SYNTHESIS AND STRUCTURE ACTIVITY STUDIES OF NOVEL H_3-RECEPTOR HISTAMINE ANTAGONISTS.

A thesis presented in partial fulfilment of the

requirements for the

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of the University of London

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Signature: Date. Sept 1991.

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Thanks finally to my parents, for their patient, generous and loving encouragement and to my friends Mr and Mrs Rafatjoo for their caring support.

For the loving memory of my grandfather.

Abbreviations

Abs. EtOH	absolute ethanol
as ym	a symmetric
Ar	aromatic
aufs	absorbance units for full scale deflection
br	broad
cAMP	cyclic-adenosine-3'-5'-monophosphate
CNS	central nervous system
d	doublet
def	deformation
DMSO	dimethyl sulphoxide
DMSO-d_6	deuteriated dimethyl sulphoxide
EC_{50}	concentration producing 50% maximal response
EI	electron impact
FAB	fast atom bombardment
fmol	femtomole, 10^{-15} mole
g	gramme
HA	histamine
HPLC	high performance liquid chromatography
Hz	Hertz
Im	imidazol-4-yl
insol	insoluble
J	coupling constant
K_i	inhibitory constant, equivalent to biological constant K_B
K_B	dissociation constant of antagonist; antagonist potency, $pA=\log_{10}K_B$
m	mutiplet (NMR), medium (IR)
M	molar
m/e	mass to charge ratio

mg	milligramme, 10^{-3} gramme
ml	millilitre, cubic centimetre cm^3
mmole	milimole
min	minute
mp	melting point
µl	microlitre, 10^{-6} litre
µM	micromole
nmol	nanomole
NMR	nuclear magnetic resonance
Nuj	Nujol; paraffin oil
Ph	phenyl
pK_i	-log Ki
p.o.	per os, by mouth
py	pyridine
q	quartet
s	singlet (NMR), strong (IR)
SKH	identification tag (S.K.Hossseini)
sp. sol	sparingly soluble
str	stretch
sol	soluble
sym	symmetric
t	triplet
THF	tetrahydrofuran
TLC	thin layer chromatography
UCL	identification tag (University College London)
UV	ultraviolet
vbr	very broad
w	weak
YK	identification tag (Y. Khalaf)

Thesis compound numbers

(1) 2-{2-[4(5) imidazolyl]ethyl}aminopyridine, SKH130A (13).

(2) 2-{2-4(5)-imidazolyl]ethyl}aminopyrimidine, SKH91B (14).

(3) 2-{2-[4(5)-imidazolyl]ethyl}aminobenzothiazole, SKH103D (15).

(4) 2-{2-4(5)-imidazolyl]ethyl}aminopyrazine (16).

(5) 2-{2-[4(5)-imidazolyl]ethylamino}-3,6-dimethylpyrazine (17).

(6) 2-{2-[4(5)-imidazolyl]ethyl}amino-3-nitropyridine (18).

(7) 2-{2-[4(5)-imidazolyl]ethyl}amino-5-nitropyridine (19).

(8) 2-{2-[4(5)-imidazolyl]ethylamino]-1H-phenyltetrazole (20).

(9) 2-{2-[4(5)-imidazolyl]ethylamino]-thiazole (21).

(10) 2-{[2-[4(5)-imidazolyl]methylthio]ethylamino]}-5-nitropyridine (23).

(11) N_1-2-[imidazol-4-yl-N_2-cyclohexyl]-thiourea (24).

(12) N_1-2-[Imidazol-4-yl-thiomethyl]ethyl-N_2-cyclohexyl (25).

(13) 2-{2-[2-pyridyl]ethyl}aminopyrimidine (26).

(14) 2-{2-[2-pyridyl)ethyl]amino}-benzothiazole (27).

(15) 2-{2-[4(5)-imidazolyl]ethylamino}-5-trifluoromethylpyridine (28).

(16) 2-{2-4(5) imidazol-4-yl]-ethylthio}-pyridine (29).

(17) 2-{2-[4(5)-imidazolyl]ethylthio}-5-nitropyridine (30).

(18) 2-{2-[4(5)-imidazolyl]ethylthio}imidazole (32).

(19) 2-{2-[4(5)-imidazolyl]-ethylamino}-4-nitropyridine-N-oxide (34).

(20) 4-{2-[4(5)-imidazolyl]-ethylamino}-2-chloropyridine (35).

(21) 1-{2-4(5)-imidazolyl]-ethylamino}-4-nitrobenzene (36).

(22) 2-{2-[1-methyl-4-imidazolyl]ethylamino}-5-nitropyridine (40).

(23) 2-{2-[1-methyl-4-imidazolyl]ethylamino}-5-trifluoro methylpyridine (41).

(24) 2-{2-[4(5)-imidazolyl]ethylamino}-5-carbomethoxypyridine (43).

(25) 2-{4-[4(5)-imidazolyl]-piperidyl}-N-5-trifluoromethylpyridine (44).

(26) 2-{2-[2-pyridyl]ethylamino}-5-nitropyridine (45).

(27) 2-{[4(5)-imidazolyl]methylthio}-5-nitropyridine (47).

(28) 2-{2-4(5)-imidazolyl]ethyl}-amino-5-aminopyridine (49).

(29) N-(N'-cyclohexyl-thiocarbamoyl)-3-(imidazol-4-yl)propylamine YK192B/UCL1053.

(30) N-(N'-cyclohexyl-thiocarbamoyl)-4-(imidazol-4-yl)butylamine YK251F/UCL1088.

(31) 2-{3-[imidazol-4-yl]propyl}amino-5-nitropyridineYK215C/UCL1073

(32) 4-[2-(1-pyrrolidinyl)ethyl]imidazol; Arrang et al.[5]

ABSTRACT

This thesis describes various novel approaches to the design and synthesis of potential H_3-receptor histamine antagonists. The goal is to provide the basis for the design of a non-toxic, brain penetrating compound which could be used as a prototype drug for investigative clinical studies in humans. The novel compounds were submitted for *in vitro* testing on rat cerebral cortex as potential antagonists. The results were used as a guide to making further modifications of structures.

Thioperamide (N-cyclohexyl-4-(4-imidazolyl)-1-piperidine carbothioamide), the published first selective H_3 antagonist, was taken as a lead compound. Early in the work, the synthesis of 4-(4-piperidyl)imidazole, the key intermediate to thioperamide, was investigated and a modified and reliable route was established. This is a useful intermediate for the preparation of other analogues of thioperamide. One such novel analogue was prepared, where the cyclohexyl thiourea was replaced by a substituted aminopyridyl moiety leading to a highly potent antagonist displaying higher *in vivo* activity than thioperamide.

Ring-opened analogues of thioperamide in which the piperidyl imidazole was replaced by histamine or a thiomethyl homologue showed a reduction in activity.

Histamine was used as the chemical starting material to prepare novel derivatives, in which the primary amine was coupled with different heterocycles, via aromatic nucleophilic substitution.

Various substituents in different positions of the heterocyclic ring were investigated by introduction of groups such as NO_2, N-oxide, NH_2, CO_2CH_3, CF_3, CH_3 and Cl. The possibility that they affected activity through an electronic effect was studied by seeking Quantitative Structure Activity Relationships.

Novel substituted heterocyclic derivatives of N^{τ}-methylhistamine and 2-pyridyl ethylamine were synthesised.

The heterocyclic ring moiety was also replaced by a substituted phenyl group.

Other compounds with the methylthioethylamine side chain replacing that of histamine have been prepared.

The side chain amino NH of some of the histamine pyridyl derivatives was replaced with sulphur. One of these compounds was as active as thioperamide. A shorter chain analogue was also prepared.

In vitro testing results showed that all these compounds are antagonists at the histamine H_3-receptor. The most potent antagonists were tested *in vivo* .

CONTENTS

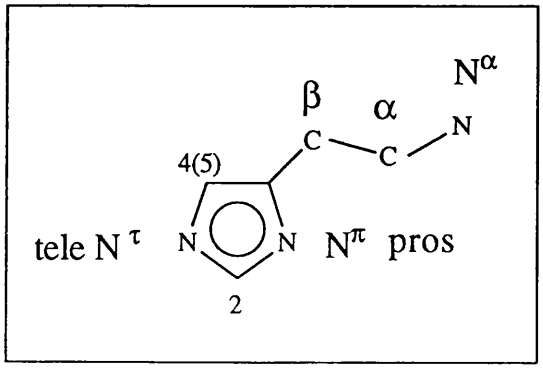
CHAPTER ONE

INTRODUCTION

1.1) Histamine history and properties:

Histamine is a biogenic amine which has many biological activities. The intensive investigation of the pharmacology of this bioactive compound in various systems and organs and its physiological and pathological role in the treatment of many diseases has resulted in many interesting agents, several of which have found therapeutic applications. Histamine [2-(4(5)-imidazolyl)ethylamine](fig. 1.1) was originally synthesised by Windaus and Vogt[81] and it was shown to occur as a constituent of ergot (a fungi growing on rye) by Barger and Dale[12]. Ackermann[1] obtained a large yield of the base by splitting off carbon dioxide from histidine by bacterial action. Dale and Laidlaw[30] demonstrated that histamine was a potent stimulant of smooth muscle contraction. The histamine release from lungs during anaphylaxis was followed and histamine was shown to be a substance liberated in response to injurious stimuli. Popielski[57] reported stimulation of the secretion of gastric acid by histamine.

Fig. 1.1: Histamine (numbering from reference 14)



1.2) Presence of histamine in organs and cells

Histamine is widely distributed in the body being present in almost all organs and body fluids. It is present in mucosal cells of the gastrointestinal tract, particularly the acid secreting parietal cells. The tissue stores of histamine in the liver, lung, skin and many other tissues are mast cells. Mast cells contain a considerable amount of histamine (lmg/g of tissue), bound in large cytoplasmic granules together with heparin and ATP[63]. Histamine-storing mast cells are present in the central nervous system of various mammals. Release of mast cell granules may be effected in several ways, for example many basic drugs containing amine, diamine, diamidine or diguanide groups are histamine releasers. Certain enzymes, such as trypsin release histamine presumably by disrupting the mast cell membrane. Hypersensitivity to certain food stuffs may involve more than antibody-antigen reactions since potent histamine liberators have been found in fresh egg-white and shell fish. Histamine is known to be synthesised in and released from a discrete set of neurons descending through the lateral hypothalamic area and widely projecting in the telencephalon from a combination of biochemical, electrophysiological and lesion studies.[7]

1.3) Histamine synthesis and metabolism:

Histamine is formed by the decarboxylation of the amino acid histidine with the release of carbon dioxide (fig 1.2). Table 1.1 shows some examples of enzyme inhibitors involved in histamine synthesis and metabolism and their *in vitro* potency.

Fig.1.2 [27]: Histamine biosynthesis and metabolism.

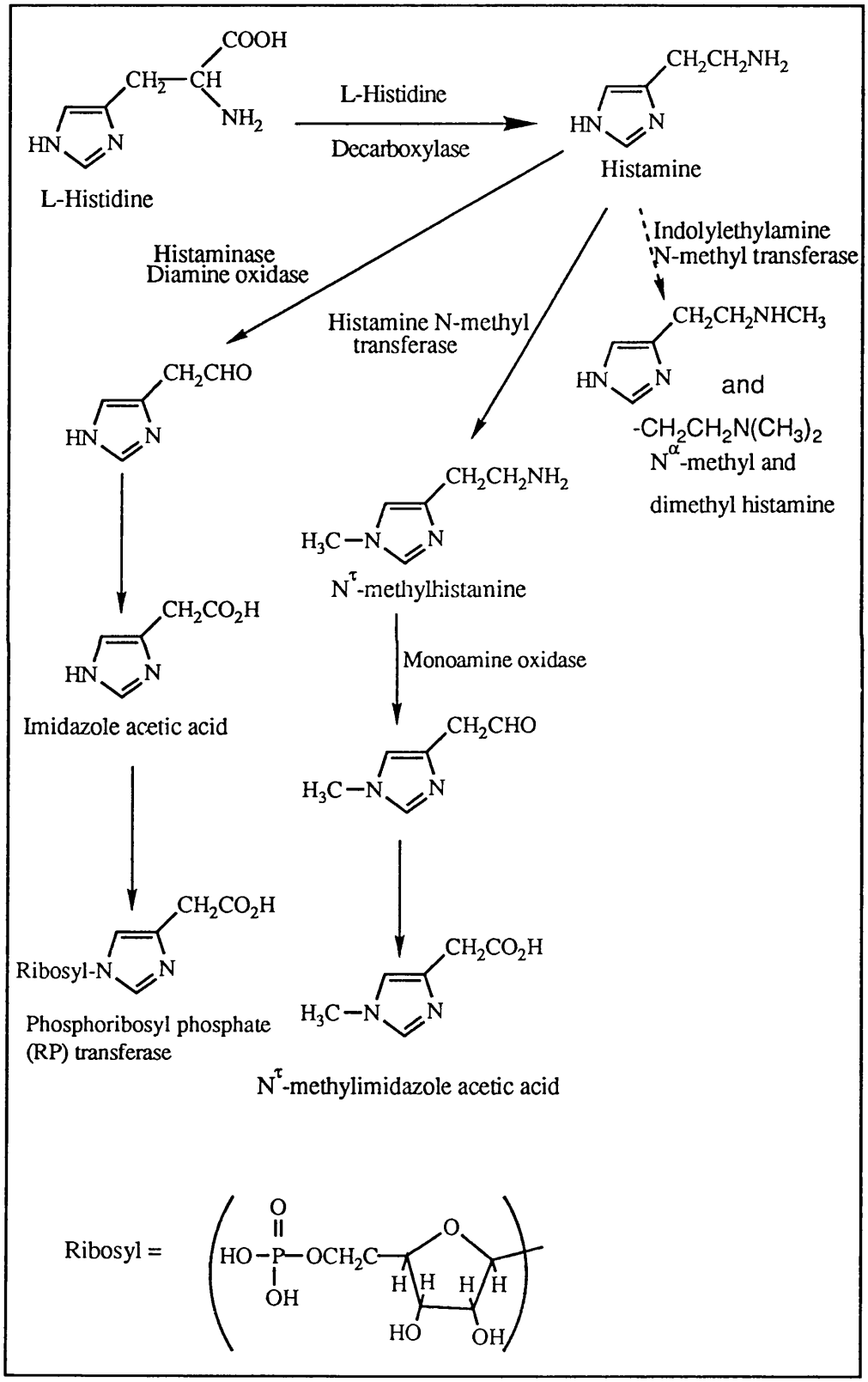
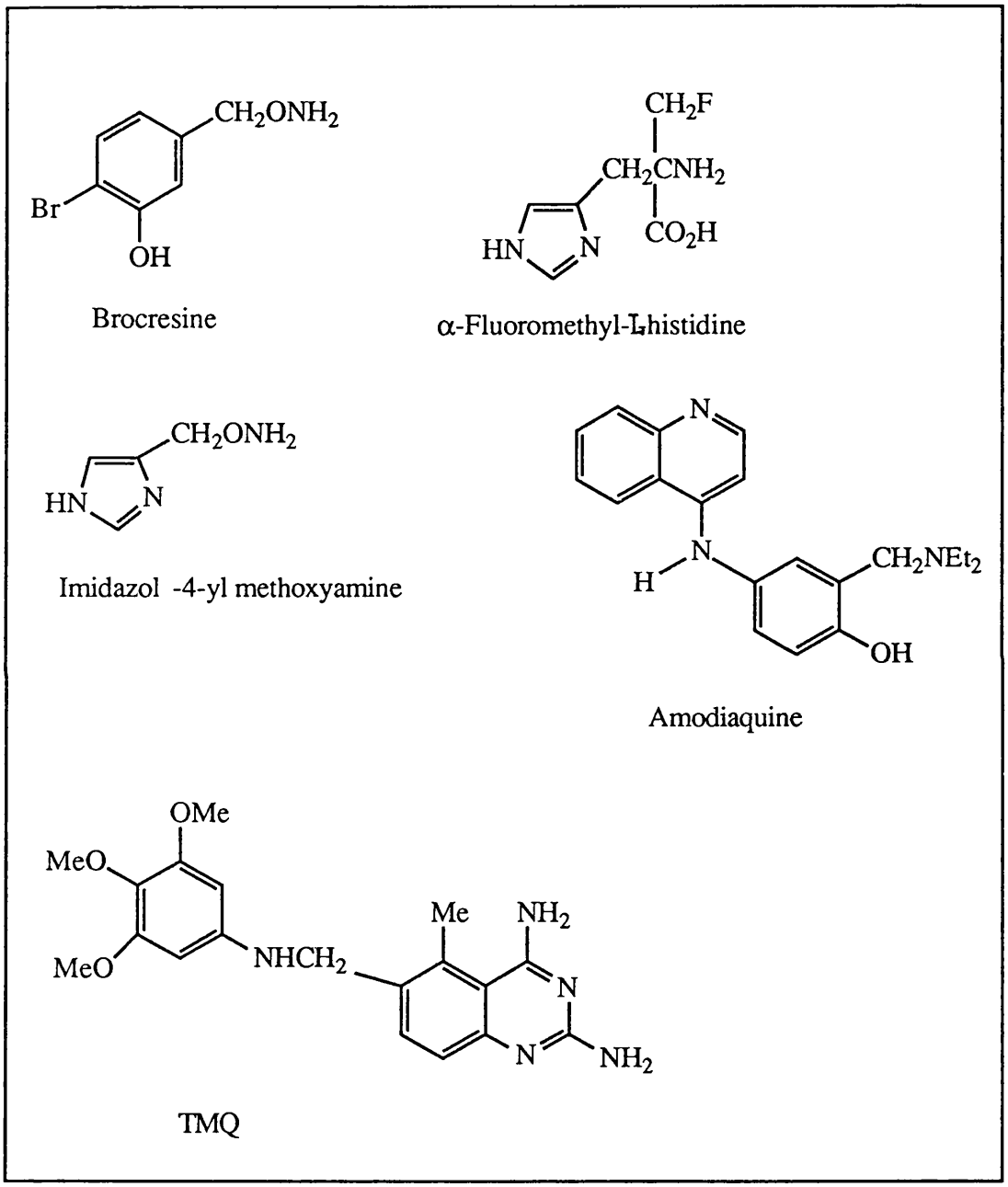


Table 1.1: Some examples of enzyme inhibitors involved in histamine synthesis and metabolism and their *in vitro* potency.[27]

Compound	K_i (M)	Function
Histidine decarboxylase		
α- Fluoromethyl-L-Histidine	3.2×10^{-5}	Most potent and useful as a pharmacological tool in vitro and measurement of brain histamine levels.
Brocresine	1.5×10^{-7}	A benzyloxyamine , non-specific, combines with pyridoxal coenzyme.
Imidazole -N-methyl transferase		
Amodiaquine	1×10^{-8}	Antimalarial.
TMQ, 2,4-diamino-5-methyl-6- [(3,4,5-trimethoxy anilino)methyl]quinazoline	7×10^{-9}	Folate antagonist.
Diamine oxidase (Histaminase)		
Imidazol-4-yl methoxyamine	9×10^{-9}	
Impromidine	7×10^{-9}	
Imidazole acetate phosphoribosyl phosphate tranferase		
sodium salicylate	2×10^{-4}	

Fig 1.3: Structures of some of the enzyme inhibitors of histamine synthesis and metabolism:



Two enzymes can catalyse the formation of histamine, the highly specific and active L-histidine decarboxylase and the less specific and less active aromatic amino acid decarboxylase (L-dihydroxyphenylamine decarboxylase). The former is widely distributed in tissues, being present in gastric mucosal cells and in the cells of the actively growing tissues.

Both enzymes require pyridoxal phosphate as a co-factor and are inhibited by substances that combine with this co-factor.

Schayer[67] has observed a decrease in tissue or urinary histamine levels or inhibition of the conversion of labelled histidine to labelled histamine *in vivo* after the administration of Brocresine (4-Bromo-3-hydroxybenzyloxamine), α-fluoromethylhistidine (a suicidal inhibitor)[36] and 4-thiazolylmethoxyamine.

Most of the histamine synthesis in the body is probably due to histidine decarboxylase. Another specific enzyme for histamine is histamine methyl transferase. Schayer and Karjalal[65] discovered that ring methylation was a major pathway for histamine metabolism *in vivo* and Brown et al[19] (1959) showed that S-adenosylmethionine acts as a methyl donor.

1.4) Histamine, a messenger:

It is the profusion of chemical signals by which cells in the body communicate with one another that provides many opportunities for a specific drug effect. Histamine is a very important endogenous compound which behaves as a neurotransmitter[70] in the mammalian brain. Upon the arrival of an action potential, histamine is released into the synaptic cleft from neuron endings interacting with different postsynaptic or presynaptic cells (fig 1.4). A variety of neurochemical, neurophysiological and neuropharmacological data implicate the presence of histaminergic synapses in the mammalian brain.[104]

1.5) Histamine receptors:

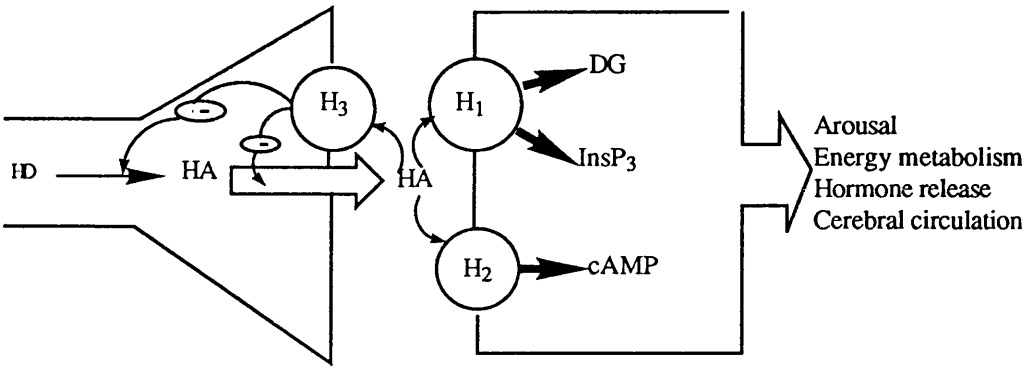
Histamine acts on target cells in the mammalian brain via the stimulation of two classes of receptors (H_1 and H_2), previously characterised in peripheral organs. H_1-receptors are linked to the phosphatidylinositol pathway and mobilization of intracellular Ca^{2+} whereas H_2-receptors mediate via adenylate cyclase activation and the production of cAMP. Stimulation of either the H_1 and H_2 system will lead to a pharmacological and ultimately a physiological effect.

The differences between H_1 and H_2 postsynaptic receptor types include localisation, function and affinity for the endogenous agonist, histamine, and exogenous agonists and the affinity for antagonists.[113]

Presynaptic receptors function rather differently. The presynaptic receptor is called an autoreceptor as these receptors modify the amount of the agonist released.

The table in fig 1.4 shows some of the selective drugs available for the study of the different classes of histamine receptors: 2-TEA, 2-thiazolylethylamine; [R]αMeHA, (R)α-methylhistamine.

Figure 1.4: Histamine receptors in the CNS.[74] Interaction between histamine (HA) and presynaptic H_3-receptors on histaminergic nerve terminals and H_1- and H_2- receptors on postsynaptic cells in the central nervous system.



Receptor	Agonist	Antagonist
H_1	2-TEA	Mepyramine
H_2	Dimaprit	Cimetidine
H_3	[R]αMeHA	Thioperamide

H_3-receptor stimulation leads to an inhibition of both the synthesis of HA from histidine (HD) and its release into the synaptic cleft. H_1- and H_2- receptor stimulation leads to various physiological responses via the action of the intracellular messengers diacylglycerol (DG), inositol trisphosphate ($InsP_3$) and cyclic 3',5'-adenosine monophosphate (cAMP).

The presynaptic autoreceptors are considered important for the regulation of neurotransmission. Such autoreceptor effects have been demonstrated in the case of noradrenaline (the α_2-receptor showing negative feedback), dopamine (D_1, D_2 and D_3 receptors), serotonin, acetylcholine and γ-aminobutyric acid (GABA) neurons.

Schwartz et al[7] in the early 1980's demonstrated the existence of such an autoreceptor for histamine. Histamine inhibits, by activation of the H_3-receptor, its own release and biosynthesis from histidine from depolarised slices of rat cerebral cortex, an action mediated by a new pharmacologically distinct class of receptor other than H_1- and H_2- receptors.

After incubation of rat cerebral cortex slices in the presence of 33μM [^{3}H]histidine, depolarisation by 30mM K^+ of the cortical slices in the absence of any added exogenous histamine resulted in the release of about 15% of tissue [^{3}H]histamine. However, when nonradioactive exogenous histamine was present in the medium, an inhibition of the K^+ induced ^{3}H-histamine release was observed[4]. The inhibitory action of histamine was concentration-dependent and saturable with maximal inhibition of 60%. The inhibition of release was reversible since it was no longer observed when slices were rapidly washed after exposure to the amine and before depolarisation. The characters of saturability and reversibility of the inhibitory effect of histamine suggests that it is a receptor mediated process[7]. The presynaptic H_3-autoreceptors are highly sensitive to histamine, being activated at histamine concentrations much lower than those required to activate postsynaptic H_1- or H_2- receptors. This suggests that *in vivo* histamine release (and synthesis) is under tonic autoinhibitory control, therefore the blockade of these H_3- autoreceptors would provide pharmacological means to enhance histamine neurotransmission in the CNS.

1.6) Histamine receptor ligands:

The differentiation between subclasses of histamine receptors has been made by the use of selective agonists and antagonists and the distinction has been supported by biochemical studies which indicate that the cellular reactions influenced by H_1-, H_2-and H_3- receptors mechanisms are fundamentally very different.

1.6.1) Agonists at histamine receptors:

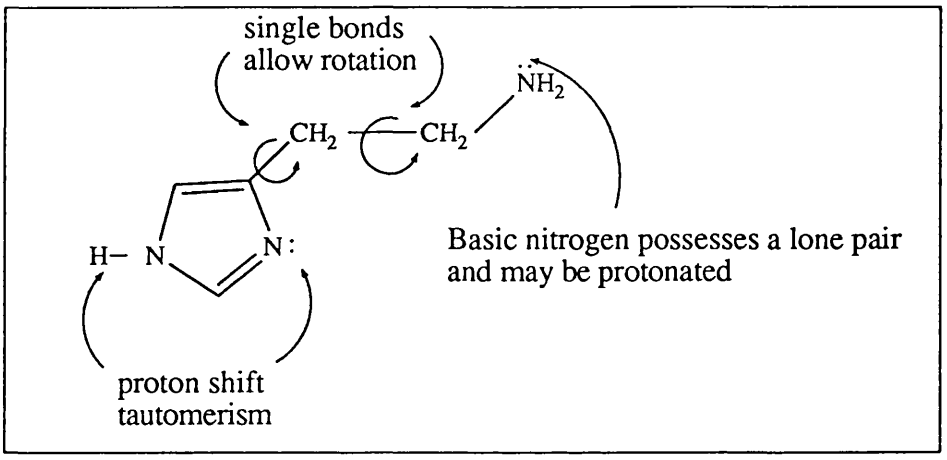
Studies of histamine pharmacology have been greatly assisted by the use of selective agonists. Identification of such agents which are used as pharmacological tools and their chemical properties have been usefully studied to reveal relationships between chemical structure and biological activity at histamine receptors. It is conceivable that when histamine interacts with its receptors, it functions in the chemical sense as a ligand binding to a specific molecular site hence some of its properties could have importance for ligand-receptor interactions.

