, addition of 1 equivalent of maleic acid for each basic centre in the molecule, evaporation, and crystallisation.

Scheme 2
A mixture of 2-nitroamino-4-pyrimidone (1 equivalent) and primary amine base (1 equivalent) was dissolved in dry absolute ethanol (200 ml / 0.1 mol) and heated under reflux for 24 h. The ethanol was evaporated at reduced pressure and the residue washed with water. Alternatively, refluxing dry pyridine (200 ml / 0.1 mol) could be used as solvent, when 12 h refluxing time was sufficient to complete the reaction.

Purification of isocytosines was achieved by crystallisation of the base or conversion to a suitable salt as described previously.

Scheme 3
To a solution of 2-[2-(2-furanylmethylthio)ethylamino]-5-(6-methyl-3-pyridylmethyl)pyrimidin-4-one **(17a)** (1 equivalent) in acetic acid (3 ml per g **(17a)**) was added the appropriate amine (1.2 equivalents) and formalin (1.2 equivalents). The reaction mixture was heated on a steam-bath until no starting material remained (1–2 h) as indicated by thin-layer chromato-graphy. The reaction mixture was then poured onto water, basified with sodium carbonate and extracted (x 3) with ethyl acetate. The combined ethyl acetate extracts were washed, dried and evaporated to give an oil. Purification by column chromatography on silica gel and / or conversion to hydrochloride salts by the method described for scheme 1 and crystallisation gave the required products.

Preparation of novel amines used in schemes 1 and 2

2-(5-Hydroxymethyl-2-furanylmethylthio)ethanamine (7)
A cold solution of sodium methoxide (5.08 g, 0.094 mol) in methanol (50 ml) was added dropwise to a cold (ice-bath temperature), stirred solution of cysteamine hydrochloride (5.35 g, 0.047 mol) in methanol (50 ml). To the resulting mixture, stirred in an ice-bath, was added a solution of 5-hydroxymethylfurfuryltrimethylammonium iodide [22] (14.0 g, 0.047 mol) in methanol (80 ml). The resulting mixture was heated at reflux temperature for 72 h, cooled, filtered and the filtrate evaporated to dryness to give a pale-green oil. This oil was dissolved in water and extracted with ethyl acetate (x 6). The combined organic extracts were dried and evaporated to dryness to give 2-(5-hydroxymethyl-2-furanylmethylthio)-ethanamine as an oil (3.95 g, 45% yield). [^{1}H NMR spectrum ($CDCl_3$), $-\delta$: 2.5–2.8, m, ($SCH_2CH_2NH_2$); 3.67, s, ($-CH_2S-$); 4.50, s, ($HOCH_2-$); 6.05–6.20, ABQ, (furan 3 and 4 protons)].

This material seemed to be relatively unstable and was used immediately in the next stage of the reaction sequence (preparation of **17h**) without further purification.

2-(5-Carbethoxy-2-furanylmethylthio)ethanamine hydrochloride (8)
To a stirred solution of sodium (2.43 g, 0.106 mol) in ethanol (150 ml) at 0°C was added cysteamine hydrochloride (6.0 g, 0.053 mol). After stirring at 0°C for 30 min, ethyl (5-chloromethyl)-2-furoate [23] (10.0 g, 0.053 mol) in sodium-dried ether (50 ml) was added dropwise at such a rate so as to maintain the temperature below 8°C. The reaction mixture was then allowed to warm up to room temperature and stir overnight. The solvent was evaporated to give an oil which was dissolved in dilute hydrochloric acid and extracted with ethyl acetate (4 x 100 ml). The aqueous solution was basified with sodium hydroxide and re-extracted with ethyl acetate (4 x 100 ml). The latter organic extracts were combined, washed, dried and evaporated to dryness to give an oil. This oil was taken up in dry ether and ethanol, saturated with hydrogen chloride gas, was added. The solid obtained was collected, dried and twice crystallised from absolute ethanol to give the title compound, mp: 151–156°C (5.62 g, 40% yield). Anal $C_{10}H_{15}NO_3S{\cdot}HCl$ (C, H, N, S, Cl).

2-(3-Dimethylaminomethyl-2-thienylmethylthio)ethanamine (9)
a). To a stirred solution of 3-dimethylaminomethylthiophene [24] (35 g, 0.247 mol) in sodium-dried ether (150 ml) at –5°C, was added dropwise a solution of n-butyllithium in ether (1.6 M, 180 ml). The reaction mixture was stirred for one hour, then paraformaldehyde (14.83 g, 0.494 mol) was added portion-wise. The reaction mixture was allowed to warm up to room temperature and stirred for 1 hour. Water was added and the organic layer separated. The aqueous layer was further extracted with ether (3 x 200 ml) and the combined ethereal extracts washed, dried and evaporated to give 3-dimethyl-aminomethyl-2-hydroxymethylthiophene as an oil (11.4 g, 25% yield). [^{1}H NMR spectrum ($CDCl_3$): $-\delta$: 2.21, s, ($-N(CH_3)_2$); 3.45, s, ($-CH_2N$); 4.63, s, ($-CH_2OH$); 6.85, d, (4H of thiophene); 7.05, d, (5H of thiophene)].
b). To a stirred solution of cysteamine hydrochloride (7.3 g, 0.064 mol) in concentrated hydrochloric acid (30 ml) at 0°C was added dropwise a solution of 3-dimethylaminomethyl-2-hydroxymethylthiophene (11.0 g, 0.064 mol) in concentrated hydrochloric acid (20 ml). The reaction mixture was allowed to warm up to room temperature and stir for 18 h. The mixture was then poured onto ice, the solution basified with 40% sodium hydroxide solution and extracted with ethyl acetate (5 x

150 ml). The combined organic extracts were washed, dried and evaporated to give the title compound as an oil (1.8 g, 12% yield). Anal $C_{10}H_{18}N_2S_2$ (H, N and S; C found: 51.5; requires 52.1%). [^{1}H NMR spectrum ($CDCl_3$): $-\delta$: 1.98, br s (NH_2); 2.21, s, ($-N(CH_3)_2$); 2.62, t, (SCH_2CH_2); 2.85, t, (CH_2CH_2N); 3.37, s, ($-CH_2S-$); 3.96, s, ($-CH_2N$); 6.92, d, (4H); 7.12, d, (5H); *J*, 4H, 5H of thiophene = 5.2 Hz].

Preparation of new isocytosine precursors used in schemes 1 and 2

5-(4-Hydroxybenzyl)-2-methylthiopyrimidin-4-one ***(10)***
5-(4-Methoxybenzyl)-2-thiouracil [1] (10.5 g, 0.0423 mol) was heated under reflux in glacial acetic acid (80 ml) and 48% aq HBr (40 ml) for 3 h. The solution was cooled to ~35°C, methyl iodide (12.04 g, 0.0846 mol) added and the mixture heated at reflux temperature for a further 2 h. Excess methyl iodide was distilled off and the resulting mixture evaporated to dryness to give an orange solid. Water (50 ml) was added, the solid broken up and the pH adjusted to ~5 with 2 N sodium hydroxide. The sandy solid was collected, washed with water and crystallised from ethanol (400 ml) to give a colourless solid (5.72 g, mp: 237–239°C). The solid was recrystallised from absolute ethanol to give the title compound, mp: 243–244°C (4.31 g, 41% yield). Anal $C_{12}H_{12}N_2O_2S$ (C, H, N).

5-(5,6-Dimethyl)3-pyridylmethyl)-2-nitroaminopyrimidin-4-one ***(12)***
a). A mixture of ethyl 3-(5,6-dimethyl-3-pyridyl)propionate [6] **(11)** (20.7 g, 0.1 mol) and ethyl formate (11.1 g, 0.15 mol) was added dropwise over 3 h to a suspension of sodium hydride (6.0 g of 50% suspension in oil, 0.125 mol) in 1,2-dimethoxyethane (35 ml) stirred in ice. The brown suspension was stirred at room temperature overnight and then poured onto ice (~150 ml). The resulting brown solution was then extracted with ether (3 x 150 ml) and the aqueous extract adjusted to pH = 7 with dilute HCl. After cooling the sandy-brown solid was collected, washed with water and dried to give ethyl 3-(5,6-dimethyl-3-pyridyl)-2-formyl propionate, 14.43 g (61%), mp = 148–149°C. Anal $C_{13}H_{17}NO_3$ (C, H, N).
b). Sodium (1.45 g, 0.063 mol) was dissolved in dry methanol (65 ml), nitroguanidine (6.05 g, 0.059 mol) added and the mixture heated at reflux temperature for 45 min. Ethyl 3-(5,6-dimethyl-3-pyridyl)-2-formylpropionate (14.3 g, 0.056 mol) was added portionwise over 30 min and the mixture heated at reflux temperature for 40 h. After cooling the methanol was evaporated to leave a red oil which was dissolved in water (40 ml) and extracted with chloroform (3 x 50 ml). The chloroform extracts were washed with water (10 ml) and all the aqueous fractions were combined and adjusted to pH = 6 with concentrated HCl. After cooling the precipitated solid was collected, washed with water and crystallised from DMF / ethanol to give the title compound, 7.06 g (46%), mp: 212–213°C. Anal $C_{12}H_{13}N_5O_3$ (C, H, N).