Histamine (figl.1) is a dynamic molecule, comprised of an imidazole ring and an amino group connected by a short chain of two carbon atoms. The amino group and the imidazole nucleus are both basic and are protonated in acidic solution. The imidazole ring is a planar aromatic system with 6π electrons. It is a cyclic amidine incorporating two types of nitrogen atoms, which are tautomeric and exist in neutral solutions as two different tautomers. The rotation of single bonds in the carbon atom chain give rise to different conformations of the molecule. Histamine in solution is a mixture of ionic forms, tautomers and conformers (fig 1.5). Histamine is a very hydrophilic molecule and does not readily cross the blood brain barrier [66], the partition coefficient for histamine free base using 1-octanol/water buffered at pH 11.8 is low (P = 0.2)[38]. Histamine has a strong capacity for hydrogen bonding, in which the ammonium and imidazolium cations act as hydrogen donors and the uncharged ring (in the monocation and the free base) act both as hydrogen donor and acceptor. The different approaches to study electronic charge distributions within the histamine molecule indicate that all the nitrogen atoms are negatively charged, that there is little net charge on the carbon atoms and that the positive charge is distributed widely all over the hydrogen atoms in the molecule, although tending to be concentrated on the N-H hydrogens.

Since various species of histamine exist, different species could be involved at the different receptors and the chemical mechanism of histamine receptor interaction could differ between

the three types of receptors.

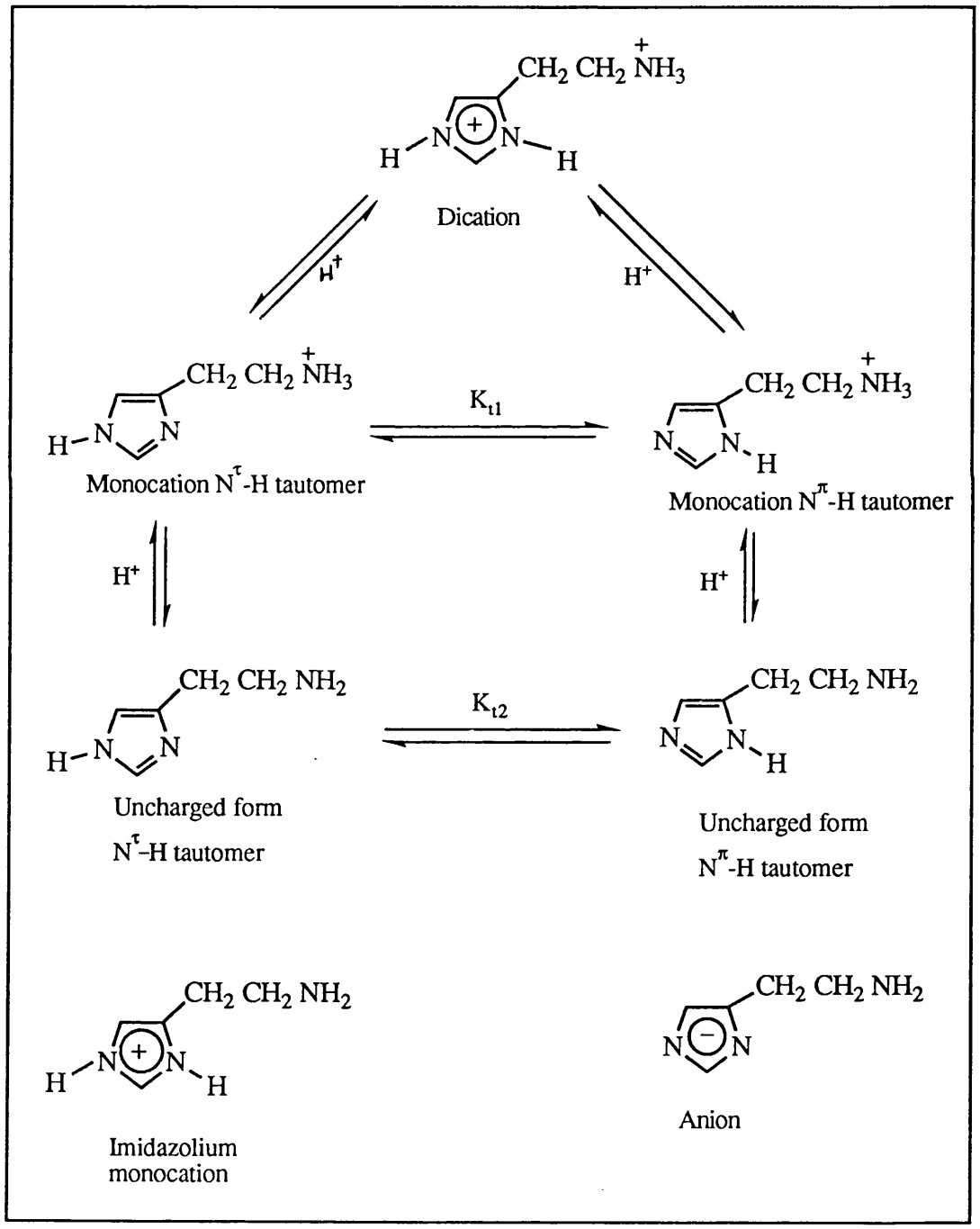
Fig(1.5)[27]: Existence of histamine in different chemical forms.



form	*Effect*	*Cause*
Ion	Differ in position of H and charge	Basic nitrogen atoms
Tautomer	Differ in position of H and and lone pair electron	Imidazole ring has N: and NH
Conformer	Differ in shape	Single bonds in side chain give flexibility

Histamine has two basic centres, and titration of histamine dication in aqueous solution (37°C) gives the first stoichiometric ionisation constant (pK_{a1} =5.80) going to the monocation (dissociation from the ring NH^+), the second (pK_{a2} =9.4) going to the uncharged molecule (dissociation at the side chain $NH_3^{+)}$ and the third in strong alkali gives an anion (pKa =14). At physiological pH (7.4), the main form is the monocation and at pH (5.8) in the vicinity of some membranes, the dication will predominate (fig 1.6) .

Fig 1.6[27]: Shows the ionic and tautomeric equilibria between histamine species. Side-chain deprotonation of the dication furnishes the imidazolium monocation and this must also be be present in solution, but at very low concentration. At very high pH (>12) significant amounts of the anion would also form.

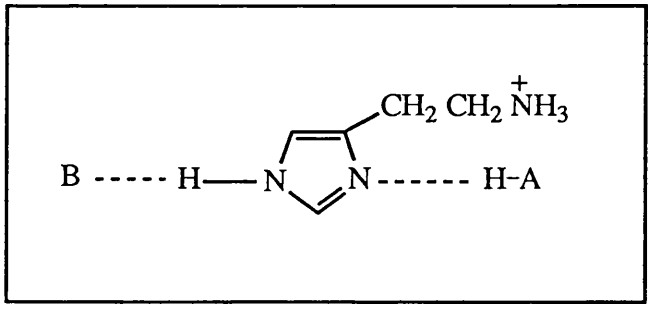


The active form of histamine at H_1- and H_2- receptors is likely to be the N^{τ}-H tautomer of the monocation (fig 1.6), possibly in the trans conformation[27] which is also the most prevalent species in water around neutrality and a side chain NH appears to be needed. However different chemical properties of histamine may be associated at these two receptor types. At the H_1-receptor imidazole tautomerism is not a functional requirement, but the presence of the nitrogen atom ortho to the ammonium ethyl group appears to have special significance. The ring may also need to achieve co-planarity with the side chain or be involved dynamically and rotate without hindrance.

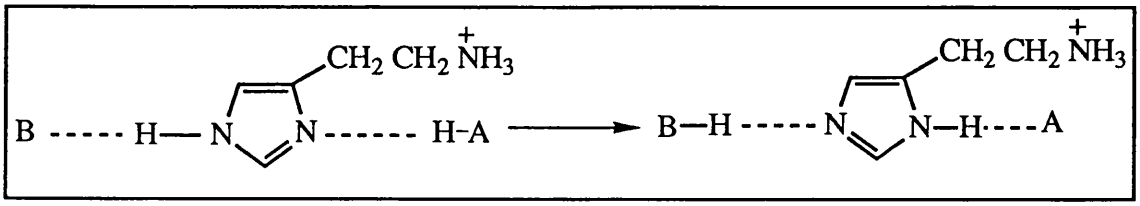
At the H_2-receptor, the tautomeric properties of the imidazole ring of histamine appear to be important and histamine might act statically, in bifunctional hydrogen bonding, or act dynamically as a proton transfer agent.

Fig 1.7: Shows the functional chemical requirements of histamine as antagonist at H_2-receptor. A and B are sites on the receptor.

a) Static hydrogen bonding:



or b) Dynamic function in proton transfer:



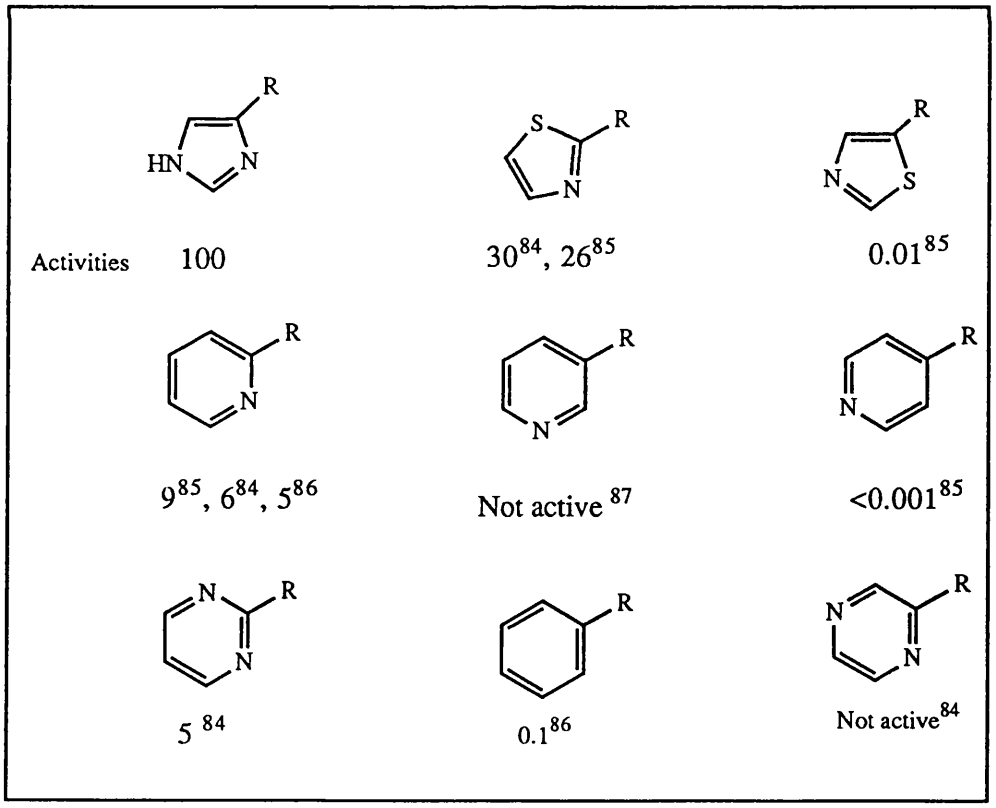
At the H_3-receptor, there appears to be a critical requirement to have an unsubstituted imidazole ring.

1.7) Selective agonists and antagonists:

1.7.1) H_1- receptor agonists:

In search of selective and potent H_1-receptor agonists, a number of heterocyclic histamine analogues has been synthesised, such as 2-methylhistamine, betahistine [N^{α}-methyl-2-(2-pyridyl)ethylamine], 2-(2-thiazolyl)ethylamine and 2-(2-pyridyl)ethylamine (table 1.2). These have become significant pharmacological tools for the selective stimulation of H_1-receptors though they are less potent than histamine. Chemical modification of the histamine molecule such as replacing the imidazole ring of histamine by other heterocycles, has resulted in many interesting observations. (Walter et al 1941).[83]

Table 1.2[27]: H_1 -Receptor agonist activity of aminoheterocyclic compounds (R = $-(CH_2)_2NH_2$). Determined *in vitro* on the guinea pig ileum.The potencies are the ratios of molar concentration of histamine/molar concentration of compounds necessary to produce equal effects relative to histamine=100



Walter[83] originally has described the finding that 2-pyridylethylamine was a histamine like stimulant of guinea pig smooth muscle contraction whereas the 4-isomer, 4-pyridylethylamine was not active. The N^{α}-methyl-2-pyridylethylamine, known as betahistine is an amino substituted derivative of 2-pyridylethylamine which has been used clinically as a peripheral vasodilator. Recently the most potent and highly selective H_1-agonists known so far have been reported[88] by Schunack et al. These are the 2-substituted phenylhistamine derivatives, 2-[2-(3-fluorophenyl)-4-imidazolyl]ethylamine and its chloro analogue,which show 80-90% relative potency compared with histamine. Various side chain alkyl histamines have been studied.[89,90] At the H_1-receptors, agonist activity falls in the series Me > Et > Pr^n ~ CH_2Ph.

1.7.2) H_2-receptor agonists:

Some of the relatively selective H_2-receptor agonists are heteroarylalkylamine derivatives (table 1.3). For example betazole, a pyrazole analogue of histamine, is a very weak but selective agent, used as a diagnostic agent for investigating gastric acid secretory capacity, however 4-methylhistamine is more selective and more potent than betazole. Other compounds, all tautomeric amidine derivatives, include dimaprit (an extremely selective agonist), which is an isothiourea derivative, impromidine (selective and very potent) and other imidazolalkyl guanidines and isothioureas.

Table 1.3[5,7,27]: Relatively selective H_1, H_2, and H_3 -receptor agonists in general use (activities relative to histamine =100)

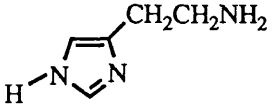
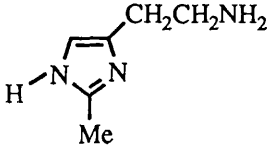
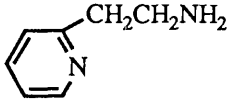
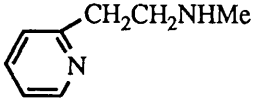
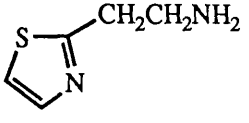
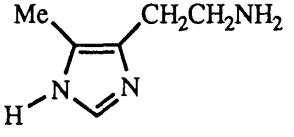
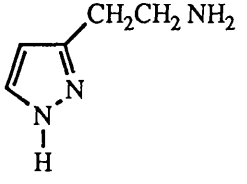
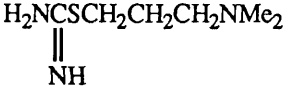
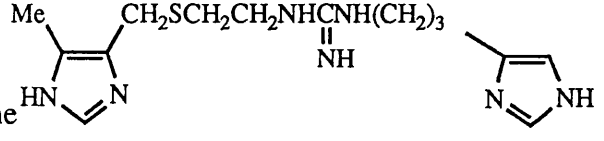
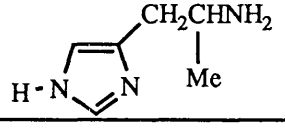
Name	Structure	H_1 Guinea pig ileum	H_2 Guinea pig atrium	H_2 Gastric acid rat	H_2 Rat uterus	H_3 Cerebral cortex rat
Histamine		100	100	100	100	100
H_1-receptor agonists						
2-Methylhistamine		16.5	4.4	2.0	2.1	<0.08
2-(2-Pyridyl)ethylamine		5.6	[2.5] 50% max	≈0.2	<0.05 20% max	
Betahistine		8.0	[1.5] 40% max	≈0.2		Antagonist K_i = 6.9 μM
2-(2-Thiazolyl)ethylamine		26	2.2	≈0.3	0.34 50% max	<0.008

Table 1.3 : continued.

Name	Structure	H_1 Guinea pig ileum	H_2 Guinea pig atrium	H_2 Gastric acid rat	H_2 Rat uterus	H_3 Cerebral cortex rat
H_2-receptor agonists						
4(5)-Methylhistamine		0.23	43	39	25	<0.008
Betazole[c]		0.12	2.1	~0.5	0.11	
Dimaprit		$<10^{-4}$	71	20	17	<0.008
Impromidine		$<10^{-3}$ Antagonist	4810	1680	930	<0.03 Antagonist K_i =0.065 μM
H_3-receptor agonists						
(R)α-Methylhistamine		0.49	1.0			1550

H_1 and H_2 receptor activities (relative to histamine = 100) determined by Blackmore, R. C.and Parsons, M. E. (Smith Kline and French Research Ltd)[91] on the following tissues: (i) ileum, tested for stimulating contraction of the isolated guinea pig iluem in the prsence of atropin; (ii) atrium, tested for stimulation of rate in the spontaneously beating isolated guinea pig right atrium in the presence of propranolol; (iii) gastric acid , tested by rapid intravenous injection for stimulation of gastric acid secretion in the atropinised and vagotomised anaesthetised rat; and (iv) uterus, tested for electrically evoked contractions of the isolated rat uterus in the presence of propranolol. H_3 receptor activities from Arrang, J.M. et al[5,7] determined from inhibition of potassium evoked [^{3}H]histamine release on slices of rat cerebral cortex. [b]Betahistine; Serc, Duphar (Holland) and Unimed Inc. (U. S.). [c]Betazole; Histalog; Eli Lilly Co.

1.7.3) H_3-receptor agonists:

All the potent substances showing H_3-agonist activity are either histamine analogues or have a close structural resemblance to histamine. Arrang et al.[5] have used a series of chiral histamine derivatives with branched side chains to elucidate the stereochemical requirements of the various receptor subclasses (table 1.4). With these compounds no stereoselectivity and limited stereoselectivity were found at H_1- and H_2- receptors respectively, whereas a high degree of stereoselectivitry, the reversal of that observed at H_2-receptors, has been found for N^{α}-methyl-α-chloromethylhistamine and α,N^{α}-dimethylhistamine at H_3-receptors. However both active enantiomers behaved as weak agonists, their potency being only 1-4% that of histamine.

Arrang et al.[5] studied the chiral analogues with a single substitution of the side chain in an attempt to reduce the conformational freedom of the histamine molecule and thereby increase its specificity with retention of the activity. Methyl substitution of histamine in the side chain led, depending on the position and the stereochemical way of substitution, to highly potent H_3-agonists with exceptional receptor selectivity. α-Methylhistamine was found to inhibit the depolarization induced release of [^{3}H] histamine from brain slices by up to 60% The (R)-α-methylhistamine exerted this effect a 100 times as potently as the S isomer and 15.5 times as potently as histamine. (R)-α-methylhistamine potency relative to histamine at H_1- and H_2-receptors are 0.5% and 1% respectively, indicating the high sensitivity of the H_3-receptors. Lipp et al [49] have shown that replacement of the methyl group by ethyl in the α- or β- position of α-methylhistamine caused a significant reduction of activity at H_3-receptors. The non-chiral compound α,α-dimethylhistamine has almost one sixth of the potency of (R)α-methylhistamine and about twenty fold potency of (S)α-methylhistamine.

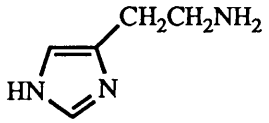
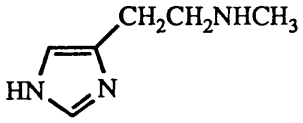
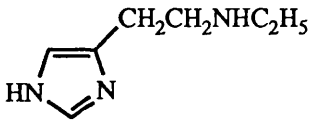
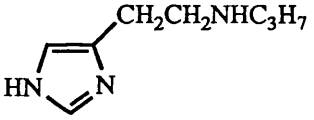
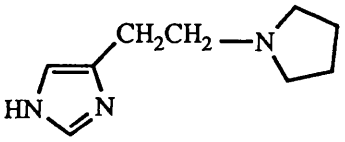
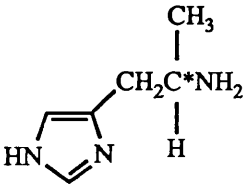
In addition to the reported stereoselectivity for α-branched histamine, Lipp et al.[49] studied the selectivity for β-branched histamines. The high stereoselectivity of the H_3-receptor was best demonstrated in the case of α,β-dimethylhistamine where the 4-stereoisomers, especially the

difference between erythro and threo racemates indicated the selectivity for β-branched histamines (see table 1.4). The comparison of the pD_2 values (negative logarithm of the EC_{50}) shows a selectivity ratio of 130,000:1, H_3 versus H_2-/H_1- agonism.

Alkylation of the histamine side-chain terminal nitrogen with bulky groups led to loss of H_3-receptor agonist activity and its introduction into a pyrrolidine nucleus led to a compound 4-[2-(1-pyrrolidinyl)ethyl]imidazole which is a partial agonist with a relative potency of 7% and a maximal response of 56% that of histamine. Arrang et al.[5] have reported K_i of 2.2μM for this compound when considered as an antagonist.

Relative potencies of histamine derivatives at three classes of histamine receptors are shown in table 1.4.[5,6,33,49,71,92,93,94,95,96,117,8,55]

Table 1.4 : shows the comparison of histamine derivatives on the guinea pig ileum contraction (H_1-receptor), atrial rate (H_2-receptor) and inhibition of rat cortical [^{3}H]histamine release (H_3-receptor).

Name	Structure	H_1	H_2	H_3
Histamine		100	100	100
N^{α}-Methylhistamine		81	185	270
N^{α}-Ethylhistamine		7	21	56
N^{α}-Propylhistamine		0.2	0	<0.6
4-[2-(1-Pyrrolidinyl)ethyl]imidazole		Antagonist K_i = 5μM	0.1 30% max	7 56% max
α- Methylhistamine	 (R)	0.5	1	1550
	(S)	0.5	1.7	13

* chiral centre

Table 1.4: continued

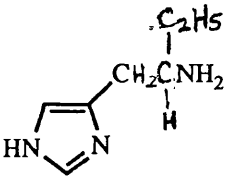
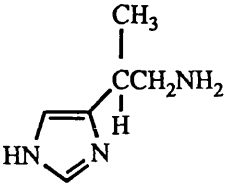
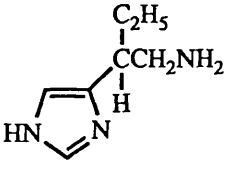
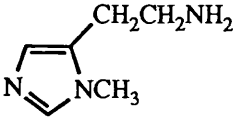
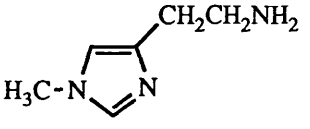
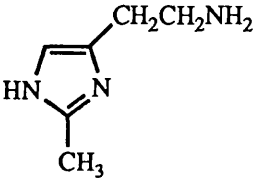
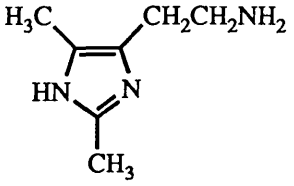
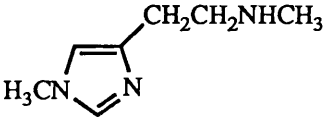
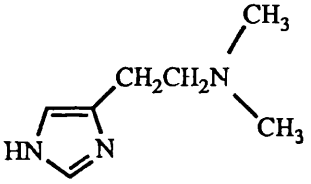
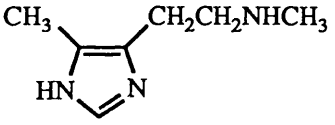
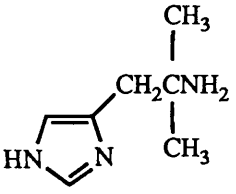
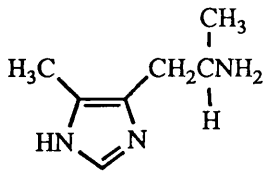
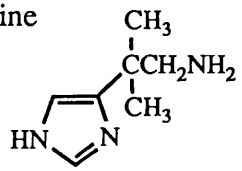
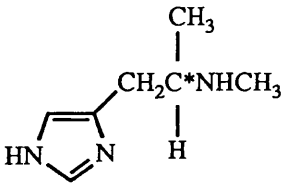
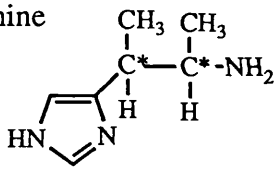
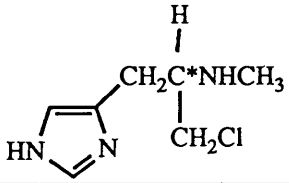
Histamine	H_1	H_2	H_3
α-Ethylhistamine 	0.11	1.2	0.6
β-Methylhistamine  (R), (S)	282	0.89	282
β- Ethylhistamine  (R), (S)	0.14	0.13	0.6
Substituted imidazole derivatives:			
N^{π}-Methylhistamine 	0.42	0.2	< 4
N^{τ}-Methylhistamine 	16.5	4.4	< 0.08
2- Methylhistamine 	16.5	4.4	< 0.08

Table 1.4: continued

Name Structure	H_1	H_2	H_3
Disubstituted:			
2,4-Dimethylhistamine 	0.3	3.8	
N^{τ}, N^{α}-Dimethylhistamine 	0.7	< 0.1	
N^{α}, N^{α}-Dimethylhistamine 	44	51	170
4, N^{α}-Dimethylhistamine 	0.16	36	
α,α-Dimethylhistamine 	0.035	0.2	270

Name	Structure	H_1	H_2	H_3
4,α-Dimethylhistamine				
	 4, α (+)-(S)-	0.26	8.7	
	4, β (-)-(R)-	0.33	4.8	
β,β–Dimethylhistamine		0.035	0.2	120
α, N^{α}-Dimethylhistamine				
	 (R)	0.7	0.4	4
	(S)	0.7	1	0.1
α,β-Dimethylhistamine		0.071	0.1	520
	Threo α, β-			33
	Erythro-α, β–			1000
	(-)-αS, βR-			18
	(+)-αS, βS-	0.035	0.25	1800
N^{α}- Methyl- α-chloromethylhistamine				
	 (R)	0.3	51	0.006
	(S)	0.3	7	1.1

1.7.4) Antagonists at H_1-receptors:

These are known as classical antihistamines. Many different structural classes of compounds show high antagonist potency at this receptor. They do not have a close chemical resemblance to histamine except apparently sharing the feature of an ammonium alkyl chain. The first H_1-receptor antagonists were discovered by Bovet[17] and collaborators in 1937. Typical H_1 antagonists (fig 1.8a) include chloropheniramine, promethazine, mepyramine and diphenhydramine. Pharmacological studies showed that, in addition to protecting guinea pigs against histamine-induced bronchospasm, these compounds also antagonised histamine-induced contraction of various other smooth muscles and lessened the symptoms of anaphylactic shock. The first H_1 antagonists were applied mainly in allergic conditions and were found to induce a strong CNS effect, including sedatory effects, thus they are used as sleeping aids. Some newer H_1 antagonists which have much reduced sedatory effects are Terfenadine, Acrivastine and Astemizole (fig 1.8b).

1.7.5) Antagonists at H_2-receptors:

Burimamide was the first H_2- receptor histamine antagonist discovered by Black and co-workers in 1972[15], followed by metiamide[97] and then the discovery and development of cimetidine[98], the first clinically useful H_2-receptor histamine antagonist. Some other examples are ranitidine,[99] famotidine,[102] tiotidine[100] and oxmetidine.[101] The structure activity studies[27] of cimetidine and analogues showed the apparent special significance of some structural features such as i) an imidazole ring or similar nitrogen heterocycle, ii) a flexible chain, especially $-CH_2SCH_2CH_2-$ and iii) a planar π-electron group which is very polar and has potential strong H-bonding (both as acceptor and donor) and contains the system -NH-C-NH-. Cimetidine, tiotidine and ranitidine are all very hydrophilic compounds which do not readily cross the blood brain barrier (fig 1.9).[82]

Fig 1.8a: Some typical H_1- antihistamines.

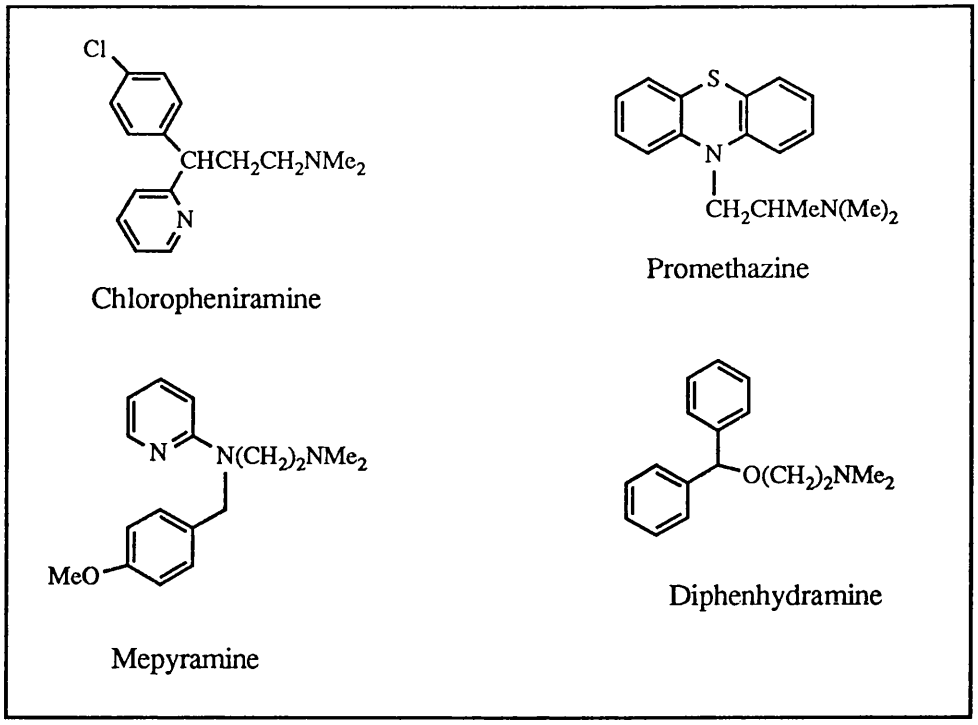


Fig 1.8b: Some H_1- antihistamines with low incidence of sedative side effects.

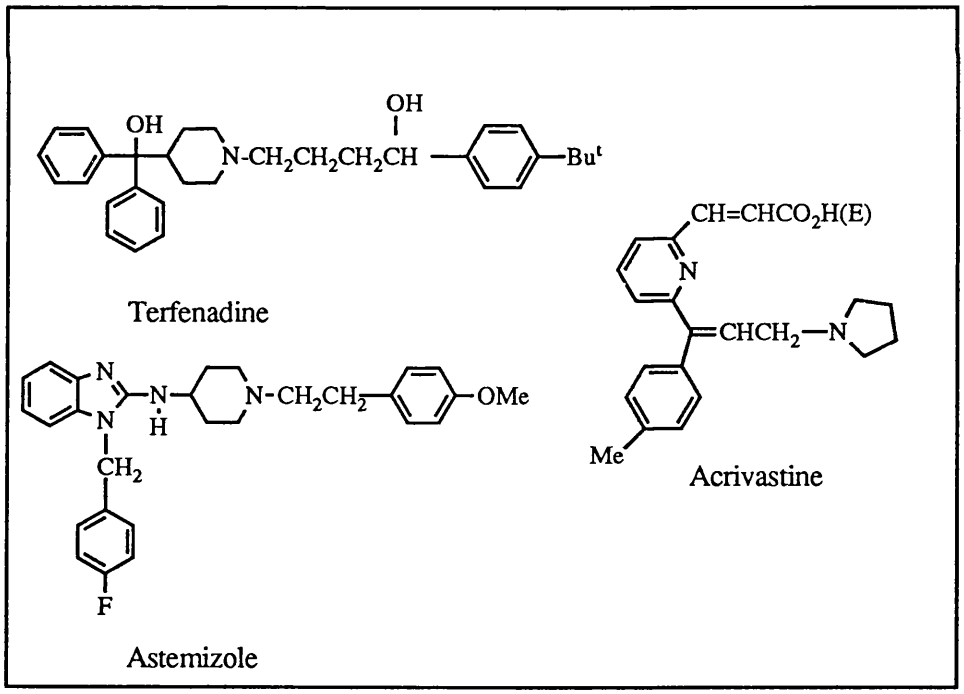
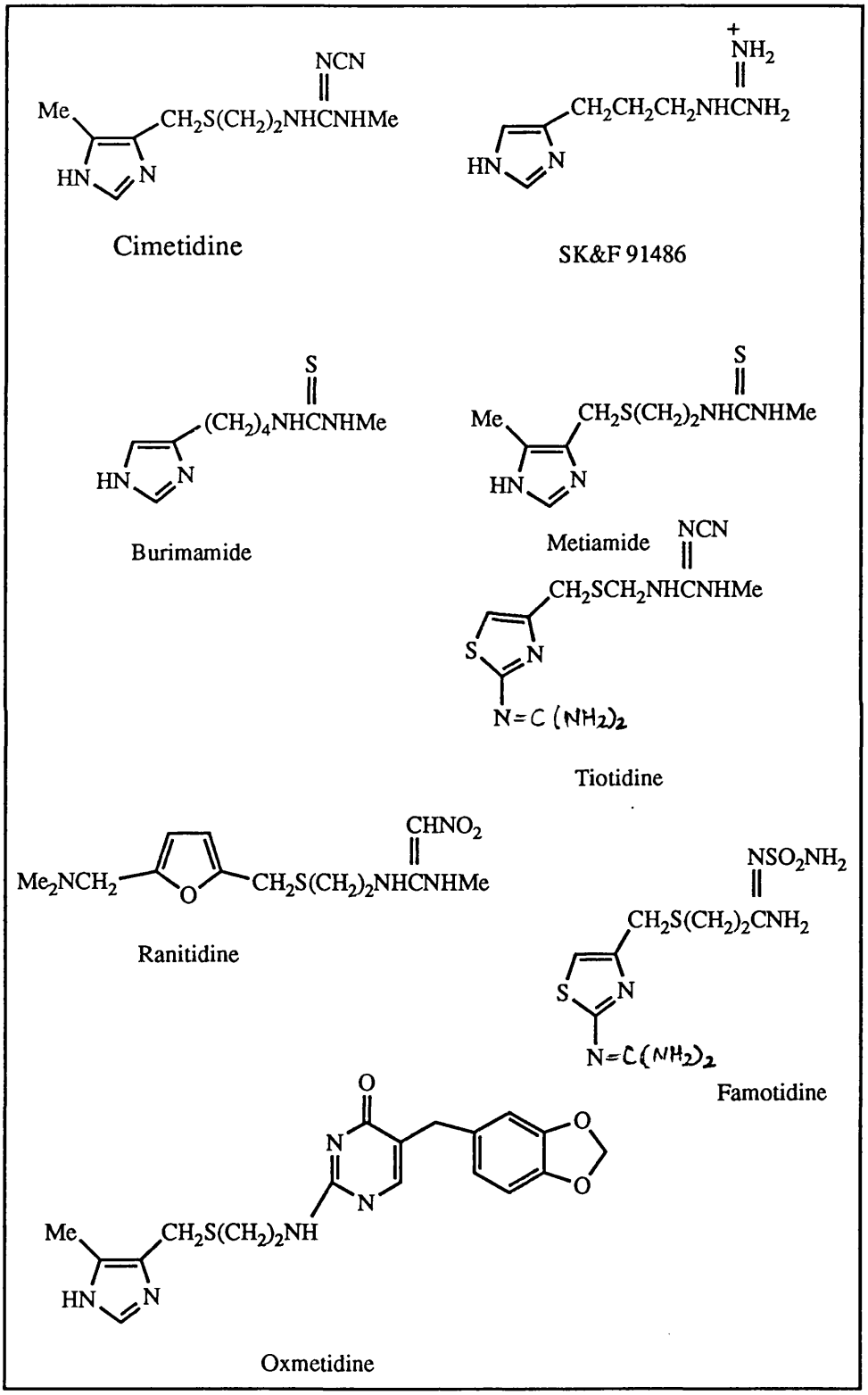


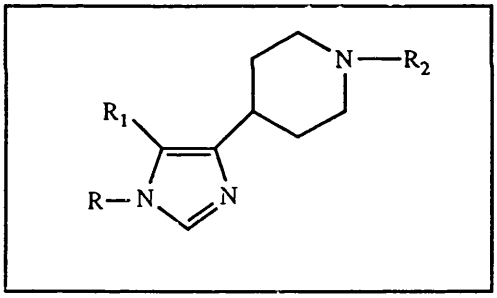
Fig 1.9: Some histamine H_2-receptor antagonists.



1.7.6) Antagonists at H_3-receptors:

Arrang et al.[5] have studied the possibility of using known H_1- or H_2- receptor ligands as chemical starting points to find a specific antagonist of histamine at cerebral H_3-receptors. The lipophilic H_1-receptor antagonists displayed negligible affinity (table 1.5) but agonists at H_1-receptors, such as betahistine, or at H_2-receptors, such as impromidine (selective and very potent) as well as H_2-receptor antagonists, such as burimamide displayed significant activity (tables 1.3 and 1.5). However these agents were generally highly hydrophilic and/or positively charged molecules with little brain-penetration ability. In their new approach[10], several analogues were made in which the ethylamine side chain was extended and its nitrogen atom included in a piperidine ring. The affinity was optimized by varying the nature of the piperidine-substituted groups with the general formula (fig 1.10)

Fig 1.10:



where R_1 = CH_3 or C_2H_5 , H. R =H or R_2.

R_2 = alkyl group, piperonyl, (benzoimidazolonyl-1)-3-propyl or -(CH_2)n-X-(C_6H_5)-R_3, n = 0-3, or -(C = Z)-NH-R_5

X = O, S, NH, CO, CH=CH or CH-C_6H_5-R_3

R_3 = H, CH_3, halogen, CN, CF_3, COR_4,

R_4 = alkyl, cycloalkyl, phenyl (CH_3 and F substituted),

Z = O, S, NH, $NHCH_3$, N-CN.

R_5 = alkyl, cycloalkyl (phenyl substituted, alkyl substituted), phenyl (CH_3, halogen or CF_3 substituted), phenyl-alkyl, naphthyl, adamantyl, *para*-toluenesulphonyl.

As a result, the first highly selective and potent competitive H_3- antagonist, thioperamide (N-cyclohexyl-4-(imidazol-4-yl)-1-piperidinecarbothioamide), was finally made. The study indicated that thioperamide competitively blocks the H_3- autoreceptor regulating [^{3}H]histamine release with a K_i = 4.3nM.

These results imply that the introduction of the nitrogen side chain into the piperidine ring reduces the conformational freedom of the side chain resulting in the loss of agonistic activity at the H_3-receptors.

Comparison of potencies of some histaminergic agents on histamine receptors.are shown in table 1.5.

Table 1.5[6,7]: Comparison of potencies of histaminergic agents on the guinea pig ileum contraction (H_1-receptors), atrial rate (H_2-receptors) and the inhibition of rat cortical ^{3}H-histamine release (H_3-receptors).

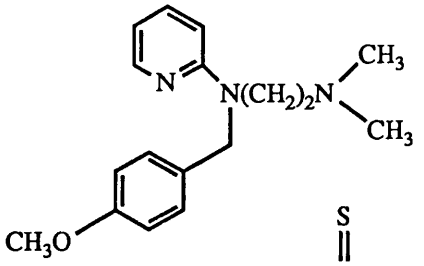
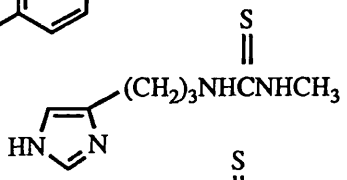
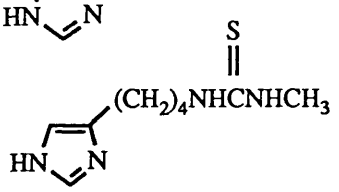
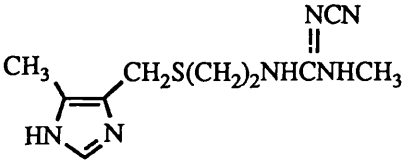
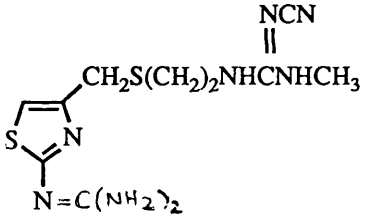
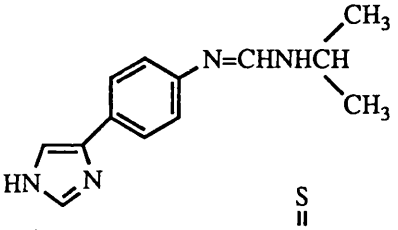
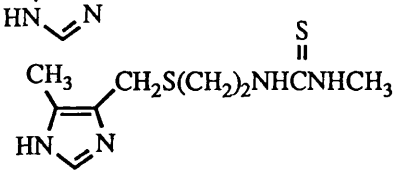
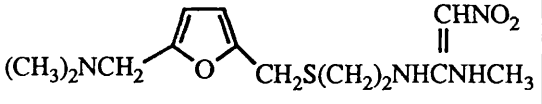
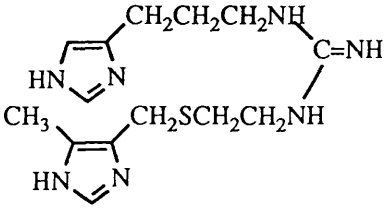
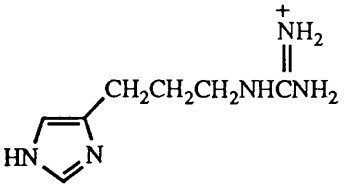
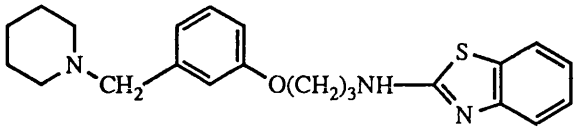
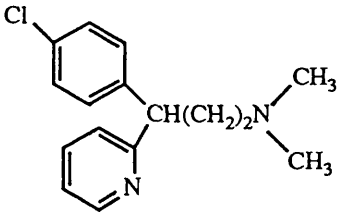
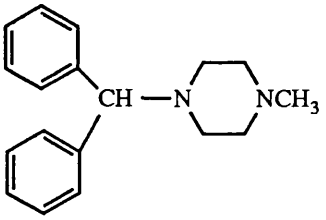
Antagonist activity (K_i 10^{-8}M)	H_1	H_2	H_3
Mepyramine 	40		>300
Norburimamide 		11500	80
Burimamide 	29000	780	7
Cimetidine 	45000	80	3300
Tiotidine 		1.5	1700
Mifentidine 		2.5	10
Metiamide 	92	>100,000	250
Ranitidine 		6.3	>120
Impromidine 		48	6.5

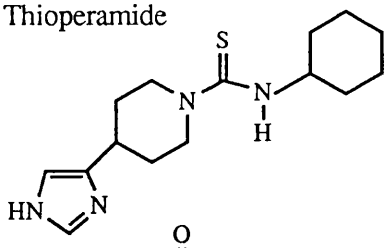
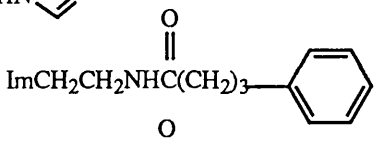
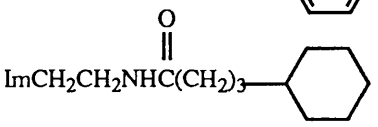
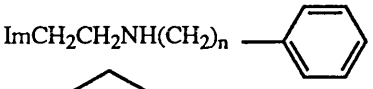
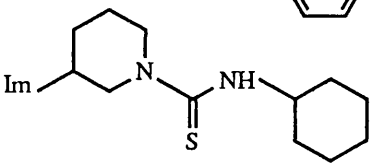
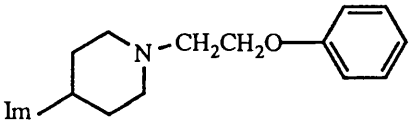
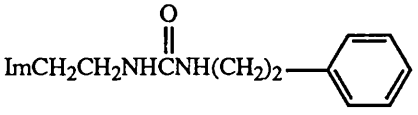
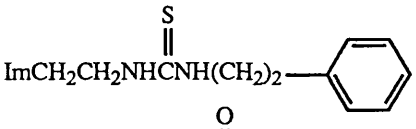
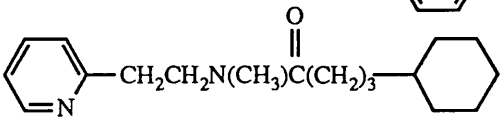
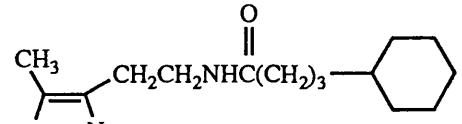
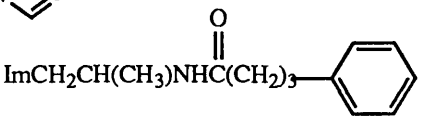
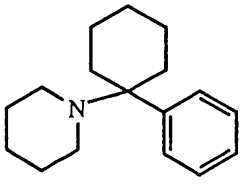
Table 1.5 contd.:

Antagonist activity (K_i 10^{-8}M)		H_1	H_2	H_3
SK&F 91486			2.2	8.8
Zolantidine		600	3.5	>1000
Chlorpheniramine		d 0.05 l 0.15		>5.8 >5.8
Cyclizine		130		>58

The thiourea-containing burimamide and the guanidino moiety in impromidine are equipotently active at H_3-receptors.

Table 1.6 shows some compounds reported to be H_3-receptor antagonists determined *in vitro* against histamine inhibition of potassium evoked [^{3}H]histamine release on rat brain slices.

Table 1.6[3,10,11,58,73]: Some reported H_3- receptor antagonists. Determined *in vitro* against histamine inhibition of [^{3}H]histamine release on rat brain cortex.

Compounds	K_i nM
Thioperamide 	4.3
	80
	50
	n =2 3200 n =3 670 n =4 700 n =5 2200
	40
	67
	240
	350
	>10,000
	>10,000
	1100
Phencyclidine 	25000

The imidazole ring is not crucial for H_3 antagonistic activity, since phencyclidine and the weak H_1 agonist, betahistine are reported to have some weak activity (see tables 1.6 and 1.3). The position of imidazole substitution in the piperidine ring plays an important role since the 3-isomer of thioperamide is about ten times less active (table 1.6).

The urea analogue of thioperamide was reported[10,27] to be eight times less active. The phenoxyethylpiperidine analogue of thioperamide was reported to be active. The phenylalkylhistamines are active with optimal chain length of propyl or butyl, substituting the phenyl ring with *para* -chloro or replacing a $-CH_2-$ in the ethyl chain with -O- or -S- resulted in similar potencies.

Thioperamide has negligible affinity for H_1- and H_2- receptors. It has been tested[22] using radioligand binding studies and *in vitro* pharmacological techniques and has been shown to have no effect at α_1, β_2, H_1, H_2, $5HT_1$-like, $5HT_2$, muscarinic or nicotinic receptors. Thioperamide was shown to have very weak affinity for α_2 adrenoreceptors, GABA receptors, dopamine and $5HT_3$ receptors, hence it is a highly selective H_3-receptor antagonist, being at least one hundred and fifty times more potent at H_3-receptors than at any other receptor tested.

A recent publication[50] of a class of new H_3-antagonists concerns a series of N^{α}-acylated histamine derivatives and their influence on H_3-antagonistic activity. The general construction pattern of these compounds was:

Histamine like----------polar linking group----------(spacer)----------cyclic residue.

The spacer has been changed from one to four methylene groups and also incorporates hetero atom linkages. In addition, the phenyl ring of the cyclic residue has been replaced by bulkier (diphenyl cyclohexyl group) or more hydrophilic substituents such as imidazol-4-yl, and 2-pyridyl.

Some of the best of the series are shown in table 1.6. The structure-activity relationship study of the series has shown that a non-hydrophilic and not too bulky cyclic residue has to be in an optimal distance (three to four methylene groups) to the amide displays the optimum antagonist activity.

1.8) Physiological role of H_3-receptors

Arrang et al[7] first reported H_3-histamine receptors in 1983 and five years later not only confirmed the existence in the brain but also located and elucidated their physiological involvement in the control of histamine synthesis and release in the living animal;[6,7,9,11,69,103-5] In this respect, the function of H_3-receptors seem to be analogous to that of other classes of presynaptic receptors mediating feedback regulation of several neurotransmitters.

The blockade of H_3-receptors eliciting a marked facilitation of histaminergic transmissions in the central nervous system should be useful in behavioural studies of sleep and wakefulness, cerebral circulation, energy metabolism and hypothalamic hormone secretion, whereas stimulation of H_3-receptors could cause sedation, producing anticonvulsant effects and diminish microvessel permeability, therefore having a therapeutic potential.[69,70,23,59,13].

Bristow and Bennett[18], reported administration in mice of selective H_1, H_2, and H_3 agonists. The H_1 agonist induced a weak hyperactivity, whereas the H_3 agonist causes hypoactivity (reduced by H_3 antagonists), and H_2 agonists did not induce any effect. These results indicate the indirect role of H_3 receptors in activity patterns.

Ishikawa and Sperelakis[41] concluded from their study on H_3-receptors using N^{α}-methylhistamine that H_3- receptors, upon stimulation, produce vasodilatation by inhibition of sympathetic tone. The presence of H_3- receptors on airway nerves has been suggested;[106] (R)-α-Methylhistamine modulates cholinergic neurotransmission at the level of parasympathetic ganglia in guinea pig airways and at postganglionic nerves in guinea pig and human airways, effects which are blocked by thioperamide. (R)-α-Methylhistamine reduces secretion and motility in cat gastro-intestinal (GI) tract, reduces histamine synthesis in the respiratory tract and inhibits bronchoconstriction mediated by release of cholinergic and peptidergic transmitters.[107]

1.9) H_3-receptor assay and visualisation:

In autoradiographic visualisation of the H_3-receptors [^{3}H](R)-α-methylhistamine was used for the rat brain and membranes of guinea-pig lung.[5] In comparison H_1- and H_2- receptors appear significantly more abundant. In binding studies, sites labelled with [^{3}H](R)-α-methylhistamine with a variety of compounds (histamine agonists), K_i values have been found which were five to ten times lower than their EC_{50} values in functional studies. Arrang et al[5] have given a possible explanation that the H_3-receptor exists in two affinity states. Like other amine receptors, it is coupled to its (still unidentified) effector system via a G-protein, while it is unusual that binding is modulated in the same directions by Ca^{2+} and guanyl nucleotides.[45] A potent radiolabelled antagonist would be more suitable and a safer probe, since it cannot be excluded that [^{3}H]R-α-methylhistamine labels more than one receptor subtype.

In autoradiographic studies[5] using [^{3}H](R)-α-methylhistamine, H_3-receptors were found to be fairly widespread in rat brain. Telencephalic areas such as the cerebral cortex, showed the highest grain densities. The receptor density in the cerebral cortex based on binding studies on cortical membranes has been reported to be approximately 30 fmol/mg of protein. In hypothalamus, a relatively low degree of labelling was observed, indicating that H_3-receptor density is not the same on all histamine neurons and/or that H_3-receptors are not restricted to the latter. The receptor density is very low (5±2 fmol/mg of protein) in guinea pig ileum.

CHAPTER 2

SELECTION OF SERIES OF COMPOUNDS FOR STRUCTURE-ACTIVITY STUDIES AS HISTAMINE H_3-RECEPTOR ANTAGONISTS

In seeking a specific antagonist of histamine at cerebral H_3-receptors able to cross the blood-brain barrier, the possibility of using known H_1- or H_2- receptor ligands as models was considered. The H_1-receptor antagonist (fig 1.8) tends to concentrate in the brain, achieving a brain-blood concentration ratio of 3.1:1, but displayed a negligible affinity (table 1.5) and cimetidine, a typically polar hydrophilic H_2- antagonist, showed some affinity but with a brain/blood concentration ratio of 0.03:1. i.e. a hundred fold lower than that for mepyramine.[82]

It is generally accepted that the most important physicochemical properties determining the rate and extent of passive entry of compounds into the brain are:[108]

i) the degree of ionisation at physiological pH (7.4)(non-ionised compounds enter readily),

ii) molecular size (there is a noticeable decrease in penetrability above molecular mass = 400).

iii) Lipid solubility; only molecules soluble in lipids are capable of traversing the membranes of the endothelial cells.

The reason is that the leaflets of the cell membrane including those of the capillary endothelium, are composed of lipid molecules. Each lipid molecule has two parts, a small head attached to two hydrocarbon long chains that form a tail. Each leaflet of the cell membrane is made up of many lipid molecules side by side, arranged with their head facing outwards. The complete membrane consists of two such leaflets in close opposition. The lipid molecules in each leaflet are not bound closely to one another. Instead, they are free to move in relation to one another, thereby forming a structure resembling a liquid in two dimensions.

Only substances capable of dissolving in lipid can diffuse through this two dimensional liquid and only molecules capable of traversing the membranes of the endothelial cells can enter the brain.

A physicochemical model for brain penetration using partitioning characteristics of the blood-brain barrier, has been developed by Ganellin et al.[82] and its application to the design of centrally acting H_2-receptor histamine antagonists shows a good inverse correlation between the logarithm of the equilibrium brain/blood concentration ratios and the partition parameter ΔlogP, defined as

logP (1-octanol/water)-logP (cyclohexane/water).

ΔlogP is related to the overall H-bonding ability of a compound by the equation (1):

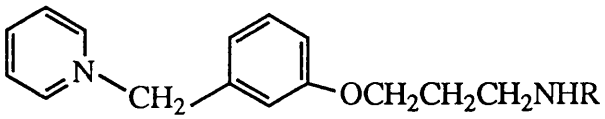
$$\Delta\log P = \log P_{oct} - \log P_{cyh} = \Sigma I_H - b \qquad (1)$$

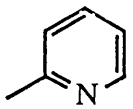
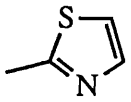
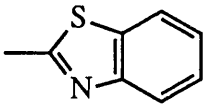
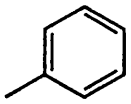
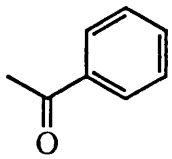
where I_H denotes the additive increment to H-bonding by a molecular fragment and b is a constant. Since brain penetration is inversely related to ΔlogP, this implies that brain penetration should be assisted by reducing overall hydrogen bonding ability.