*2-Nitroamino-5-(4-pyridylmethyl-*N*-oxide)pyrimidin-4-one* ***(13)***
2-Nitroamino-5-(4-pyridylmethyl)pyrimidin-4-one [2], (8.63 g, 0.0349 mol) was dissolved, with warming, in glacial acetic acid (300 ml). To the stirred solution at ~50°C was added meta-chloroperbenzoic acid (8.4 g, 0.0487 mol) batchwise over a period of 60 min. The mixture was then stirred at room temperature overnight and then warmed, with continued stirring, at 60°C for a further 5 h. On cooling the title compound crystallised as a yellow solid and was collected, washed with cold glacial acetic acid and dried *in vacuo*, mp: 256–258°C (Dec), (8.27 g, 90% yield). Anal $C_{10}H_9N_5O_4$ (C, H, N).

*2-Nitroamino-5-(3-pyridylmethyl-*N*-oxide)pyrimidin-4-one* ***(14)***
2-Nitroamino-5-(3-pyridylmethyl)pyrimidin-4-one [2] (12.35 g, 0.05 mol) was dissolved with warming in glacial acetic acid (300 ml), cooled to room temperature and meta-chloro-perbenzoic acid (10.35 g, 0.06 mol) added over 5 min. The solution was stirred at room temperature for 18 h and then at 60°C for 5 h. The mixture was cooled and the precipitated solid collected, washed with glacial acetic acid and water and dried to give a light-green powder (11.37 g, 86%). A small quantity of this material was purified for analysis by dissolution in dilute sodium hydroxide (→pH~7.5) and re-precipitation by addition of dilute hydrochloric acid (→pH~6.5). The precipitated solid was collected, washed with water and dried to give a colourless powder, mp: 271°C (Dec). Anal $C_{10}H_9N_5O_4$ (C, H, N).

*2-Nitroamino-5-(6-methyl-3-pyridylmethyl-*N*-oxide)-pyrimidin-4-one* ***(15)***
2-Nitroamino-5-(6-methyl-3-pyridylmethyl)pyrimidin-4-one [2] (10.0 g, 0.038 mol) was dissolved with warming in glacial acetic acid (200 ml). The solution was cooled to room temperature and meta-chloroperbenzoic acid (6.6 g, 0.038 mol) added portionwise over 5 min. The solution was stirred for 1.5 h and then a second portion of *m*-chloroperbenzoic acid (6.6 g, 0.038 mol), was added over 5 min. After standing in the cold overnight the mixture was stirred at room temperature for 6 h, the precipitated solid filtered off and washed with ether. The solid was dissolved in 5% sodium bicarbonate solution (200 ml), the solution filtered and adjusted to pH 4 with N·HCl (~115 ml). The precipitated solid was collected, washed with water and dried *in vacuo* to give the title compound, 7.46 g (70%), mp: 212–213°C. Anal $C_{11}H_{11}N_5O_4 \cdot H_2O$ (C, H, N).

Acknowledgments

We gratefully acknowledge the contributions of Dr GR White and his colleagues for the preparation of some starting materials. Also our colleagues in the Physical Organic Chemistry Department of SK&F Research Ltd for microanalyses (Mr MJ Graham), NMR spectra (Dr ES Pepper) and IR spectra (Dr RC Mitchell). Toxicological data were provided by the SK&F Toxicology Department (TF Walker).

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