The known H_2-receptor antagonists are usually highly polar, hydrophilic molecules with high hydrogen-bonding ability showing poor ability to enter the CNS. e.g. ranitidine (Fig 1.8). Recently, some. compounds containing aminophenoxyalkyl fragment, such as the [(piperidinylmethyl)phenoxy]propyl fragment, (Table 2.1) were introduced. These H_2-

antagonists are potent compounds able to cross the blood-brain barrier. e.g. Zolantidine, having an unusually low ΔlogP value of 1.69.

Table 2.1:[82] Shows a series of aminoalkylphenoxyalkyl substituted heterocycles able to cross the blood-brain barrier readily.



R	H_2 - receptor antagonism (Guinea -pig atrium) K_B M	C_{brain}/C_{blood}	ΔlogP
H	6.45×10^{-6}		
	8.13×10^{-7}	4.89	1.06
	3.89×10^{-8}	2.75	
	3.46×10^{-8}	1.38	1.69
	1.99×10^{-6}		
	1.62×10^{-8}	0.57	

High levels of brain penetration were reported for the aminoaromatic derivatives.

The histamine molecule was selected as the starting point because all H_3-agonists showing high potencies (table 1.4) are histamine derivatives and some of the highly potent antagonists (table 1.6) have strong structural resemblance to this molecule.

The agonist-antagonist relationships among various H_2-receptor ligands were considered as an analogy in case similar structure-activity patterns should apply to the histamine H_3-receptors.

Another lead used as a guide in the selection of compounds was the most potent and highly selective H_3-antagonist , thioperamide (N-cyclohexyl-4-(imidazol-4-yl)-1-piperidine carbothioamide (Fig 2.1)

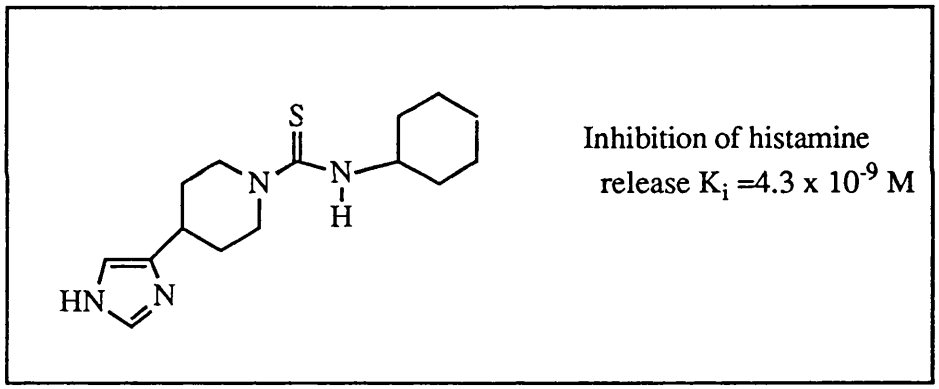


Fig 2.1: Thioperamide

In spite of high *in vitro* potency, thioperamide was shown to have a toxic effect *in vivo* observed by Schwartz et al at Inserm. The thiourea group could be the cause of toxicity; when metiamide (fig 1.9) was administered, the H_2 antagonist caused reversible granulocytopenia (a drop in the number of circulating white cells in the blood) in a small number of patients[109] and it was considered that this effect could be related to the thiourea group in the structure of metiamide.

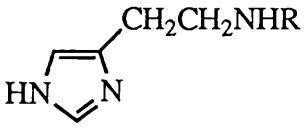
It was observed at Inserm that relatively high doses were required for an *in vivo* effect indicating an inadequate brain penetration ability.

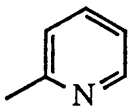
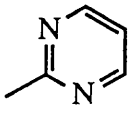
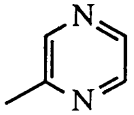
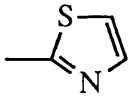
Brain penetration should be improved by reducing overall hydrogen bonding ability; hence removal of the -NH- group of the cyclohexyl thiourea could improve brain penetration and removal of C=S could eliminate the possible toxic effect observed. In our approach, the amino substituted heterocycles were chosen by analogy with the brain-penetrating H_2 antagonists (Table 2.1), since:

1) they are planar and have a π-electron system which could affect the interaction of the receptor-ligand in an electronic way.

2) In order to minimise the overall hydrogen bonding ability for targeting the molecules into the brain, aromatic amino groups ($I_H = 0.61$) were considered. The compounds (1), (2), (4) and (9) were prepared (fig 2.2).

Fig 2.2: 2-aminoheterocyclic derivatives of histamine



Compound No	R
(1)	
(2)	
(4)	
(9)	

The heterocycles are all less basic than the ammonium group of histamine. The lone pair electron of nitrogen is in the sp^2 orbital rather than in sp^3 orbital (aliphatic amines) and is less available for bond formation.[44]

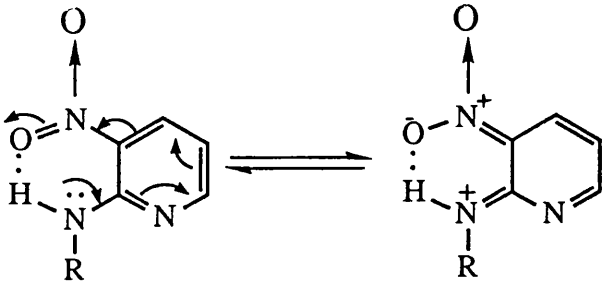
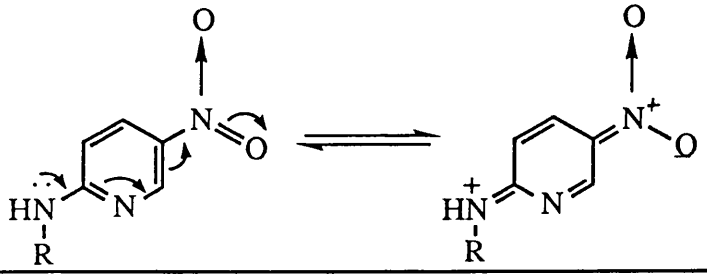
The necessity of polar functional groups incorporated into the heterocyclic ring system for enhanced antagonist activity was explored. In order to enhance the activity by introduction of these groups with low I_H values (i.e. the additive increment to hydrogen bonding by a molecular fragment), the 2-amino-5-nitropyridine derivative of histamine (7) was prepared.

Table 2.2: Contribution of I_H of some molecular segments to ΔlogP according to P.Seiler[72] is as follows:

Molecular Segment	I_H
$-NH_2$ aliphatic	1.33
$-NH_2$ aromatic	1.18
-NHR, R = aromatic	0.61
$-NO_2$	0.45

With the nitro group, hydrogen bonding ability would be probably reduced at the *ortho* position as in 2-amino-5-nitropyridine (6) due to intramolecular hydrogen bonding between the nitro and the 2-amino group.

Fig 2.3 : Possible intramolecular hydrogen bonding in compound (6):

Compound No	R = 4(5)-ImCH$_2$CH$_2$
(6)	
(7)	

An other electronic difference between these two isomers is indicated by their UV absorption. In the *para* isomer, the two electronically complementary groups, i.e. 2-amino and 5-nitro, produce a red shift in the absorption band due to extension of the chromophore from the electron-donating group to the electron withdrawing group through the pyridine ring. When these two groups are situated *ortho* to each other, as in compound (6), the observed spectrum is closer to that of the separate, non-interacting chromophores.

Compounds(6) and (7) differ also in the dipole orientation of their nitro groups relative to the side chain.

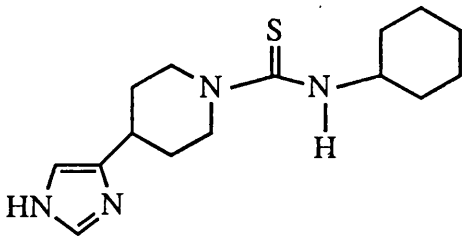
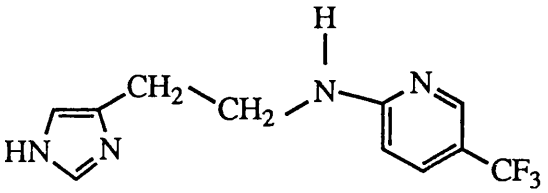
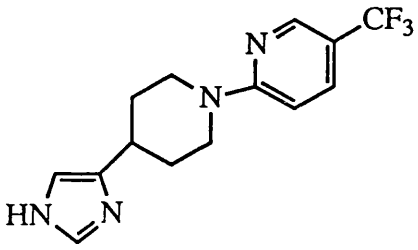
Other groups were introduced as shown in table 2.3.

Table 2.3: substituents and their effects:

Compound No	Group	Electronic Effect
(6),(7),(21)	*ortho* and *para* NO_2	Mesomeric and inductive: Electron withdrawing.
(19)	4-NO_2- 1-oxide	Mesomeric and inductive groups: Electron withdrawing.
(15)	*para* -CF_3	Strong inductive effect: Electron withdrawing
(24)	*para* -CO_2Me	Mesomeric and inductive: Electron withdrawing
(29)	*para* -NH_2	Inductive and mesomeric effect: Electron releasing.
(20)	2-chloro	Mesomeric and inductive: Electron releasing.
(5)	methyl	Inductive: Electron releasing.

The ethylamine side chain of compound (15), was also extended and the nitrogen was introduced into a constrained ring. i.e. piperidine ring as in thioperamide to give (25). Here the conformational freedom has been reduced and the secondary amine group has been replaced by a tertiary one.

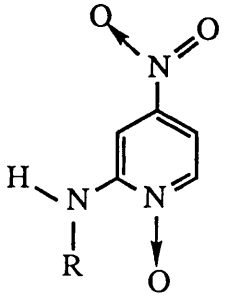
Fig 2.4: Analogues of thioperamide

Compound No	Structure
Thioperamide	
(15)	
(25)	

In compound (19), a dipolar interaction has been introduced with a nitro group at the *meta* position relative to the side chain and an N-oxide at the pyridine nitrogen. This could alter the dipole orientation from that of compounds (6) and (7).

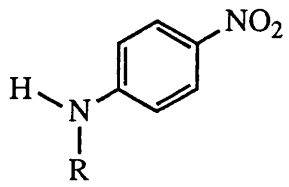
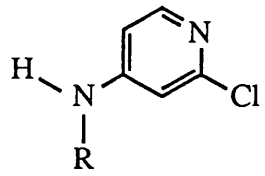
Fig. 2.5: Dipolar substituent

R = 4(5)-ImCH_2CH_2R

Compound No	Structure
(19)	

The importance of the pyridine type nitrogen *ortho* to the side chain for activity was explored when in compound (7), the nitrogen was removed leading to a phenyl analogue (21) or a 4-pyridyl group having an electron withdrawing group (Cl) *meta* to the side chain (20).

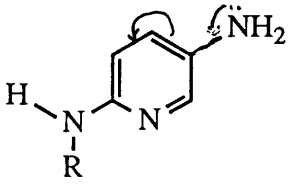
Fig 2.6: The *ortho* nitrogen was removed ; R = 4(5)-$ImCH_2CH_2$

Compound No	Structure
(21)	
(20)	

The *para* nitro (I_H = 0.45) in compound (7) was replaced by *para* amino group

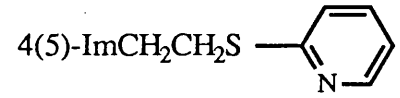
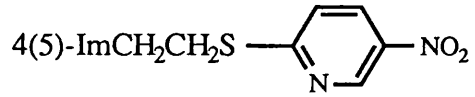
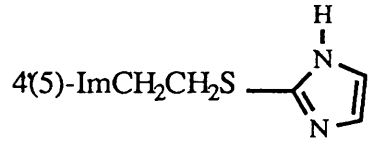
(I_H = 1.18) as in compound (28) in order to compare the effects of a powerful electron withdrawing group with an electron -releasing group.

Fig 2.7: Polar electron releasing substituent

Compound No	Structure
(28)	

Electron withdrawing groups in the pyridine ring would acidify the pyridyl -NH- hence increase its hydrogen bond donating ability. This is likely to reduce brain-penetrability. The possibility was explored of replacing the -NH- by another heteroatom. In the series nitrogen was replaced by sulphur as in 2-thioimidazole (18), 2-thiopyridine (16) and 2-thio-5-nitropyridine (17) analogues.

Fig 2.8: Replacing the -NH- by -S- :

Compound No	Structure
(16)	
(17)	
(18)	

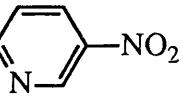
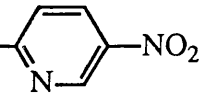
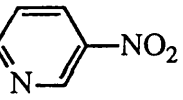
The extension of the side chain length had been observed to increase the activity during the development of H_2-antagonist, cimetidine. e.g. in the imidazolylalkyl guanidines, the increase in chain length from two methylene groups to three methylene groups increased the antagonist activity about five times[37].

Similarly the extension of the side chain length in the thiourea containing norburimamide (K_i =800 nM) led to burimamide (K_i = 70 nM) (table 1.5, chapter 1) resulting in ten fold increase in activity at the H_3 receptors.

The higher homologue (31) of compound (7) was prepared by my colleague Ms. Y.Khalaf..

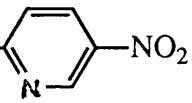
The longer side chain length analogue (10) of (31) was prepared by insertion of a sulphur atom into the side chain at the carbon atom next but one to the ring which is known to be similar in size and isosteric in nature with the $-CH_2-$ group[35].

Fig 2.8: Extension of the chain length.

Compound No	Structure
(7)	4(5)- $ImCH_2CH_2NH$—  —NO_2
(31)	4(5)- $ImCH_2CH_2CH_2NH$—  —NO_2
(10)	4(5)- $ImCH_2SCH_2CH_2NH$—  —NO_2

The lower homologue (27) of compound (17) was also prepared.

Fig 2.9: Minimising the chain length

Compound No	Structure
(27)	4(5)- $ImCH_2S$—  —NO_2

From the early structure-activity studies on systems, known to be mediated by the H_1-receptor which investigated imidazole related heterocyclic compounds by Walter et al.[83] (1941), it has been shown that imidazole can be replaced by other heterocycles often with retention of activity, provided that a pyridine type nitrogen is present in a position *ortho* to the side chain (chapter 1, table 1.2).

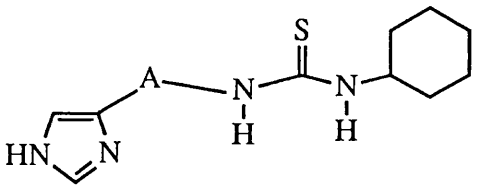
The need to remove an NH to assist brain penetration led to investigation of pyridine or N^{τ}-methyl analogues, hence the compounds (13), (14) and (26) were made which are the 2-pyridylethylamine analogues of the aminoheterocyclic derivatives (2), (3) and (7) respectively.

Pyridine provides only a hydrogen-bond acceptor and additionally, betahistine (N^{α}-methyl-2-pyridylethylamine) a 2-pyridyl analogue of N^{α}-methylhistamine (an agonist) is known to act as an antagonist at the H_3-receptor (see chapter 1, table 1.3, $K_i = 6.9x10^{-6}M$).

As an alternative route to remove >NH, N^{τ}-methylimidazole analogues were made as in the aminoheterocyclic derivatives; 2-(5-nitro)pyridine (22) and 2-(5-trifluoromethyl)-pyridine (23) which may be compared closely with compounds (7) and (15) respectively.

In part of a study in collaboration with Ms.Y.Khalaf, the steric relationship between the imidazole ring and the thiourea group was explored by synthesising lower and higher homologues of thioperamide in which the semi-rigid piperidine ring (capable of boat-chair interconversions) was replaced by a more flexible alkyl chain, and the tertiary amine has been replaced by a secondary one, having the general strucure shown.

Fig 2.10: Sterically modified analogues of thioperamide



Compound No	A
(11)	$-CH_2-CH_2-$
(29)	$-CH_2-CH_2-CH_2-$
(30)	$-CH_2-CH_2-CH_2-CH_2-$
(12)	$-CH_2-S-CH_2-CH_2-$

The compounds (29) and (30) were made by Ms. Y.Khalaf.

Replacement of a methylene group (-CH_2-) by its isosteric thioether linkage (-S-) at the carbon atom next but one to the ring in compound 12 was done, hoping for an increase in acivity as has been observed for burimamide and thiaburimamide at the H_2-receptor.

The list of the compounds prepared is shown in table 2.4.

Table 2.4: Shows compounds (1) to (11).

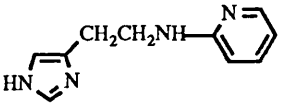
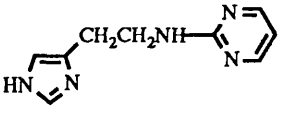
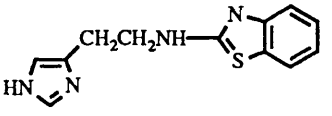
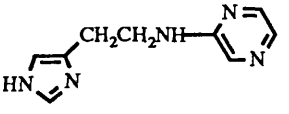
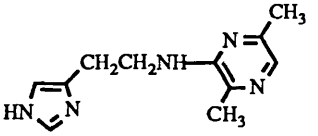
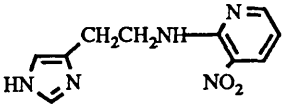
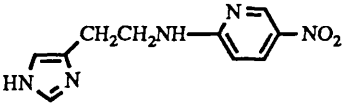
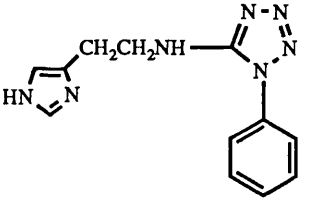
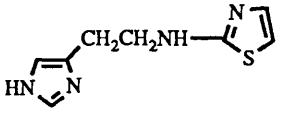
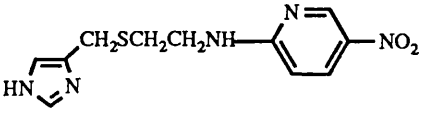
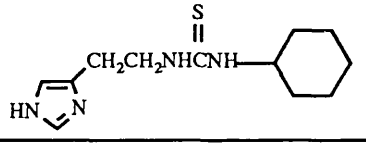
Compound No			Structure
(1)	UCL1038	SKH130A	 $2.2\ C_4H_4O_4$
(2)	UCL1017	SKH91B	 $C_2H_2O_4,\ H_2O$
(3)	UCL1029	SKH103D	 $C_4H_4O_4$
(4)	UCL1093	SKH176A	 $1.5C_2H_2O_4,\ 0.75H_2O$
(5)	UCL1094	SKH179A	 $2C_2H_2O_4,\ H_2O$
(6)	UCL1039	SKH117A	
(7)	UCL1040	SKH126B	
(8)	UCL1079	SKH135A	
(9)	UCL1090	SKH173A	 $2C_2H_2O_4$
(10)	UCL1068	SKH156A	
(11)	UCL1108	SKH198B	

Table 2.4: Compounds (12) to (22).

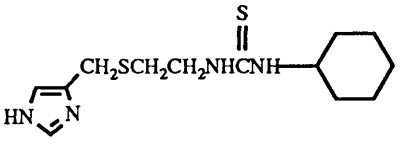
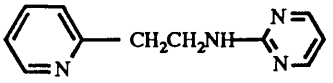
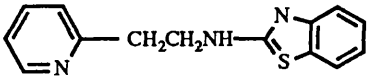
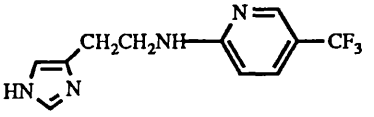
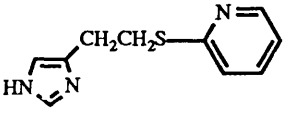
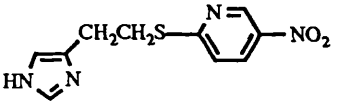
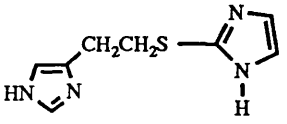
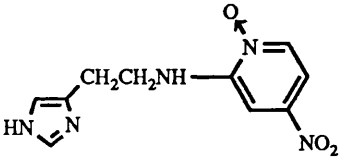
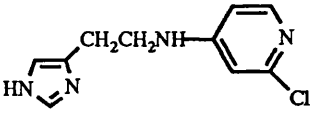
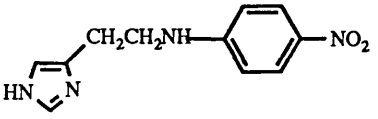
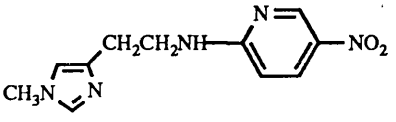
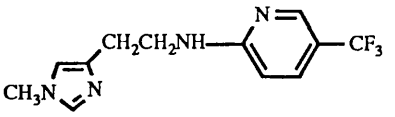
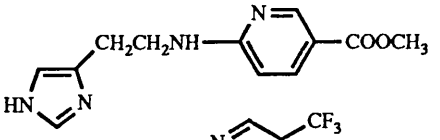
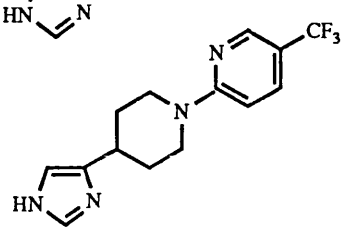
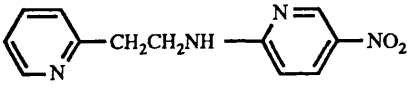
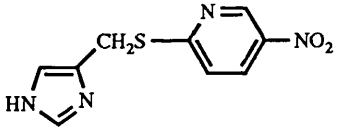
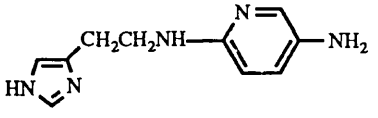
Compound No			Structure
(12)	UCL1109	SKH188A	 0.9 $C_2H_2O_4$
(13)	UCL1030	SKH108A	 $C_4H_4O_4$
(14)	UCL1031	SKH111B	 $2C_4H_4O_4$
(15)	UCL1235	SKH329B	 2 $C_2H_2O_4$, 0.5 H_2O
(16)	UCL1162	SKH238B	 2 $C_2H_2O_4$, H_2O
(17)	UCL1199	SKH298A	 0.25 H_2O
(18)	UCL1210	SKH280A	 1.61 $C_2H_2O_4$
(19)	UCL1256	SKH380A	 1.45 CF_3CO_2H, 0.1 C_3H_8O
(20)	UCL1236	SKH377B	
(21)	UCL1205	SKH299C	 1.25 $C_2H_2O_4$
(22)	UCL1264	SKH418A	

Table 2.4: shows compounds (23) to (28).

Compound No			Structure
(23)	UCL1265	SKH421A	 2 $C_2H_2O_4$
(24)	UCL1249	SKH394A	 1.7 $C_2H_2O_4$
(25)	UCL1283	SKH456A	
(26)	UCL1200	SKH307B	
(27)	UCL1191	SKH277C	
(28)	UCL1334	SKH452A	 2 $C_2H_2O_4$, H_2O

CHAPTER 3

SYNTHESIS DISCUSSION

The discussion of synthesis of the compounds can conveniently be divided into two sections:

3.1) Synthesis of 4-(4-piperidyl)imidazole involving formation of the imidazole nucleus attached to a pyridine ring via the corresponding α-aminoketone obtained from the Neber rearrangement of the appropriate tosyl ester.

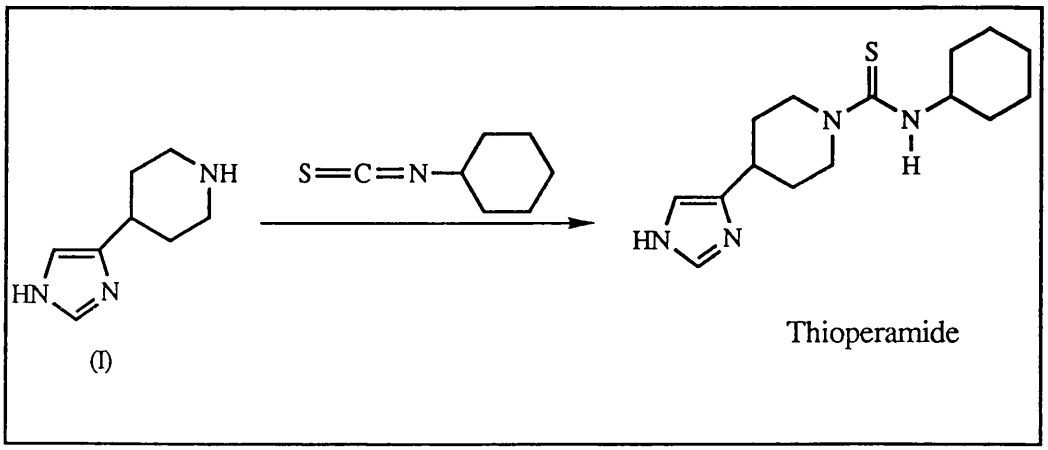
3.2) Synthesis of heterocyclic compounds by aromatic nucleophilic substitution.

3.1) Synthesis of 4-(4-Piperidyl)imidazole

4-(4-piperidyl)imidazole (I) is the key intermediate in the synthesis of thioperamide and the derivatives which were prepared (see chapter 1) and has played an important role in the understanding of the structural requirements for ligand-H_3-receptor interactions.

The synthesis of thioperamide from this intermediate has been described in a patent.[10]

Scheme 3.1:

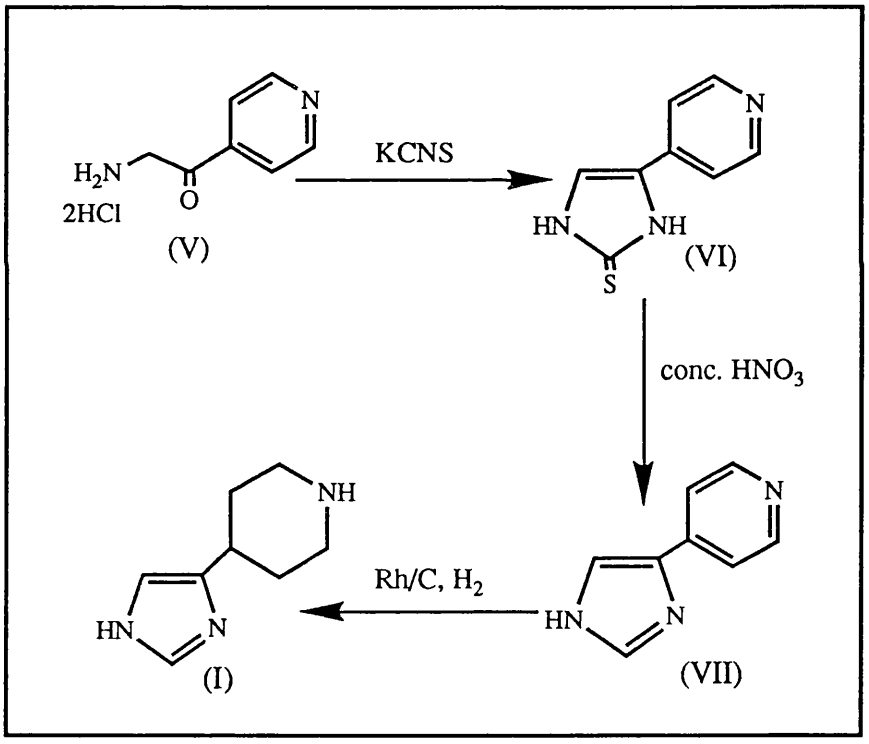


(I)

Thioperamide

Two groups of chemists[56] have encountered problems with the synthesis of the key intermediate (I). The syntheses of the key intermediate has been described by Schunack[68] starting from the α-aminoketone (V), which is reacted with potassium thiocyanate to give the corresponding imidazole-2-thione (VI). Desulphurisation of (VI) using the classical nitric acid oxidation affords the pyridyl imidazole (VII), which is then reduced to (I) by catalytic hydrogenation using Rh/C (A summary of the synthesis is shown in scheme 3.12 at the end of this section).

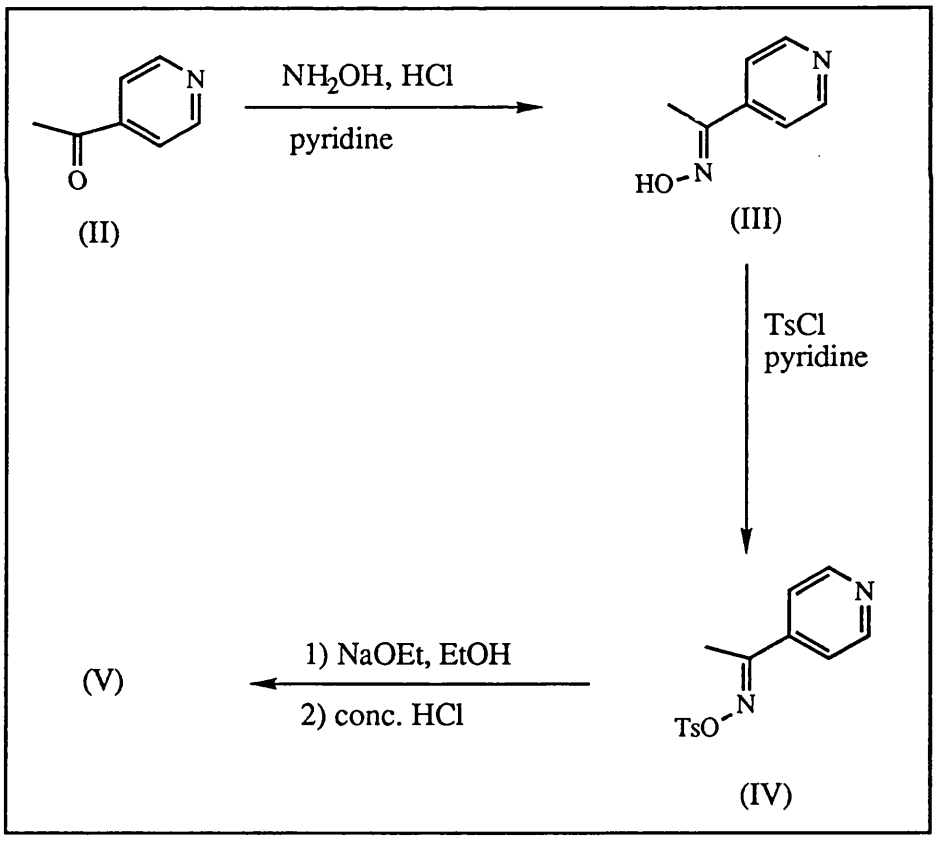
Scheme 3.2:



Schunack refers to (V) as the dihydrochloride, but apart from the melting point, gives no other characterisation. The synthesis of the 2- and 3- pyridyl isomers have been described by Clemo et al.[26] The 4-pyridyl isomer has been prepared following the same scheme from 4-acetylpyridine(II)

The synthesis was repeated several times and each intermediate was analysed completely. The oximation of the methyl ketone (II) in pyridine and crystallisation of the crude product from water afforded pure E-isomer.(III) in quantitative yield. The ^1H NMR (DMSO-d_6, 200MHz) shows the hydroxy group proton at 11.75ppm; LaMattina[47] has reported the preparation and ^1H NMR of both isomers and has observed the OH proton of the E-isomer and the Z-isomer at δ 11.65 and 10.97 ppm respectively.The tosylation of the oxime using tosyl chloride in pyridine gave a quantitative yield of the desired tosyl ester (IV).

Scheme 3.3:

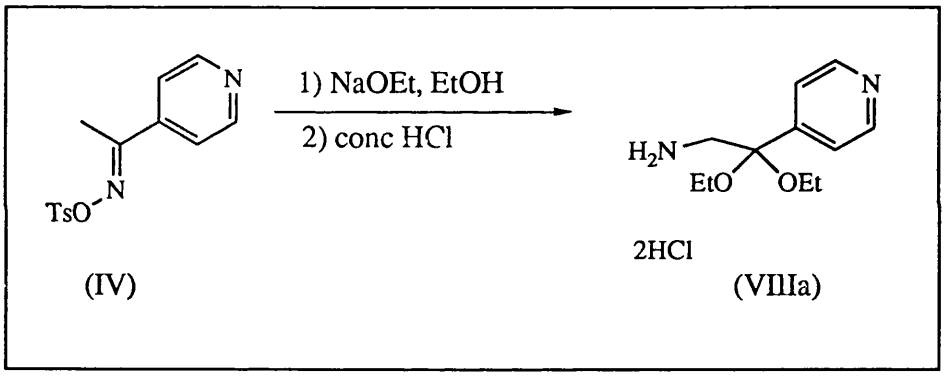


The final step involves the Neber rearrangement of the pyridine oxime-O-*p*-tosylate (IV) to the corresponding α-aminoketone (V) in the presence of a base followed by acidic hydrolysis.

3.1.1) Synthesis of the α-aminoketone

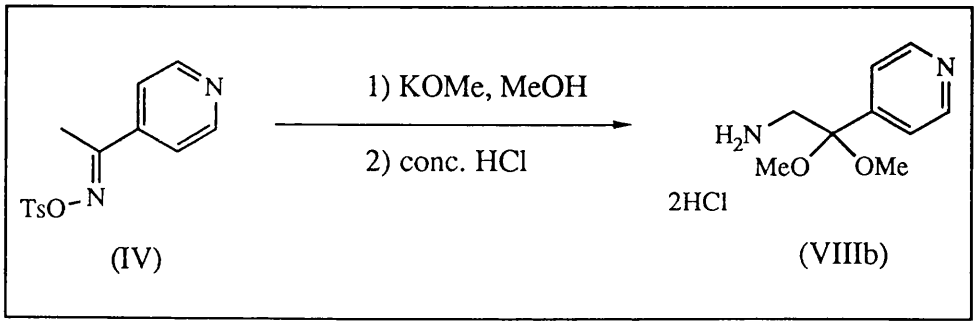
One of the methods used for the preparation of imidazoles involves α-aminoketones. A simple entry into α-aminoketones is the Neber rearrangement of the keto-oxime tosylates. Thus the key synthetic problem for the preparation of the target molecule (I) can be reduced to the preparation of the α-aminoketone (V). On attempting to reproduce this synthesis (scheme 3.4), we obtained poor yields of the ketone (V), but found that the main product was the acetal (VIIIa).

Scheme 3.4:



Subsequently we found that this product (VIIIa) had been described by La Mattina and Suleske in 1980[47] and Van der Meer et al in 1953.[79] No substantial difference was observed between the yields or the product composition obtained from using either sodium or potassium ethoxide (76% and 81% respectively).With methanol as solvent and potassium methoxide as base, the methyl acetal (VIIIb) was produced in 90% yield.

Scheme 3.5:



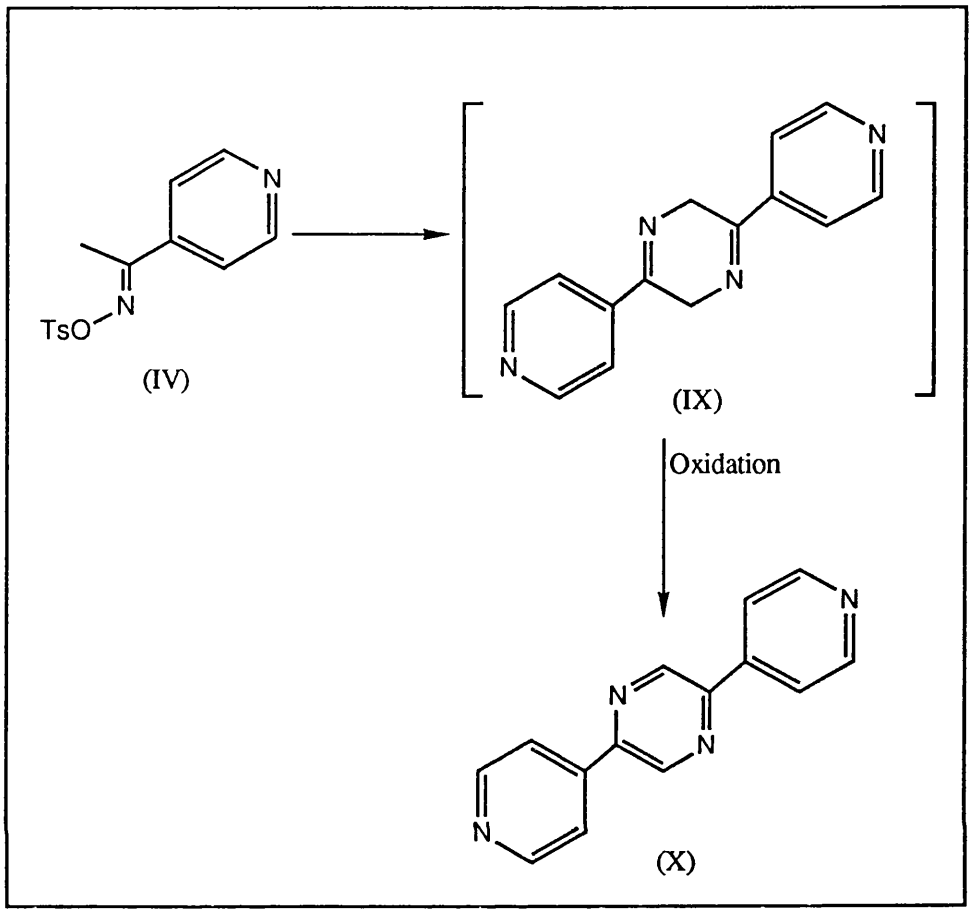
The acetals (VIIIa and VIIIb) were analysed, and the IR showed two characteristic acetal absorbance bands at 1068 and 1048 cm^{-1} (C-O str) and absence of C=O absorption. The ^{1}H NMR and ^{13}C studies confirmed the presence of dialkoxy groups at the α-carbon.

Since several attempts to form the imidazole -2-thione (VI) using the acetal (VIIIa or VIIIb) and KCNS were unsuccessful, preparation and characterisation of the pure α-aminoketone (V) was crucial.This had not been reported in the previous literature.

Characterisation of the α-aminoketone base by ^{1}H NMR (DMSO-d_6, 200MHz) was not possible due to its facile dimerisation, but the dihydrochloride showed the expected resonance for the α-CH_2 group and absence of OMe or OEt groups. The IR showed the characteristic carbonyl band at 1705 cm^{-1} and absence of the acetal absorbance bands. Good diagnostic evidence was provided by the UV spectrum of the product (see selected spectra, page 89, table 3.3) showing an absorption at 313nm. Initially, when working up the reaction mixtures, this absorption band was used to indicate whether the α-aminoketone was present in the crude acetal (after it had been isolated), but subsequently reverse phase HPLC proved to be more a sensitive assay method and traces of the α-aminoketone were detected.

Attempts to circumvent the formation of the acetal in the Neber rearrangement by using aqueous NaOH led to dimerisation of the aminoketone and immediate cyclisation to 2,5-di-(4-pyridyl)-3,6-dihydropyrazine (IX), which oxidised in the atmosphere to 2,5-di-(4-pyridyl)pyrazine (X).

Scheme 3.6:



Van der Meer et al [79] have reported that they have been unable to isolate the α-aminoketone (V) since in the presence of an amine (in this case another molecule of the compound), the cyclic pyrazine was always formed.[25] However, formation of the acetal appeared to be unavoidable and in fact, a way to prevent dimerisation leading to pyrazine (X) formation.

Various methods have been used to hydrolyse acetals.[24,28,40,46,48,54,76,80] Use of these methods to hydrolyse the acetal (VIIIa) were unsuccessful such as

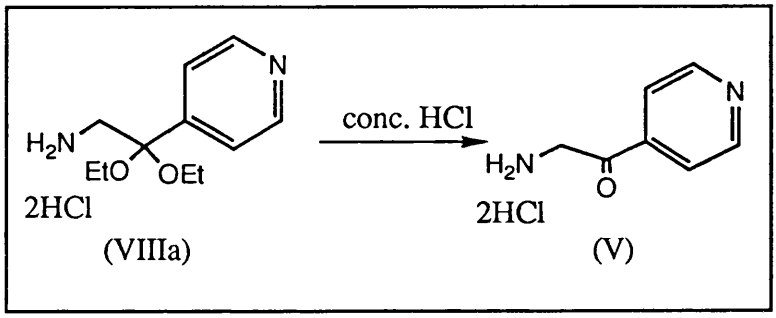
1) Using Amberlyst-15, a sulphonic acid based polystyrene cation exchange resin in acetone and water at 40°C under reflux for 20h ;

2) Conc. sulphuric acid and water, heating for 24h and using an ultrasonic bath for 4h;

3) Using *para* -toluenesulphonic acid in acetone and water, for 2 days.

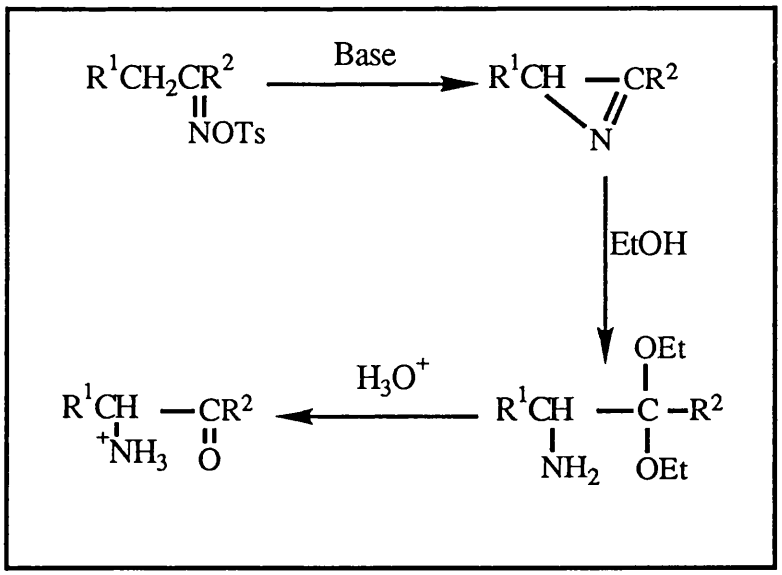
However, heating in conc. HCl at 50°C afforded a quantitative yield (98%) of the aminoketone (V).

Scheme 3.7:



Neber[52] has described the mechanism of the rearrangement of the tosyl oximes to α-aminoketones according to the following scheme.

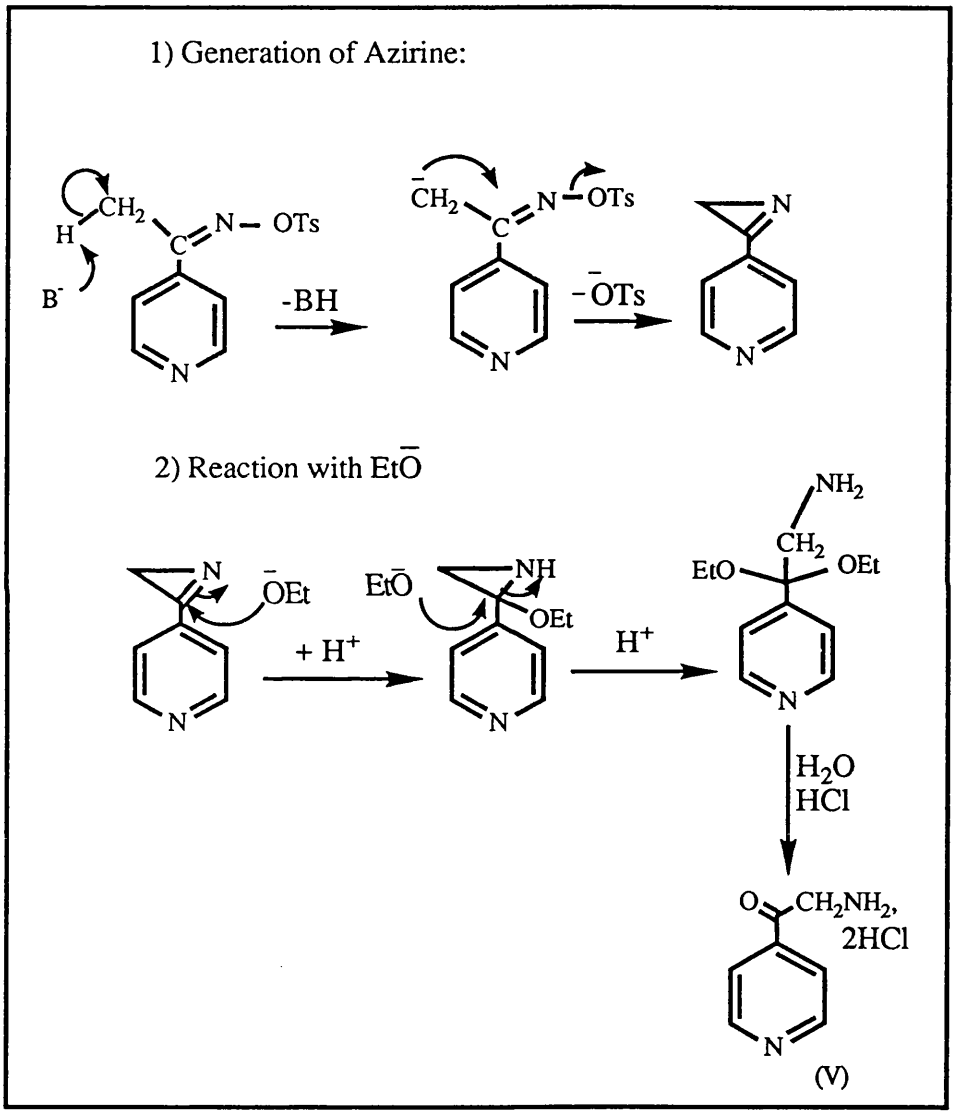
Scheme 3.8:



The general mechanism involving the formation of the three membered heterocyclic ring has been justified for many reactions of this type.[29,52]

In view of Neber's rearrangement mechanism, the overall reaction can be formulated as occurring via the following mechanism:

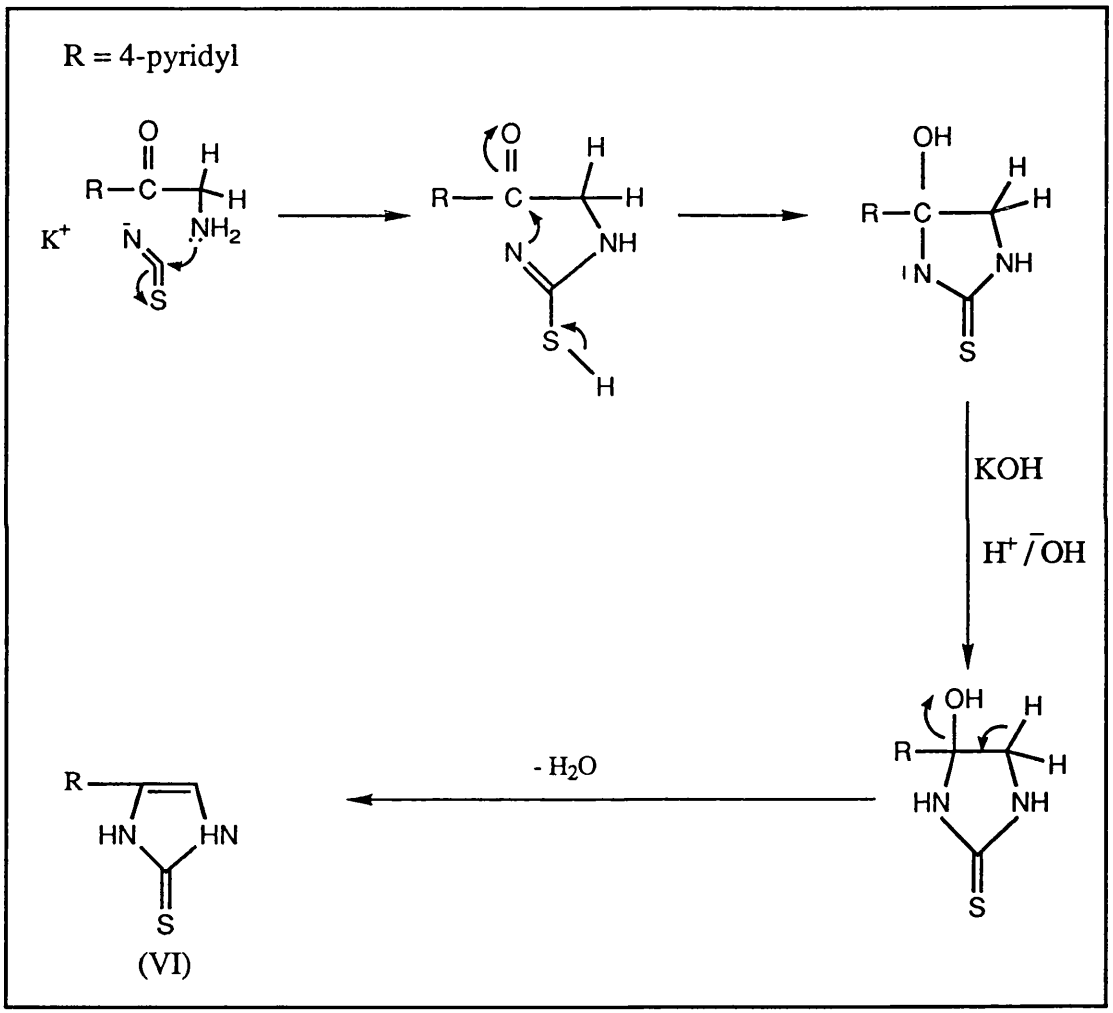
Scheme 3.9:



Treatment of (V) with KCNS gave the imidazol-2-thione (VI) in 65% yield

The mechanism[39] involves initial nucleophilic attack by the primary amine at the electron deficient carbon of KCNS followed by nucleophilic attack of the C=N- at the carbonyl group and subsequent elimination of water (scheme 3.10).

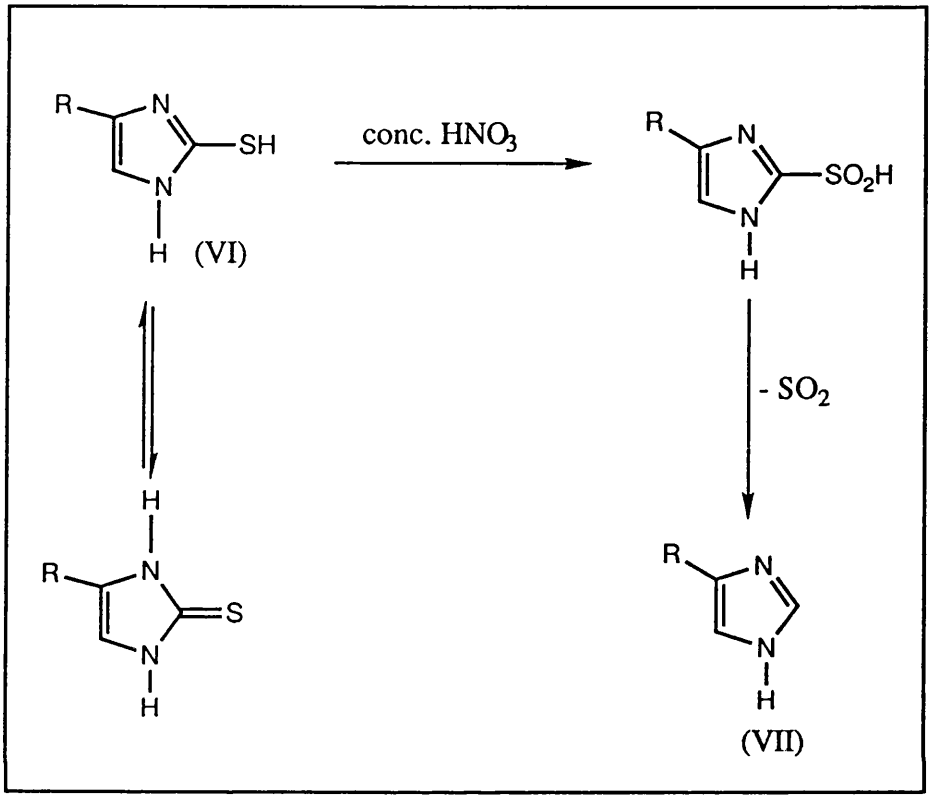
Scheme 3.10:



The imidazole-2-thione was oxidatively desulphurised with conc. HNO_3 yielding the pyridylimidazole (VII) in 56% yield.(Wohl and Marckwald method).[37]

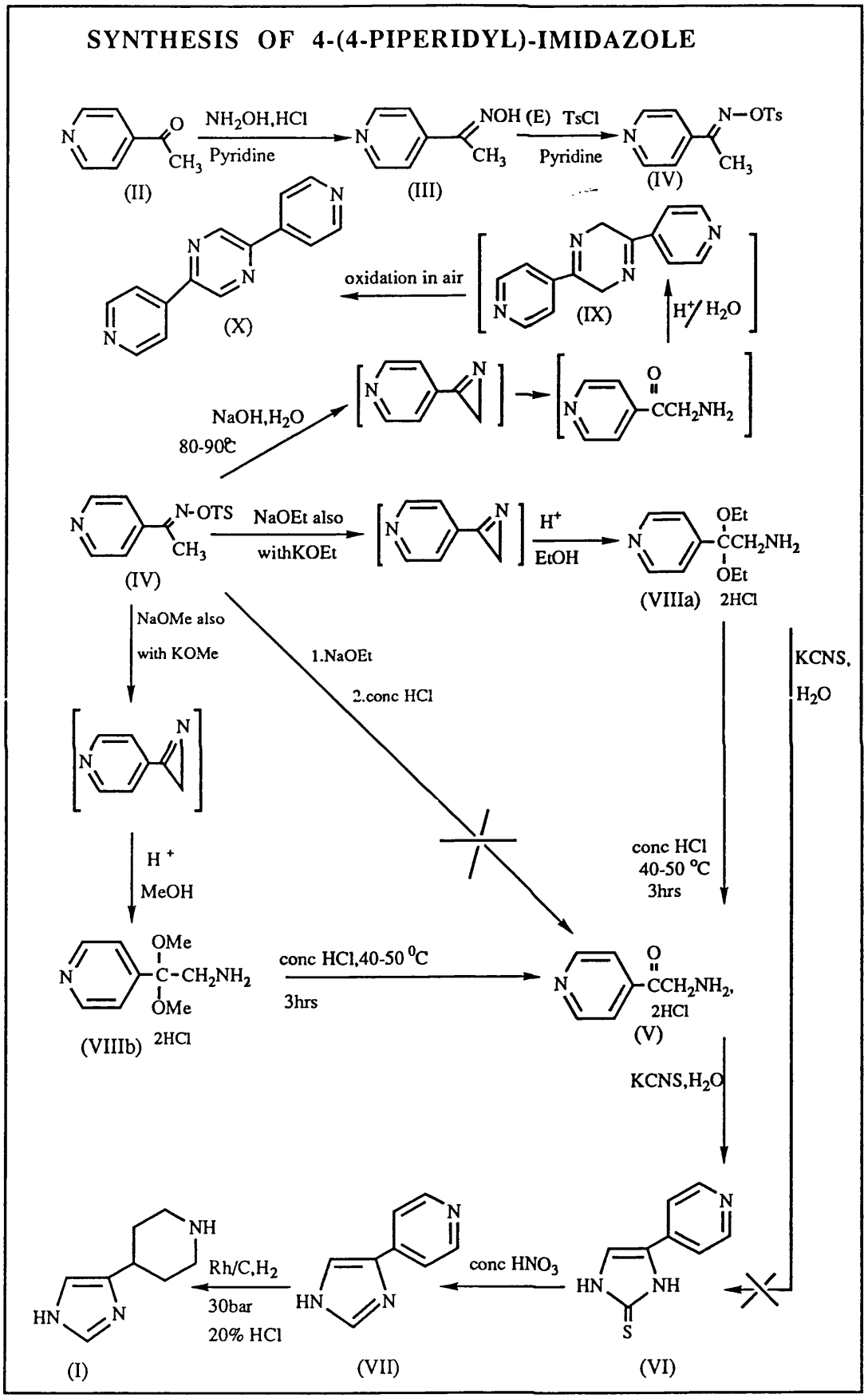
The oxidative desulphurisation involves the initial formation of a 4(5)-(4-pyridyl)imidazole-2-sulphinic acid followed by fission of the carbon-sulphur linkage with the formation of 4(5)-pyridylimidazole and sulphur dioxide (scheme 3.11).

Scheme 3.11:



Initially, the reduction of (VII) using hydrogen and Rh/C, which has been described by Schunack[68], was unsuccessful (scheme 3.12). The use of Adam's catalyst (PtO_2), or aluminium alloy, or Raney-Nickel was also unsuccessful. Only after traces of the thione(VI) were rigorously excluded, was the pyridyl imidazole (VII) successfully reduced by Rh/C affording a quantitative yield of (I) (97%). Presumably the presence of traces of thione poisons the Rh/C catalyst.

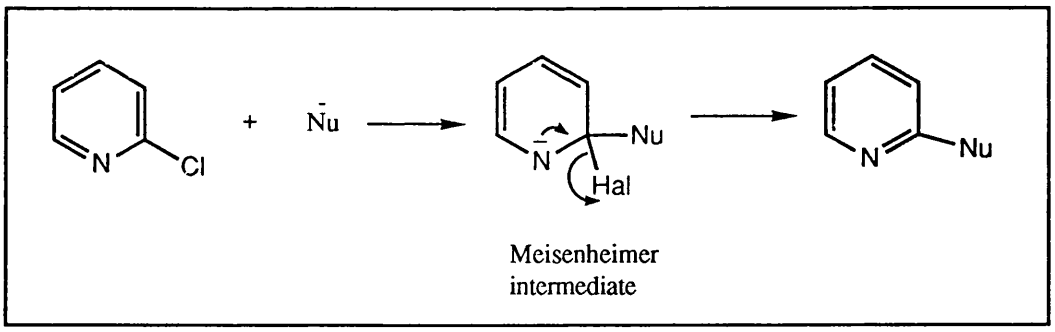
Scheme 3.12: Shows the summary of the synthesis of compund (I).



3.2) Synthesis of heterocyclic compounds by aromatic nucleophilic substitution.

Synthesis of compounds (1-4, 8,9,13) and (14) involve nucleophilic attack specifically at an α-ring carbon by a primary amine. Although the primary amine itself is insufficiently nucleophilic to react with pyridine or similar heterocycles, hence 2-halo (Cl or Br)-heterocycles were chosen to aid the substitution. Nucleophilic displacement of an α-halogen atom by the classical S_{AE} mechanism of nucleophilic displacement via a Meisenheimer intermediate is faciliated by mesomeric stabilisation of the transition state.[44]

Scheme 3.13:



The aromatic nucleophilic substitution reactions at the α-position of the heterocycles are assisted also by the typical effect of the heteroatom(s) which attract the electrons away from the carbon atoms of the ring.

Halides are the most common leaving groups in nucleophilic substitution for synthetic purposes.SN_2 reactions require powerful nucleophiles (which are generally strong bases) most often take place under basic conditions.

The conditions used for similar reactions have been described by Brown et al.[20] They were coupling the reactants in a high boiling point protic solvent or in a mixture of protic and aprotic solvents or in the absence of solvent i.e. in a melt.

At first using a high boiling point solvent e.g. DMF, DMSO seemed advantageous. The dipolar aprotic solvents having a large dielectric constant coupled with the solvation by hydrogen bonding i.e. poor anion solvation was expected to be a good choice of solvent. However due to their marked hydrophilicity, the small amounts of water absorbed could diminish the nucleophilicity. As in the synthesis of compound (1) in DMF, the reaction afforded a very poor yield. The other solvent tried was 4-picoline. The synthesis was carried out in i-PrOH and potassium bicarbonate which proved to be a better medium. Potassium bicarbonate was added as a weak base (pH= 8-9) to neutralise the acid produced (H^+Cl^- or H^+Br^- from the displaced chloro or bromo) but not such a strong base to initiate nucleophilic attack at the -NH- of imidazole.

Table 3.1 and table 3.2 show some of the compounds prepared .

Table 3.1: Strucure of the reactants, solvent, duration of the reaction ,yield of the 2-aminoheterocyclic derivatives of histamine and 2-pyridylethylamine analogues. The salts are a =dimaleate; b =hydrogen oxalate monohydrate; c =dioxalate; d =maleate

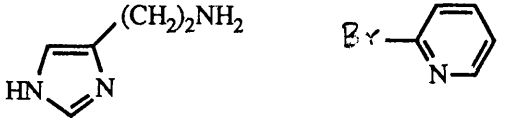
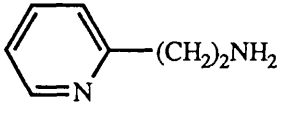
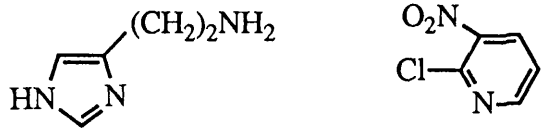
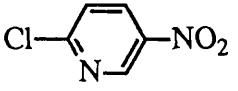
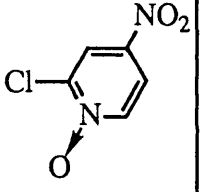
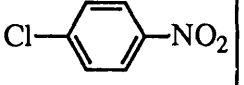
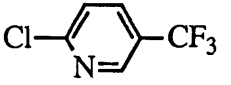
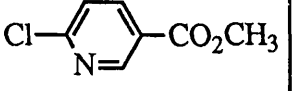
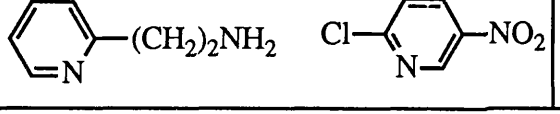
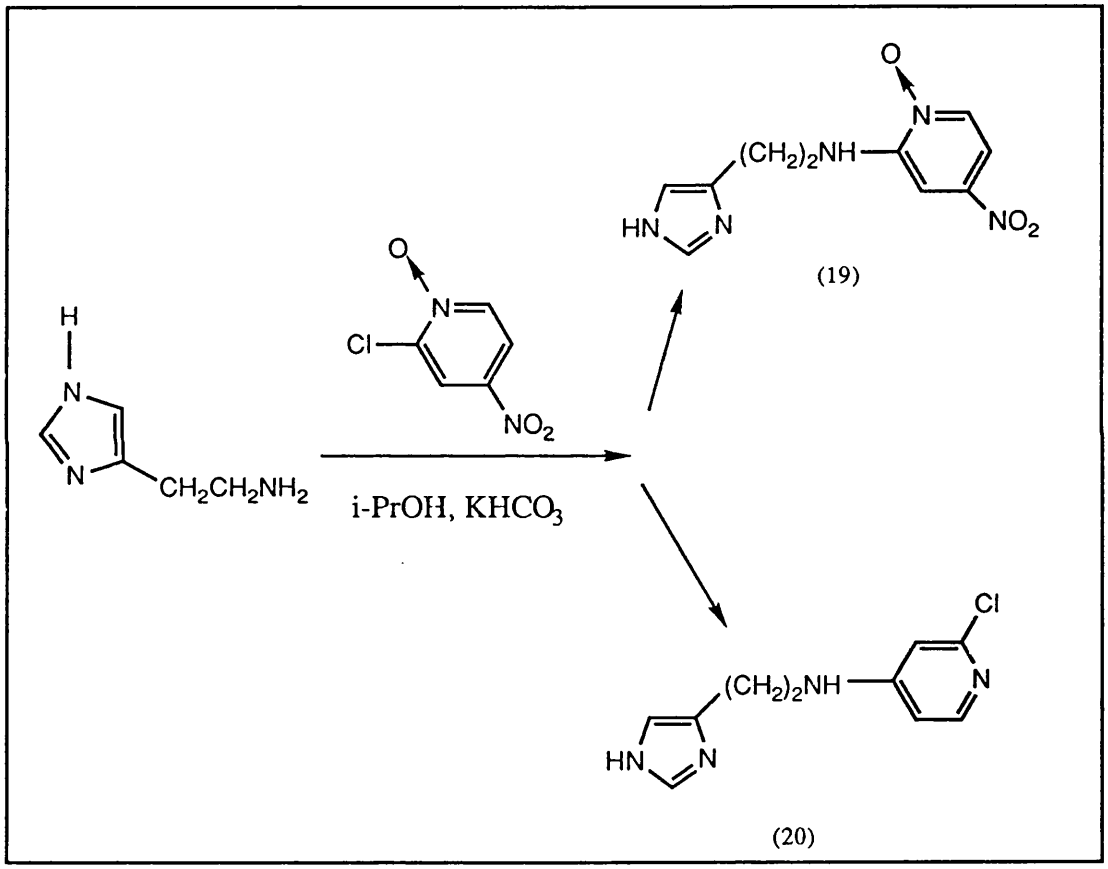
Compound No	Reactants	Solvent	temp. °C	Reaction Time hrs	Salt	M.P. °C	Yield %	Recrystall. solvent
(1)		4-picoline	145	26	a	137-8	8	Abs.EtOH
(2)		i-PrOH	80	36	b	200-2	27	Abs.EtOH and water
(3)		i-PrOH	80	48	d	137-8	66	Abs.EtOH and Ether
(4)		i-PrOH	80	72	b	201-2	25.6	i-PrOH and ether
(9)		i-PrOH	80	96	c	198-9	31	i-PrOH
(8)		pyridine	80	5	–	272-3	13.5	Abs.EtOH
(13)		i-PrOH	80	19	d	132-3	59	i-PrOH
(14)		i-PrOH	80	30	a	114-6	9[illegible]5	i-PrOH

Table 3.2: Structure of the reactants, solvents, duration of the reactions and yields of the substituted 2-aminoheterocyclic derivatives of histamine and 2-pyridylethylamine analogues. The salts are a = oxalate, b = dioxalate, c = trifluoroacetate.

Compound No	Reactants	Solvent	temp. °C	Reaction Time hrs	Salt	M.P. °C	Yield %	Recrystall. solvent
(6)		i-PrOH	80	3	–	177	64	Abs. EtOH and water
(7)		i-PrOH	80	48	–	161-2	26	Abs. EtOH and water
(19)		i-PrOH	21	72	c	193-4	15	i-PrOH and ether
(21)		i-PrOH	80	72	a	185-6	87	Abs. EtOH and $CHCl_3$
(15)		i-PrOH	80	3	b	174-5	46	i-PrOH and ether
(24)		THF	21	24	a	209-10	32	MeOH and ether
(26)		i-PrOH	50	2	–	143-4	44	i-PrOH

The synthesis of compound (19) involved the synthesis of the intermediate 2-chloro-4-nitropyridine-N-oxide which was prepared by the method described by Brown[21]. The hydrochloride salt of the 2-chloropyridine-N-oxide was nitrated at the 4-position in 90% yield by heating in conc. H_2SO_4 and fuming HNO_3. In the reaction of 2-halogeno-4-nitropyridine-1-oxide with histamine two products were isolated:

Scheme 3.14:



In the first attempt, the reactants were stirred at 21°C for 3h and then heated for 12h at 80°C. The two products isolated were compounds (20) as the major component (38%) and compound (19) as the minor product (15%).

When the reaction was repeated at 21°C for 8h, the major product was compound (20) and when the histamine base was added after the N-oxide had been treated with potassium

bicarbonate in i-PrOH and then stirred at 21°C for 3 days, the major compound was found to be the replaced halogen derivative i.e. compound (19).

The reactivity of the halo and nitro group in the presence of nucleophilic reagents has been investigated.[42,43,62,75,110,118,121]. In the reaction of 2-halogeno-4-nitropyridine-1-oxides with methanolic sodium methoxide, the nitro group is lost first but subsequent conversion into 2,4-dimethoxypyridine-1-oxide also occurs. However with dimethylamine or diethylamine, the halogen atom in 2-halogeno-4-nitropyridine-1-oxide is replaced preferentially, hence compound (19) was obtained. But consequences leading to formation of compound (20) could be:-

(i) time dependent;

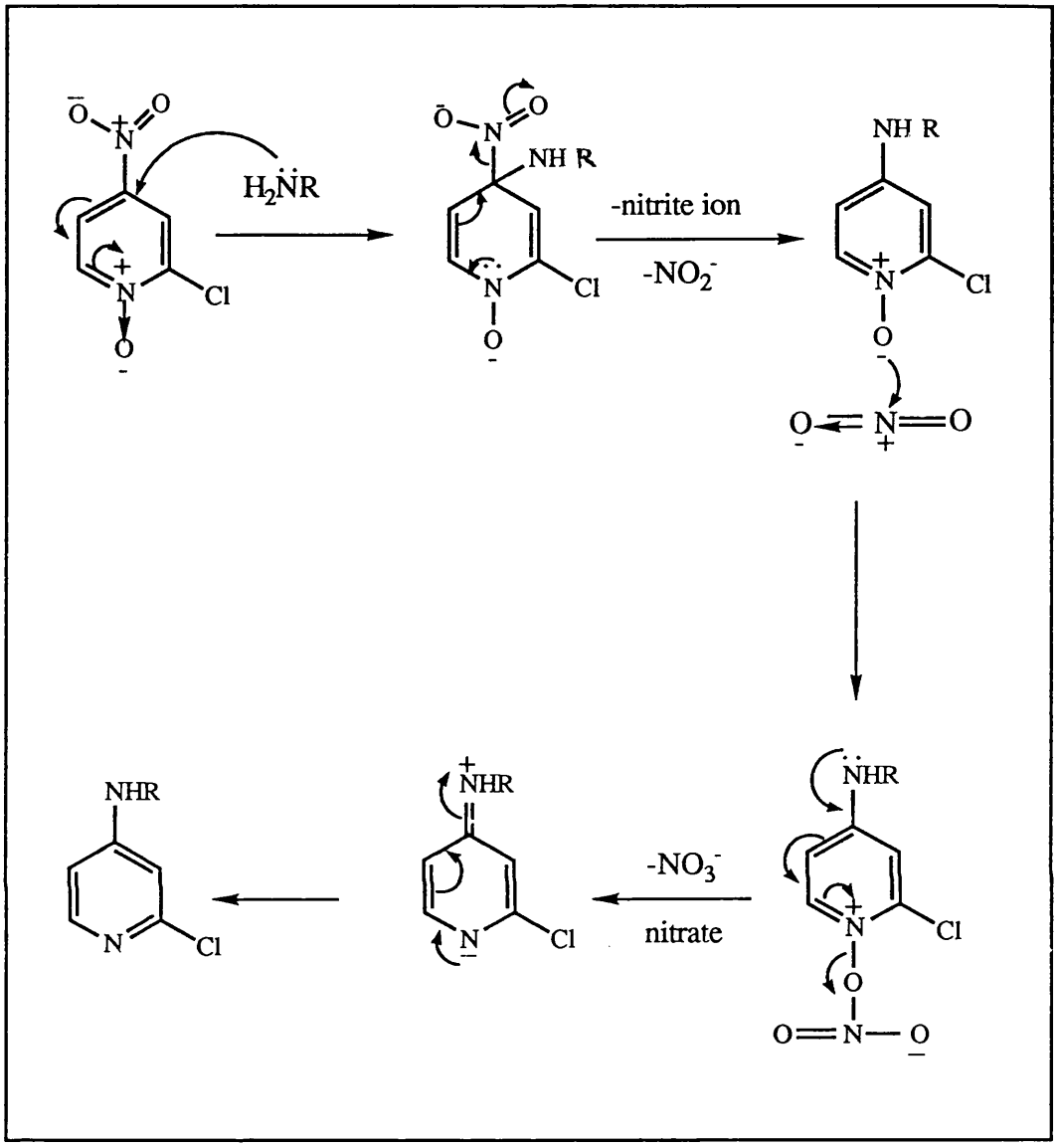
(ii) an excess of the histamine base increases percentage of (20) and

(iii) a higher temperature increases the ratio of compound (20).

In a recent report by Bolhofer et al[118], use of 2-chloro-4-nitropyridine instead of the N-oxide in a coupling reaction with a primary amine resulted in a preferential displacement of the nitro group suggesting that the N-oxide affects the reactivity at 2-position more than the 4-position.

The mechanism for formation of compound (20) could be described as the initial nucleophilic attack of the amine at the 4-position of the pyridine ring (addition) and elimination of the nitro group as nitrite ion (NO_2^-) is a good leaving group from sp^2 - hybridized carbon atoms. Relyea et al[62], in a survey of potential deoxygenating agents for pyridine 1-oxides, diphenylamine, pyrrole and i-PrOH were heated with pyridine 1-oxide and in no case could any free pyridine be detected. It could be speculated that the nitrite ion reduces the N-oxide and forms the nitrate ion which is neutralised by the potassium bicarbonate present in the reaction mixture. The mechanism leading to elimination of the nitro group and reduction of the N-oxide could be shown as in Scheme 3.15.

Scheme 3.15:



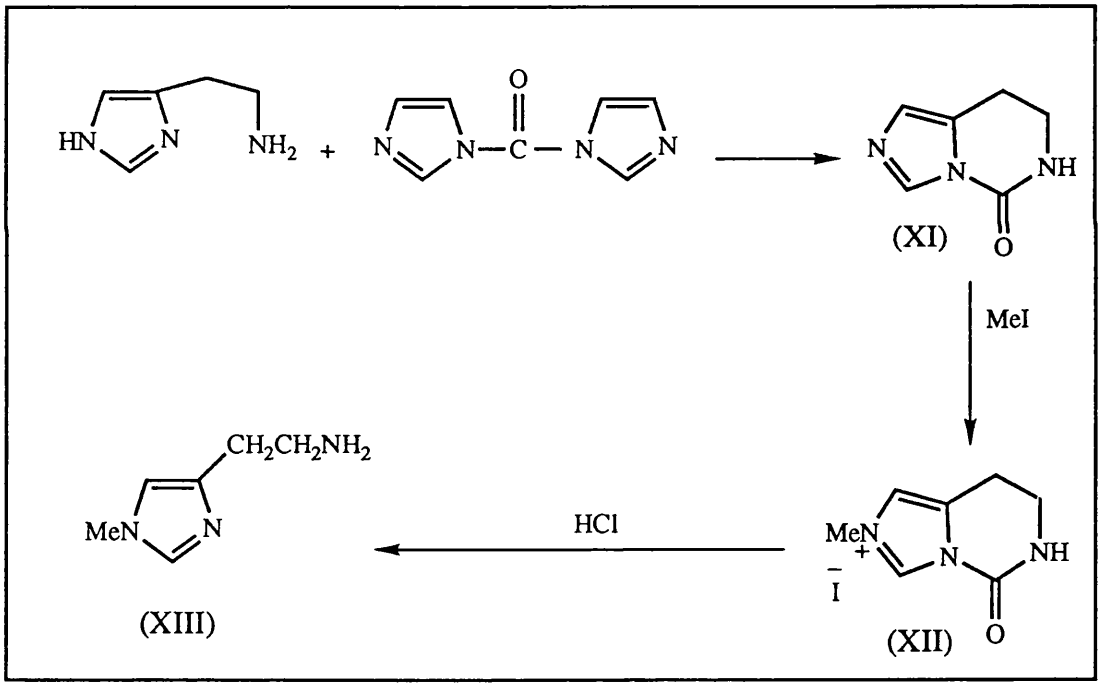
The nitrate formed is neutralised by potassium bicarbonate present in the reaction mixture.

3.2.3) Synthesis of N$^{\tau}$-Methylhistamine Derivatives

In the synthesis of the histamine derivatives described so far, due to the labile hydrogen of the -NH- of the imidazole nucleus, which is a possible reaction site in these reactions, in some cases poor yields were obtained. Extension of the reaction time led to only a small increase in the yield of the products, indicating that non-quantitative yield is not due to the short duration of the experiments. Using N$^{\tau}$-methylhistamine, apart from its importance for structure-activity relationship analysis, it was coupled with two other 2-halo substituted

pyridines under the similar condition for quantitative comparisons and relatively higher yields were obtained. For example the preparation of compound (22) gave 68% yield whereas that of compound (7) afforded 26% yield. N^{τ}-methylhistamine was first synthesized by Pyman[60] together with N^{π}-methylhistamine, from 4(5)-imidazolyl-acetonitrile. Methylation of N-acetylhistamine by Rothschild and Schayer[64] afforded N^{τ}-methylhistamine. Martin and co-workers[51] described the synthesis from 1-methyl-4-hydroxymethylimidazole which may be made from the corresponding imidazoline carboxylate by dehydrogenation and reduction. In a more recent paper by Durant et al.[33], the synthesis of N^{τ}-methylhistamine is described involving selective methylation of histamine at the N^{τ} position.

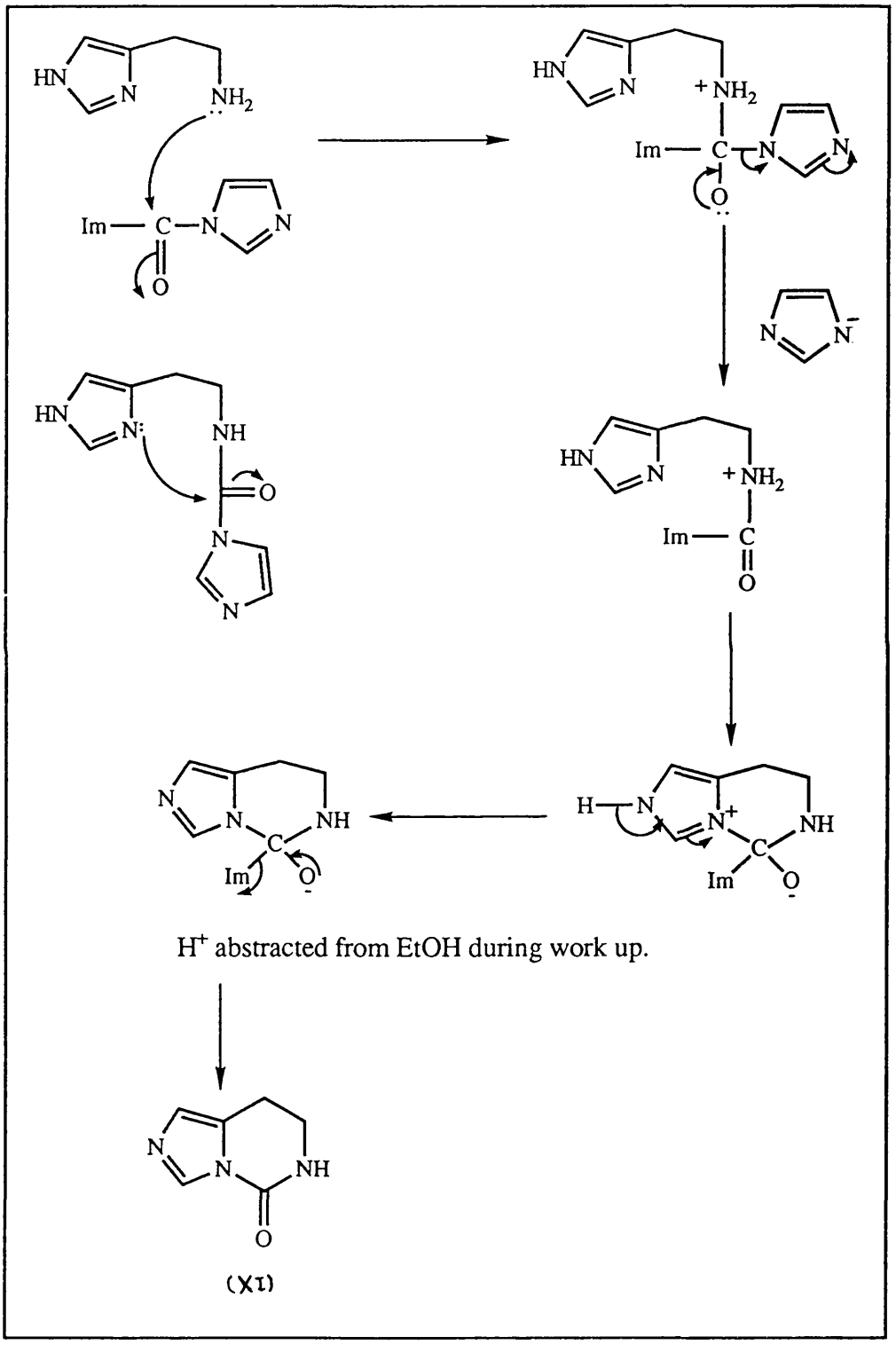
Scheme 3.16: Synthesis of N^{τ}-methylhistamine:



The histamine base was generated from the dihydrochloride by passing through a column of DOWEX IRA-400 OH^- form. The reaction between histamine and carbonyl diimidazole gave 5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidine (XI) in 76.5% yield. The Compound (XI) was also prepared by a method described by Potvin et al.[58] when the histamine base was treated with 1.1 equivalent of N, N'-carbonylimidazole in chloroform under reflux for 6h. The N-carbamoylated intermediate (scheme 3.17) precipitated immediately and slowly redissolved over 6h to afford, after work up by trituration of the

solvent free residue with ethanol, a 90% yield of the 5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidine (XI).The mechanism is shown in scheme 3.17.

Scheme 3.17:



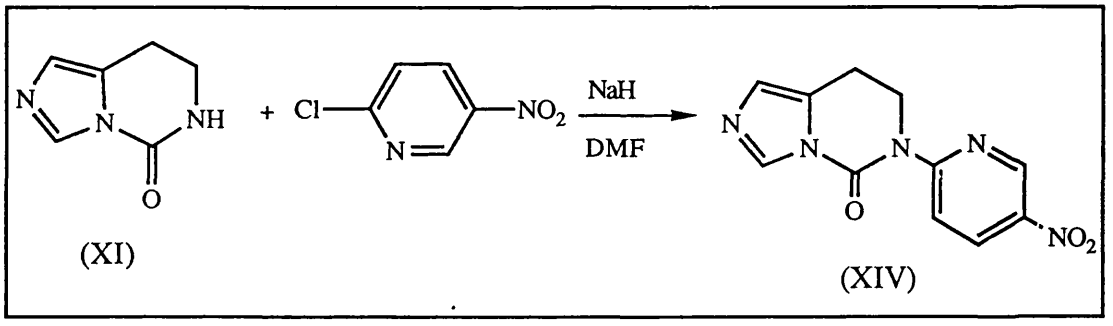
Methylation of the cyclic urea (XI) by MeI in dimethylformamide (21°C, 24h) produced the methoiodide (XII) in 90% yield, after removal of solvent and washing the residue with cold

methanol. Recrystallisation of the product from MeOH-Et_2O lowered the yield (56%). The reaction involves the conversion of a tertiary amine to a quarternary salt which is an example of the Menschutkin reaction.[111] Hydrolysis (refluxing 12M HCl under Ar) followed by neutralisation (NaOH) and trituration of the dried reaction mixture with $CHCl_3$ afforded a quantitative yield of very pure N^{τ}-methylhistamine (XIII). For characterisation, the oxalate salt was prepared.

The reaction of N^{τ}-methylhistamine with 2-chloro-5-nitropyridine and 2-chloro-5-trifluoromethylpyridine gave 68% and 46% yield respectively.

The 5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidine (XI) was coupled with 2-chloro-5-nitropyridine to give compound (XIV) using sodium hydride to generate the nucleophile.

scheme 3.18:



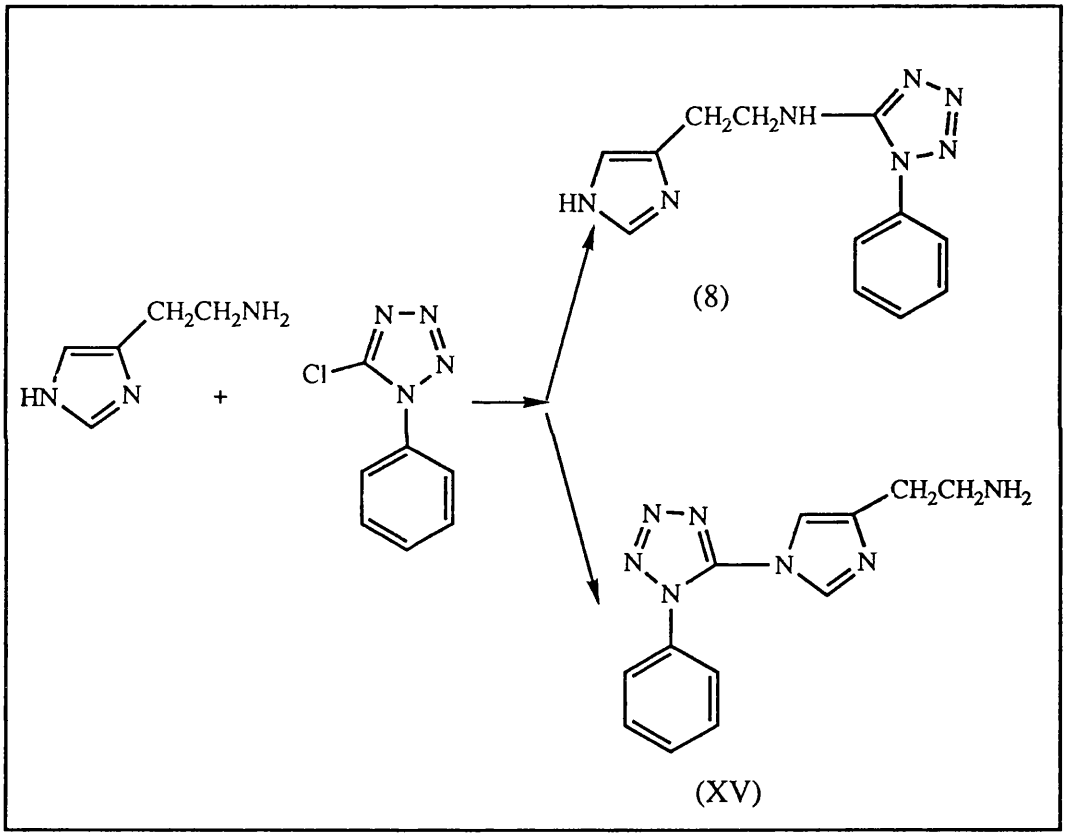
(XI) (XIV)

The reaction gave a higher yield (56%) in comparison with the reaction of the unprotected imidazole with 2-chloro-5-nitropyridine (26%).

Further evidence that -NH- of imidazole could react to give a by-product was provided when the reaction of histamine and 5-chloro-1-phenyl-1H-tetrazole gave two products. The mass spectra of the products were identical and ^1H NMR of compound (8) showed two triplets for the two side chain methylene groups at δ 3.06 and 4.58 whereas the ^1H NMR of compound (XV), showed two triplets at δ 2.8 and 3.51 for the corresponding groups, and

also the phenyl resonance of compound (8) was observed at δ 6.98 (t, J = 7.30Hz, 1H, para-H); 7.34 (t, J = 7.62Hz, meta-H) and 7.62 (d, J = 7.86, ortho-H) whereas the phenyl resonance of compound (XV) was observed at δ 7.1 (t, J = 5.45Hz, 1H, para-H) and 7.51-7.61 (m, J = 6.5 and 4.6Hz, 4H, meta and para-H). Apparently in compound (8), the aminogroup of the ethylamine side chain affects the chemical shifts of the phenyl protons through mesomeric effect.

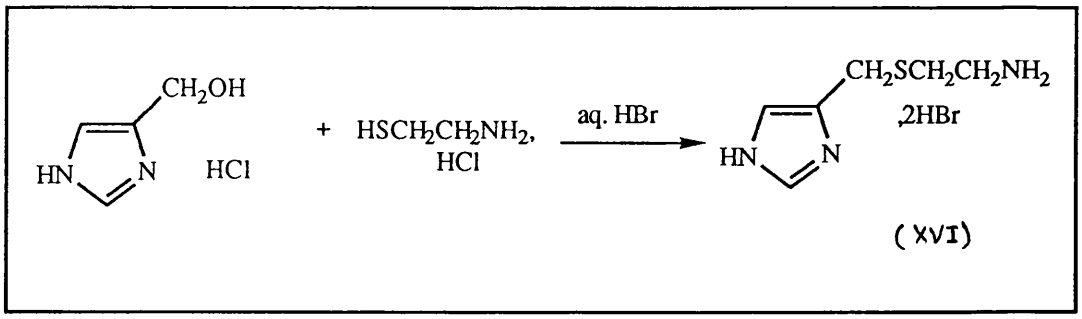
Scheme 3.19:



The synthesis of compounds (10) and (12) involves synthesis of the intermediate,

4-[(2-aminoethyl)thiomethyl]imidazole, which was prepared by the method described by Durant et al.[32]

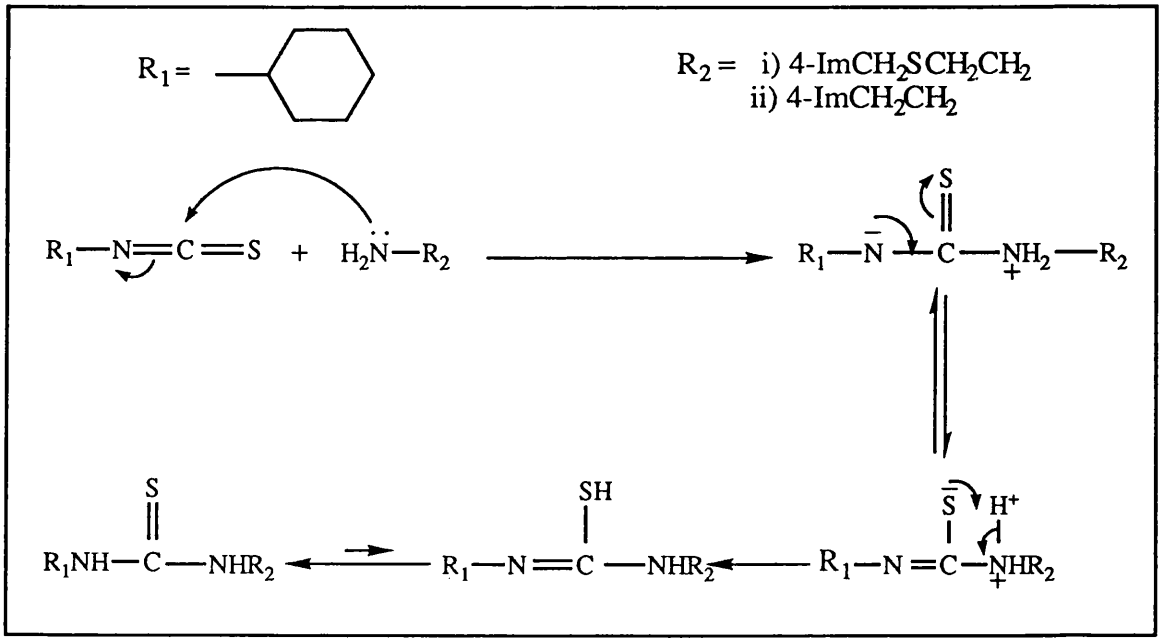
Scheme 3.20:



The synthesis of compound (xvi) involves acid catalysed elimination of the hydroxyl group followed by nucleophilic attack of the thiol group. The synthesis of compound (10) involves the similar mechanism for compounds in table 3.2 i.e. a nucleophilic aromatic substitution mechanism is involved with nucleophilic attack by the amine at the 2-pyridine position.

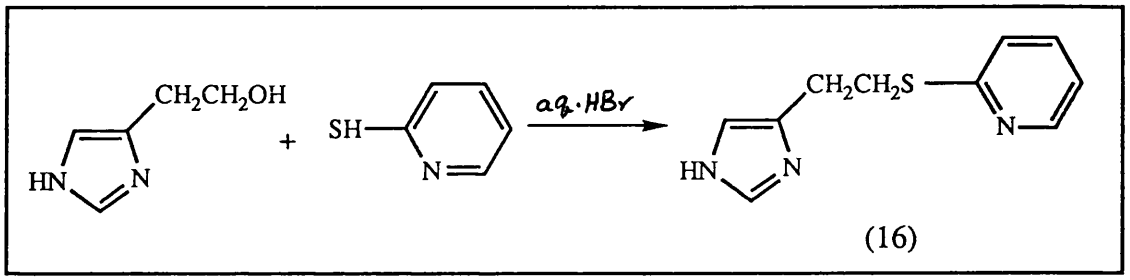
The synthesis of compound (12) and (11) involves the nucleophilic attack of the primary side chain amine on the isothiocarbonyl carbon.

Scheme 3.21:



The compounds were synthesised in quantitative yield in absolute ethanol under reflux for 1h. The compound (16) was prepared by reaction of hydroxyethylimidazole (provided by Mr W.Tertiuk) and 2-mercaptopyridine in aq.HBr (47%).The method followed was described in a patent[34] for the preparation of isothioureas by reaction of hydroxy or chloroethylimidazole hydrochloride and thiourea or substituted thiourea in aq.HBr.

Scheme 3.22:

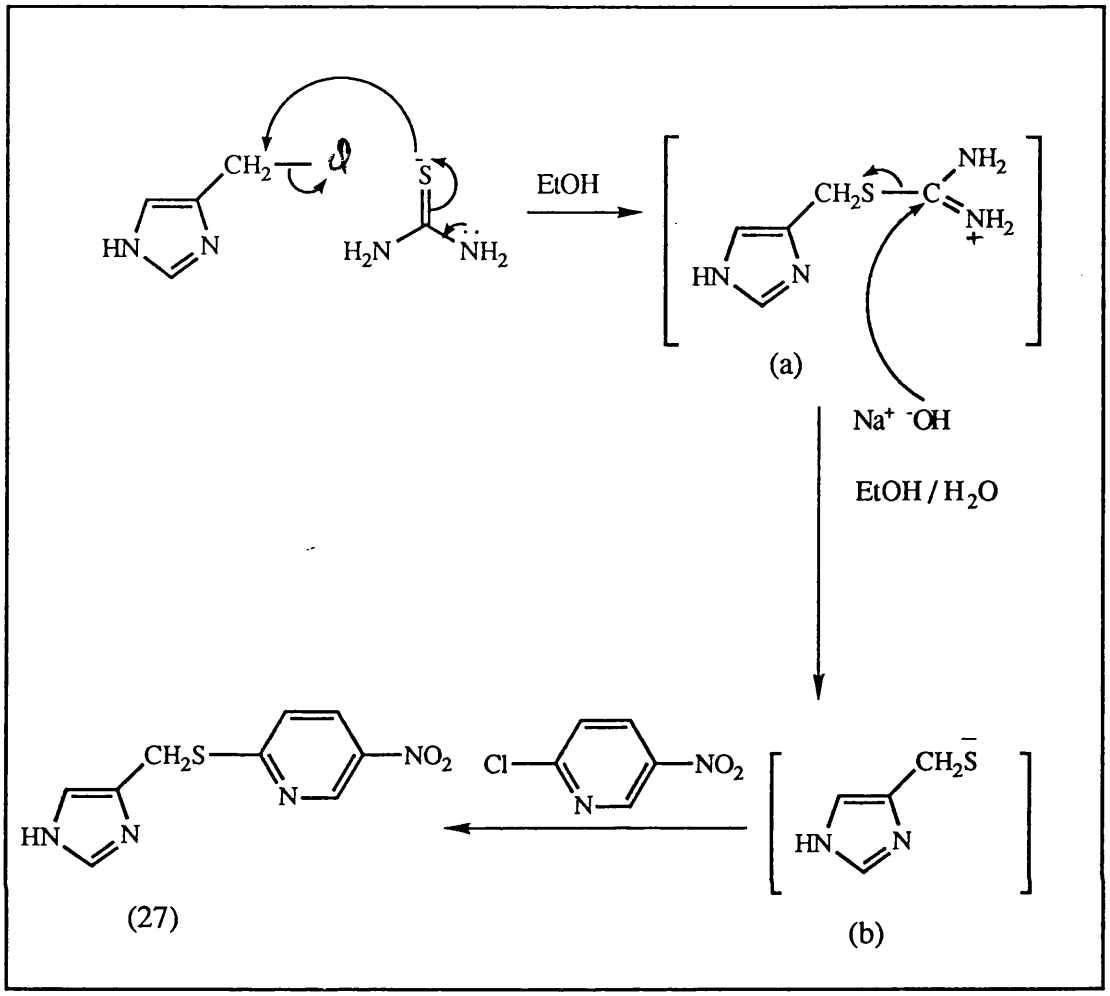


(16)

The reaction involved protonation of the hydroxyl group hence generating a good leaving group ($^{+}OH_2$) and formation of 2-(4-imidazolyl)ethylbromide hydrobromide salt followed by -S-alkylation (SN_2).

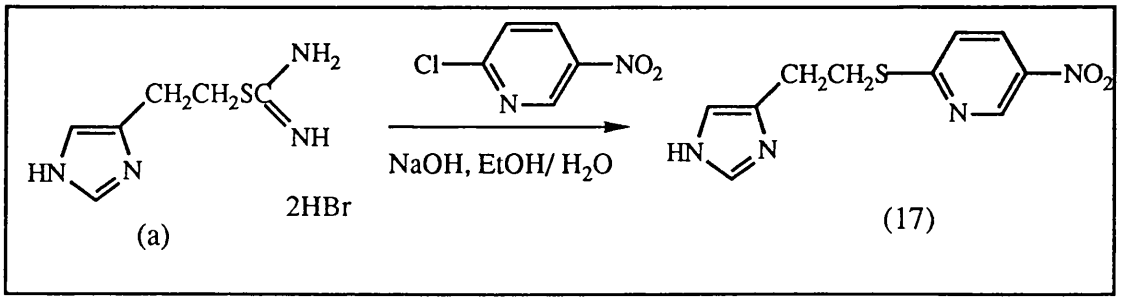
Synthesis of compound (27) involved generation of the unstable intermediate (a) followed by aromatic nucleophilic substitution at 2-pyridine in presence of NaOH. Sodium hydroxide (^{-}OH) reacts with the intermediate (a) by a nucleophilic mechanism to generate the 2-[4(5)-imidazolyl]-methylthiolate anion (b) which subsequently replaces the 2-chloro group by a nucleophilic aromatic substitution mechanism.

Scheme 3.23:



In the synthesis of compound (17), the analogue of intermediate (a) was provided by Mr W.Tertiuk and the mechanism is similar to that of compound (27) in scheme 3.23.

Scheme 3.24

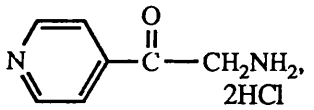
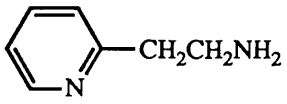
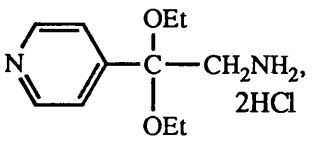
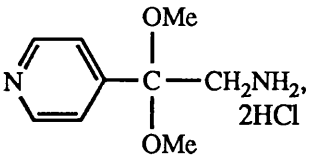


SELECTED SPECTRA

Comparing the UV spectra of four similar pyridine compounds in order to establish a distinguishable absorbance characteristic for α-aminoketones.

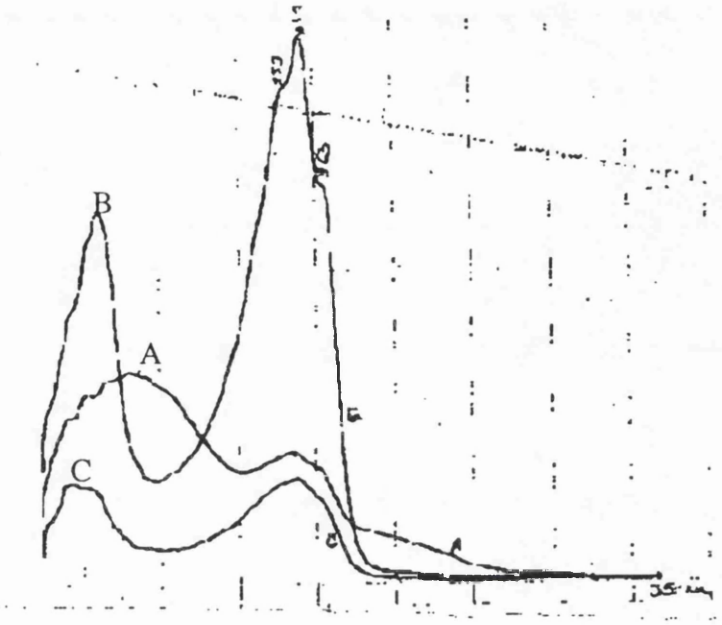
The four compounds were studied by UV and their maximal extinction coefficients are shown.

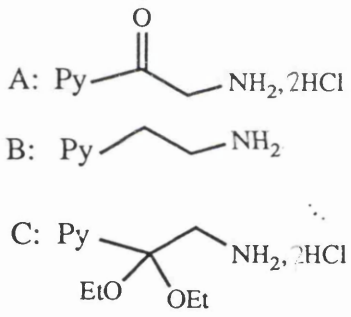
Table 3.3:

No	Structure	λ_{max}nm	A	ε_{max}	logε_{max}	C mdm^{-3}
(A)		313 253 206	0.025 0.137 0.168	416.6 22833 2800.0	2.61 3.35 3.44	C= 6.0 x10^{-5}
(B)		263 257 253 205	0.423 0.55 0.503 0.380	1311.32 1705.02 1559.32 1178.01	3.1 3.23 3.19 3.07	C= 3.22 x10^{-4}
(C)		259 200	0.128 0.056	3018.86 1320.75	3.47 3.12	C= 4.24 x10^{-5}
(D)		254 200	0.085 0.135	1634.61 2596.15	3.21 3.41	C =5.2 x10^{-5}

The conclusive evidence is that the carbonyl group of the α-aminoketone in 4-(2-aminoacetyl)pyridine shows an absorbance at 313 nm in the UV spectrum (see page 68).

Fig 3.1: The UV spectra of the compounds in table 3.3 are shown below:





The UV spectrum of ortho and para nitro aminopyridines:

The UV spectra of the two isomers, namely 2-amino-3-nitropyridine and 2-amino-5-nitropyridine derivatives of histamine, compounds (6) and (7) (see chapter 2, table 2.4), are interesting. The electronically complementary groups, the 2-amino and the 5-nitro are situated para to each other as in compound (7), there is a pronounced red shift in the main absorption band, compared to the effect of the substituents when they are ortho to each other, due to the extension of the chromophore from the electron donating group through the ring.

When the two groups are *ortho* to each other, the observed spectrum is closer to that of the separate non-interacting chromophores.

Table 3.4: Shows the absorption bands of both isomers in MeOH.

X	λ_{max}nm	log ε_{max}
ORTHO-NO_2	223 265 404	4.39 3.47 3.85
PARA-NO_2	218 357	4.10 4.27

Considering the resonance structure of the ortho isomer, it can be represented in two forms. The first with a shorter conjugation effect compared to the *para* -isomer hence shorter chromophore and the second having a much longer conjugative form hence having a band (though a weaker one) at longer wavelength than the *para* one.

Figure 3.2: Shows the resonance structure of the *para* isomer:

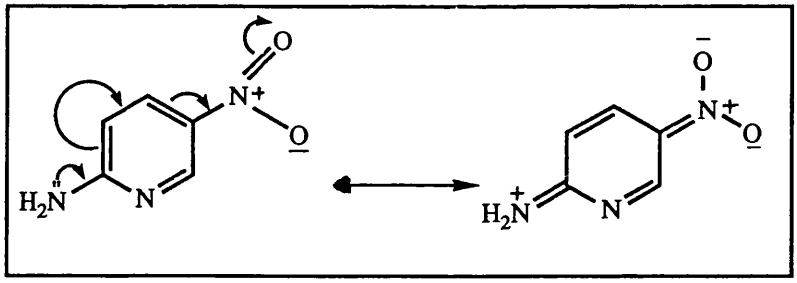
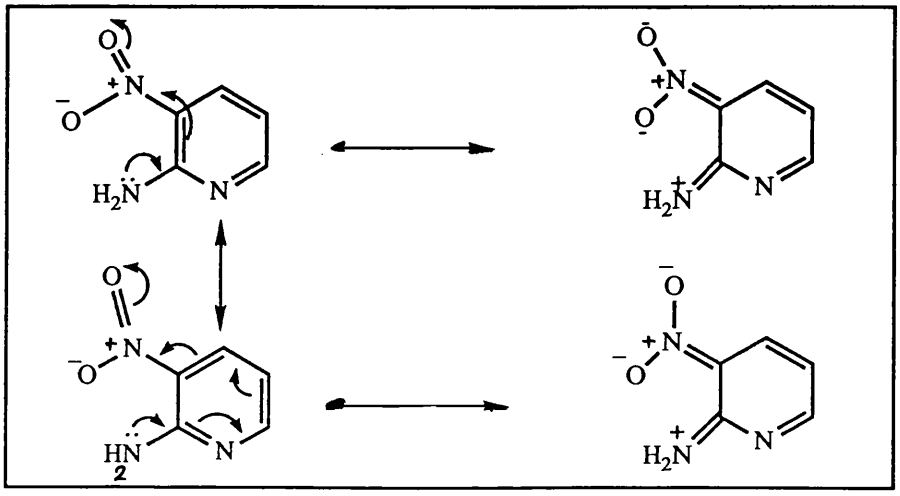


Fig 3.3: Shows the two possible resonance structures of the ortho isomer.

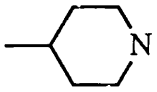
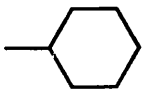
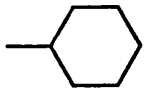


CHAPTER 4

STRUCTURE-ACTIVITY RELATIONSHIP DISCUSSION

The twenty eight novel compounds synthesised can be categorised according to their structural features. A general structure for all compounds is shown.

Table 4.1.:

Heterocycle ———	chain A ———	X ———	Ring
Imidazole	$(CH_2)_2$	N	het
Pyridine	$(CH_2)_2$	N	het and subst. het
Imidazole	$(CH_2)_2$ and $CH_2S(CH_2)_2$	N	Subst. het
Imidazole	$(CH_2)_2$	S	het. and subst. het.
N^{τ}- Methylimidazole	$(CH_2)_2$	N	Subst. het.
Imidazole	CH_2	S	Subst. het.
Imidazole		—	Subst. het.
Imidazole	$(CH_2)_2$		
	and		
	$CH_2S(CH_2)_2$		
Imidazole	$(CH_2)_2$	N	Subst. phenyl.

The biological results are shown in table 4.22 at the end of this chapter.

The chapter is divided into five parts:

4.1) 2-Aminoheterocyclic derivatives.

4.2) Substituted 2-aminoheterocycles.

4.3) Replacing imidazole.

4.4) Replacement of ethylamine -NH- by -S-. and 4.5) Chain length.

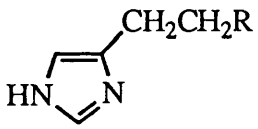
4.1) 2-Aminoheterocyclic derivatives

The compounds constituting the first group are 2-aminoheterocyclic derivatives of histamine.

The significant observation that there may be a progression from agonist through partial agonist to antagonist was first made by Professor C.R.Ganellin. One example of such progression is 4-[2-(1-pyrrolidinyl) ethyl]-imidazole where the side chain terminal nitrogen of histamine has been introduced into a pyrrolidine nucleus.This shows the maximal response of 56% that of histamine,[5] suggesting that this compound (32) represented a partial agonist and when considered as an antagonist, a K_i value of 2200 nM (see table 4.2).

All the aminoheterocyclic derivatives of histamine showed moderate antagonist activity.

Table 4.2: Shows the antagonist activity of 2-aminoheterocycle derivatives of histamine.



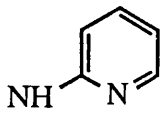
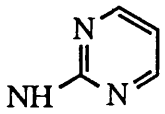
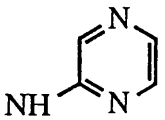
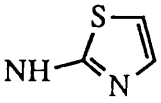
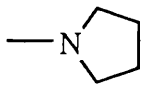
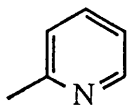
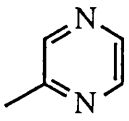
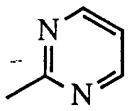
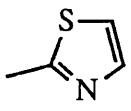
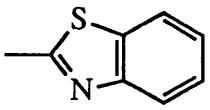
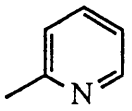
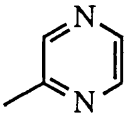
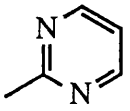
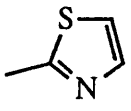
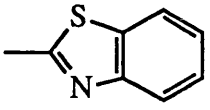
Compound No	R	Histamine release K_i nM
(1)		200
(2)		2100
(4)		200
(9)		340
(32)		2200

Table 4.3 [2]: Shows the substitutent constant (σ_p) and pK_a values of some heterocycles.

Compound No	(1)	(4)	(2)	(9)	(3)
heterocycle					
σ_p	0.81	0.26		0.72	1.61
pK_a of 2-aminoheterocycle	6.86	3.14	3.54	5.39	4.51
K_i nM	200	200	2100	340	330

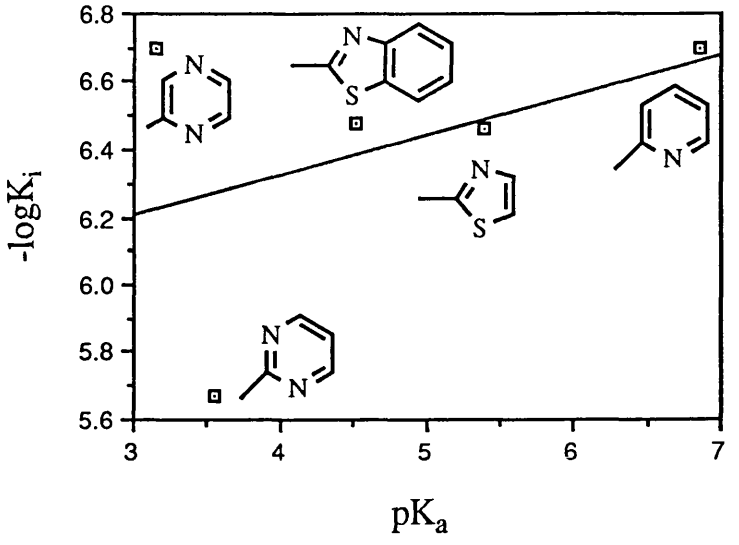
Searching for a correlation between the basicity (pK_a) and the activity of the aminoaromatic heterocycles derivatives , the pK_a values and the activities (-log K_i) of the compounds were tabulated and plotted as follows:

Table 4.4:

Compound No	(1)	(4)	(2)	(9)	(3)
heterocycle					
pK_a of 2-aminoheterocycle	6.86	3.14	3.54	5.39	4.51
-log K_i	6.7	6.7	5.67	6.46	6.48

Graph 4.1 showing the poor correlation between basicity (pK_a) and activity (-log K_i).

$$y = 5.8508 + 0.11758X, R = 0.41$$



There is no apparent correlation between the basicity and the activity of the aromatic heterocycle (the correlation coefficient was found to be equal to 0.41).

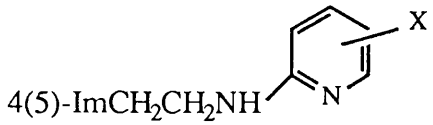
The pK_a's of the aminoheterocycle derivatives, (1), (2) and (9) increases with the activity, the 2-aminopyrazine (4) which has the lowest pK_a is as active as the aminopyridine (1) which has the highest pK_a. Clearly other factors must also affect the activity.

4.2) Substituted 2-aminoheterocycles

The second group consists of the compounds in which various substitutuents were introduced into different positions of the aminoheterocyclic moiety in order to investigate their effect on activity.

Polar substituents were found to be necessary for enhanced antagonist activity. The most polar substituent was the nitro group (σ_p =0.81).[112]

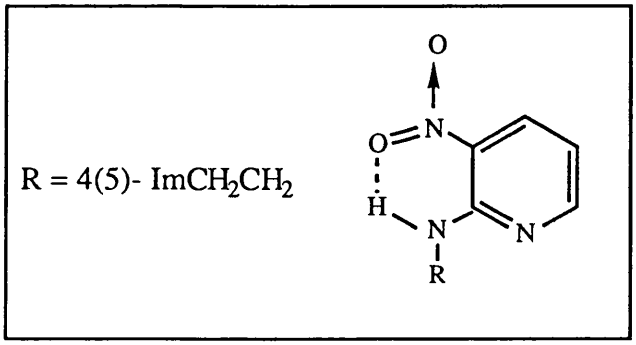
Table 4.5: Shows the nitro derivatives of 2-aminopyridine derivatives of histamine.



Compound No	X	K_i nM
(1)	H	200
(6)	*ortho*-NO_2	240
(7)	*para* - NO_2	29

The electronic effect of polar substituents on activity is apparent from compound (7). The importance of orientation of the substituent was confirmed by the decreased activity displayed by compound (6), where the nitro group is at *ortho* position (with respect to the amino substituent). The nitro group is capable of forming an intramolecular hydrogen bond between the amino and the nitro group as shown below.

Fig 4.1: Intramolecular hydrogen bond formation by *ortho* - NO_2 in 2-amino-3-nitropyridine derivatives of histamine.



Apparently encouraging intramolecular hydrogen bonding reduces antagonist activity (see table 4.5).

The reduced antagonist activity could be due to at least three factors:

1) One possibility is that **dipole-assisted hydrogen bonding** may be important to the antagonist-receptor interaction and that encouraging intramolecular hydrogen-bonding reduces antagonist activity.

2) The **steric effect** of the *ortho* substituent [which could be compared to compounds (5) and (4)].

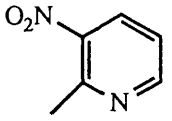
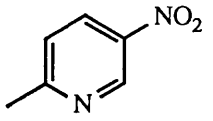
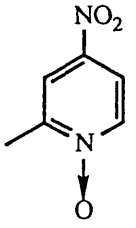
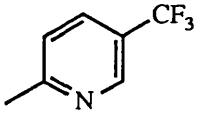
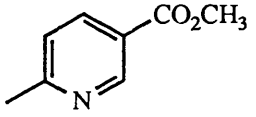
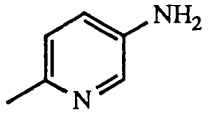
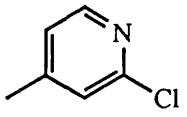
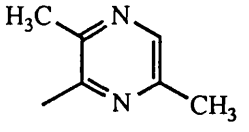
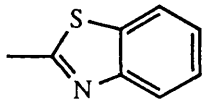
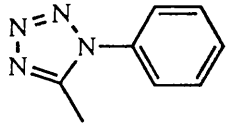
3) Antagonist activity could be related to **dipole orientation** and change in dipole direction could have more effect than its magnitude.

The *para* substituted analogues were chosen according to their effect on acidity or basicity of the heterocyclic head of the molecule (table 4.6).

They could be involved in a direct **dipole-dipole** or **dipole-charge** interaction at the receptor.

These groups are expected to be a determinant of the electron density on the side chain -NH- , thereby influencing its ability to hydrogen-bond to an acceptor group on the H_3- receptor.

Table 4.6: Shows the antagonist activity of substituted aminoheterocyclic analogues.

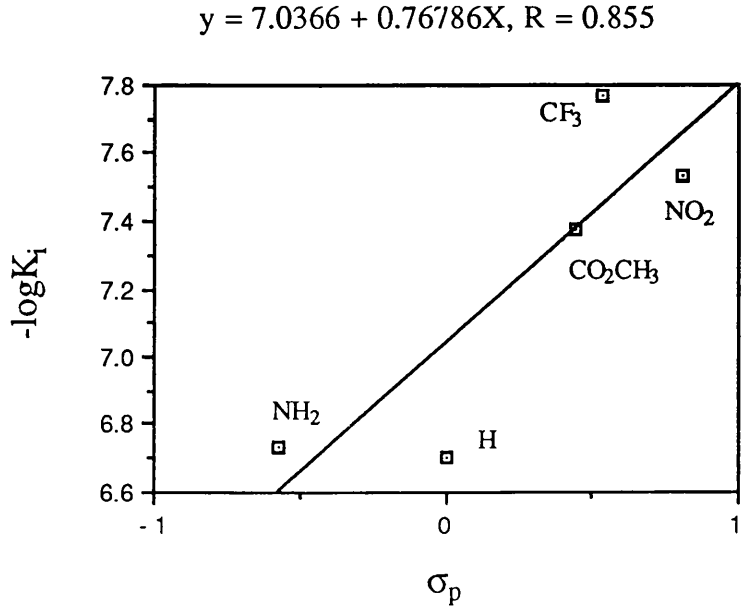
Compound No	4(5)-ImCH_2CH_2NHR R	Histamine release K_i nM
(6)		240
(7)		29
(19)		53
(15)		17
(24)		42
(29)		186
(20)		160
(5)		1300
(3)		330
(8)		11000

The substituents could affect the side chain -NH- or the pyridyl -N= or provide a **dipolar effect**.

Table 4.7 [77,112] : Shows the physicochemical parameters (π) and (σ) relating to hydrophobic and electronic effects of the substituents.

groups / parameter	H	NO_2	CF_3	CO_2CH_3	Cl	Ph	Me	NH_2
σ_p	0.0	0.81	0.53	0.44	0.24	0.05	-0.14	-0.57
σ_m	0.0	0.71	0.46	0.35	0.37	0.05	-0.06	-0.09
π_p	0.0	0.24	1.07	—	0.76	—	0.84	-1.23
π_m	0.0	0.11	1.07	-0.5	0.76	—	0.51	-1.23

Graph 4.2: Shows the correlation (r = 0.855) between the substituent constant (σ_p) and antagonist activity ($-\log K_i$).

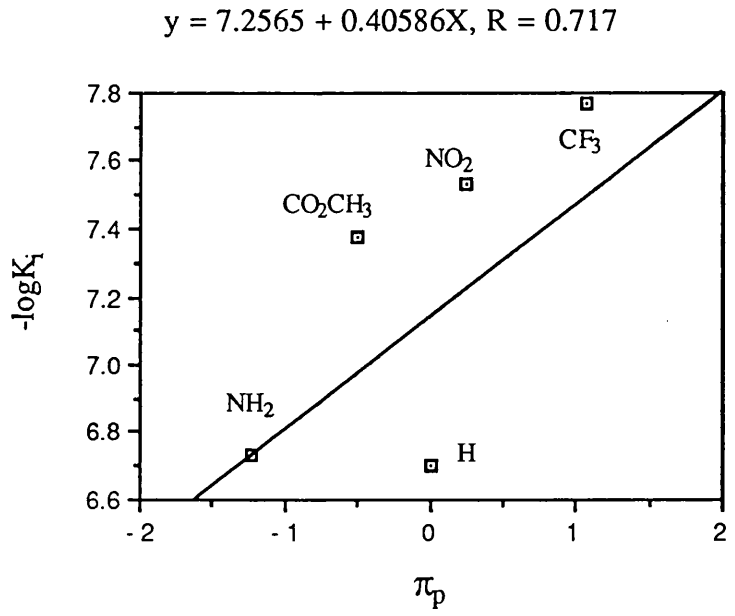


Plotting the partition coefficient parameter (π_p) of the substituents against -logK_i gives a moderate correlation indicating clearly that hydrophobicity is a cofactor of antagonistic activity (see table 4.8 and graph 4.3).

Table 4.8: Shows the antagonist activity (-log K_i) and the partition coefficient of the substituents in the *para* substituted 2-aminopyridine derivatives of histamine.

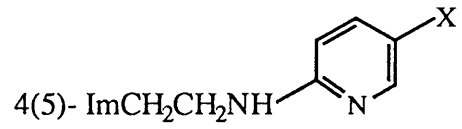
Compound No	(1)	(7)	(15)	(24)	(28)
Substituents	H	NO_2	CF_3	CO_2CH_3	NH_2
π_p	0.0	0.24	1.07	-0.5	-1.23
-logK_i	6.7	7.537	7.769	7.376	6.730

Graph 4.3: Shows the correlation (r = 0.71) between partition coefficient of the substituents and the antagonist activity.



Comparing the antagonist activity of the 2-amino-*para* substituted pyridine derivatives are tabulated as follows:

Table 4.9: Comparison of some *para* substituted aminopyridine derivatives:



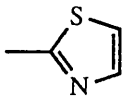
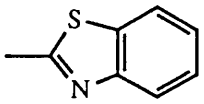
Compound No	X	Histamine release K_i nM
(1)	H	200
(7)	NO_2	29
(15)	CF_3	17
(24)	CO_2CH_3	42
(28)	NH_2	186

Comparing the antagonist activities (table 4.9) indicates that the nitro group is not critical for improved activity and the other analogues although less polar, are still equipotent antagonists. The *para* CF_3, a larger group is slightly more active (K_i = 17nM) and the CO_2CH_3 group (K_i = 42nM), more hydrophobic, in which a combination of electronic effect and hydrophobicity might be responsible for the increased activity (see compound 1). The important results with compounds (19) and (20)(table 4.6) clearly indicates that presence of an *ortho* nitrogen is not essential.

A substituent with an intermediate electron withdrawing power such as a fused benzene ring in 2-benzothiazole (σ_p =0.3-0.5),[82] which is a benzologue of compound (9) has no significant influence on activity.

Table 4.10: Shows the effect of an intermediate electron withdrawing substituent.

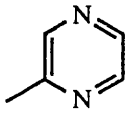
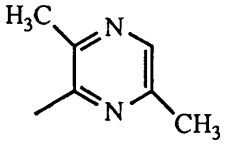
4(5)-ImCH_2CH_2NHR

Compound NO	R	Histamine release K_i nM
(9)		340
(3)		330

Methyl substituents are weakly base strengthening due to hyperconjugative and inductive effects (σ_p = -0.14).[112]

Table 4.11: Shows possible '*ortho* effect' causing reduced activity in 3,6-dimethyl substituted pyrazine:

4(5)-ImCH_2CH_2NHR

Compound No	R	Histamine release K_i nM
(4)		200
(5)		1300

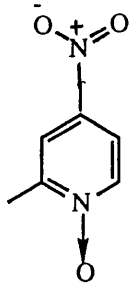
The decreased activity due to the presence of *ortho* methyl substituents could be attributed to an ***ortho* effect** which was observed in *ortho* nitropyridyl analogues.

The orientation of the dipole moment could be a factor determining the enhanced activity in *para* -nitropyridyl (7) compared to the ortho isomer (6) and in pyrazine (4) compared to pyrimidine (2), yet in compound (19) having 4-NO_2 and 1-N-oxide polar groups on the

pyridyl moiety could represent a different **orientational function** which has resulted in an increased antagonist activity.

Fig 4.11: Shows the dipole interaction in the meta nitropyridine-N-oxide derivative of histamine:

4(5)-ImCH_2CH_2NHR

Compound No	R	Histamine release K_i nM
(19)		53

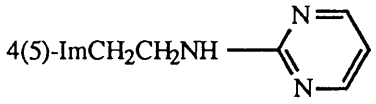
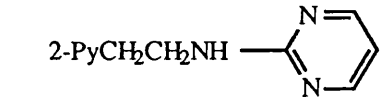
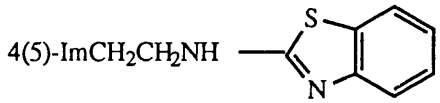
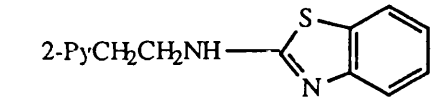
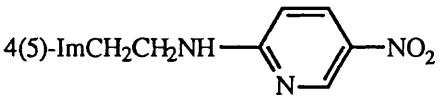
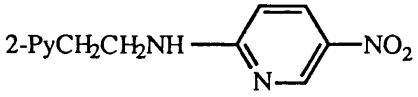
4.3) Replacing the imidazole

Once an additional binding site with the receptor which is sufficiently strong had been located, the attention was turned to establish the necessity of the imidazole group for the binding to the receptor.

It is known that imidazole can be replaced by other heterocycles (see chapter 1, table 1.2) with retention of activity as agonists at the H_1-receptor.

At the H_2-receptors, replacing imidazole ring in metiamide and cimetidine by other heterocycles such as pyridine, thiazole and isothiazole gave active compounds[38,114,113] but for H_3-receptors it appears otherwise.

Table 4.13: Shows H_3-affinity ratios for 4(5)-imidazole and 2-Pyridyl compounds:

Compound No	Structure	Histamine release K_i nM	ratio $\frac{K_i \text{ (2-Py)}}{K_i \text{ (4-Im)}}$
(2)		2100±400	4.8
(13)		>10,000	
(3)		330±100	9.4
(14)		3100±900	
(7)		29±11	34.4
(26)		1000±700	

In the 2-Pyridyl analogues (see.table 4.13), pyridine contains just one acceptor hydrogen-bonding heteroatom instead of the two hydrogen-bonding acceptor and donor nitrogen atoms in imidazole (=N- and -NH-; respectively).

In the *para* nitropyridine analogues (7 and 26), the ratio of antagonist activity is more significant suggesting that replacing imidazole with pyridine in some particular cases is not possible without a reduction in activity.

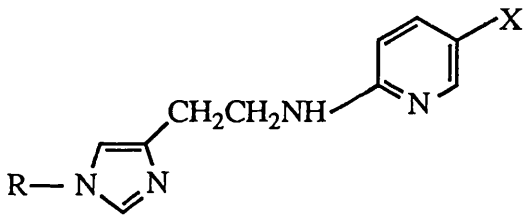
In another group of compounds, the hydrogen -bond acceptor =N- feature was retained and the -NH- of imidazole which is capable of a strong donor hydrogen-bond was converted to N^{τ}-methyl which reduces the hydrogen-bonding ability of the imidazole group.

This resulted in a more substantial reduction of activity confirming that not only imidazole is necessary for enhanced antagonist activity but also the -NH- could be contributing to the activity (see chapter 1, phencyclidine, 1.7.6).

It is known that in the imidazoles related to cimetidine and metiamide, methyl substitution in the ring at the 4(5)- position gave compounds having enhanced potency whereas N^{τ}-methylation considerably reduces potency at the H_2-receptors.[32]

In compounds (22) and (23), the methyl group could also exert an inhibitory steric effect.

Table 4.14 : Shows the ratios of H_3-antagonist activity of N^{τ}-methylimidazole and imidazole analogues of 2-aminoheterocycle derivatives.

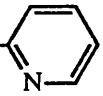
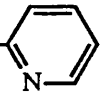
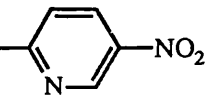
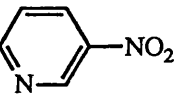
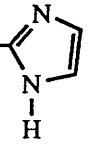


X	NO_2	NO_2	CF_3	CF_3
Compound No	(7)	(22)	(15)	(23)
R	H	CH_3	H	CH_3
K_i nM	29	2500	17	1300
ratio	$\frac{K_i\ N^{\tau}\text{-}CH_3}{K_i\ N\text{-}H} = 86$		$\frac{K_i\ N^{\tau}\text{-}CH_3}{K_i\ N\text{-}H} = 76$	

4.4) Replacement of ethylamine -NH- by 'S'.

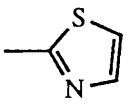
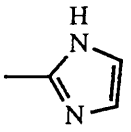
The replacement of -NH- of the ethylamine side chain by sulphur in compounds (1) and (7) resulted in enhanced antagonist activity, indicating that the side chain nitrogen is not needed. This might suggest that a reduction in hydrophilicity increases activity.

Table 4.15: Shows the effectiveness of the thioheterocycles at the H_3-receptors for the first time:

Compound No	Structure	Histamine release K_i nM	ratio $\frac{K_i\text{-NH-}}{K_i\text{-S-}}$
(1)	4(5)-ImCH_2CH_2NH- 	200	2.2
(16)	4(5)-ImCH_2CH_2S- 	92	
(7)	4(5)-ImCH_2CH_2NH- 	29	6.0
(17)	4(5)-ImCH_2CH_2S- 	4.8	
(18)	4(5)-ImCH_2CH_2S- 	400	

There is some resemblance between compound (9) and compound (18)(table 4.16). The two compounds have similar activities; possibly the advantage of replacing the side chain NH- by -S- is cancelled by replacing the ring -S- by -NH-.

Table 4.16: Interchanging the 'S' atom with 'NH'.

Compound No	Structure	Histamine release K_i nM
(18)	4(5)-ImCH_2CH_2NH 	340
(9)	4(5)-ImCH_2CH_2S 	400

4.5) Chain length:

The other structural variable considered was the chain length. The two methylene C-C bond length was found to be ideal. Activity decreases with increasing chain length. This effect was observed in both mercapto and aminopyridines.

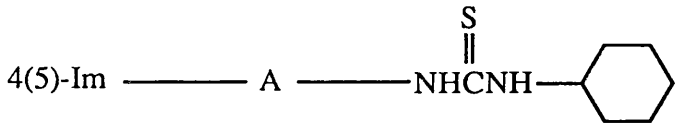
Table 4.17: Two methylene chain length is the ideal chain length.

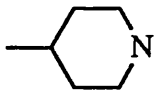
Compound No	A	X	Activity K_i nM
(7)	$(CH_2)_2$	NH	29
(31)	$(CH_2)_3$	NH	170
(10)	$CH_2S(CH_2)_2$	NH	1300
(17)	$(CH_2)_2$	S	4.8
(27)	CH_2	S	600

* Compound (31) was made by Ms. Y. Khalaf.

Opening up the piperidine ring in thioperamide (three methylene group) resulted in a more flexible molecule but a drop in activity.The H_3-antagonist activity in this series changes with distance as shown in table 4.18.

Table 4.18: The steric relationship between the imidazole and the thiourea group.

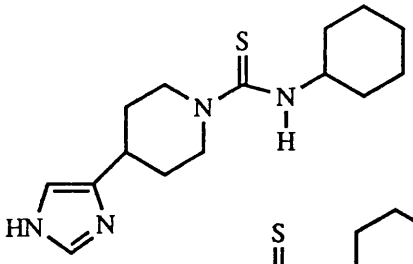
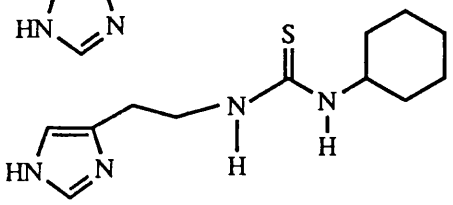
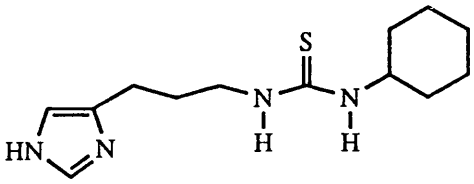
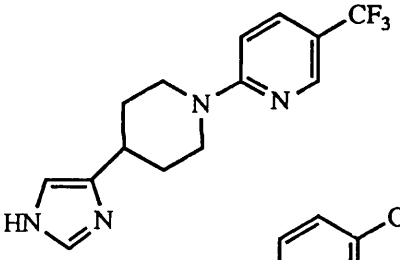
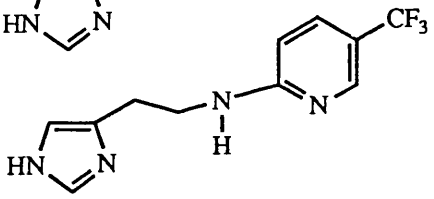


Compound No	A	Histamine release K_i nM
Thioperamide		4.3
(11)	$(CH_2)_2$	200
(29)	$(CH_2)_3$	13
(30)	$(CH_2)_4$	20
(12)	$CH_2S(CH_2)_2$	470

* Compounds (29) and (30) were made by Ms. Y. Khalaf.

The activity varies with the chain length. It is interesting to note that the thioether linkage -S- is not equivalent to the methylene group (-CH_2) for activity in compounds (12) and (30), although they have similar sizes and are known to be bioisosteric in some cases.[35] The removal of the isothiourea cyclohexyl group and introduction of 2-(5-trifluoromethyl)pyridine at the tertiary nitrogen of piperidine ring resulted in compound (25).

Table 4.19:

Compound No	Structure	Histamine release K_i nM
Thioperamide		4.3
(11)		200
(29)		13
(25)		42
(15)		17

Compounds (15) and (25) could be compared. Both are almost equipotent. The *para* - CF_3 substituent is expected to be a determinant of the electron density on the side chain -NH- in (15) and extensive charge delocalisation results not only in a dipole moment (which is common in both) but also serves to acidify the -NH- in (15).

The compound (25) has far less conformational freedom in comparison with compound (15).Both compounds have imidazole alkylamine chain as their common feature and their difference in activity clearly, is the result of replacing cyclohexyl thiourea with 2-(5-trifluoromethyl)-pyridyl. The increased activity could also be due to a combination of electronic and hydrophobic effects.

However these results were not additive since one would have expected the same increase in activity in compound (25) compared to thioperamide but it was found to be otherwise. The trend observed in open chain thiourea analogues of thioperamide are :

1) Increase in the chain length from two methylene groups to three methylene groups increased the activity,

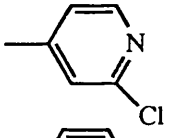
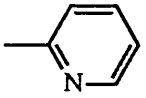
2) Cyclisation of the side chain into a piperidine ring improved the activity, but was not additive in comparing compounds (15) and (25). However removal of the -NHC(=S)NH- linkage proved advantageous due to

i) Possible toxicity of C=S;

ii) Improving brain penetration since the NH's in thiourea are hydrogen bonding donors.

The position of the amino substituent was changed to the 4-position where the amino substituent enhances the basicity of the ring but having a meta chloro substituent (electron-withdrawing) results in a slightly more active (though not significant) analogue of the 2-aminopyridine derivative (1).

Table 4.20:

Compound No	Structure	Histamine release K_i nM
(20)	4(5)-ImCH_2CH_2NH 	160
(1)	4(5)-ImCH_2CH_2NH 	200

Since compound (20) indicates that an ortho nitrogen atom is not required in the aminoheterocycle, a benzenoid compound was investigated. The 2-aminoheterocyclic end of the molecule in compound (7) was replaced by 1-amino-4-nitrobenzene which showed no decrease in antagonist activity(table 4.21) indicating that removal of =N- and insertion of =C- does not affect activity but would encourage lipophilicity. This is a very important result which opens up a new area for exploration.

Table 4.21:

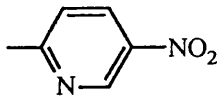
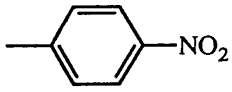
Compound No	Structure	Histamine release K_i nM
(7)	4(5)-ImCH_2CH_2NH 	29
(21)	4(5)-ImCH_2CH_2NH 	23

Table 4.22: H_3-receptor histamine antagonist activities of the compounds *in vitro* on rat cerebral cortex .

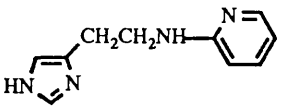
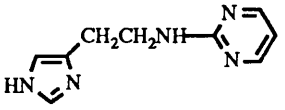
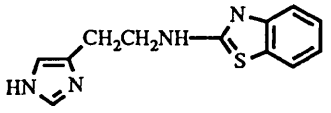
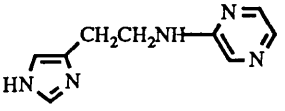
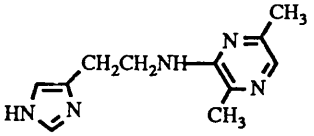
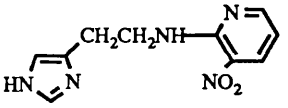
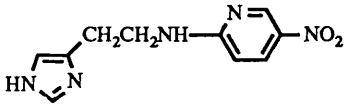
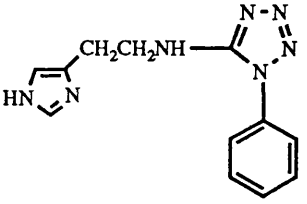
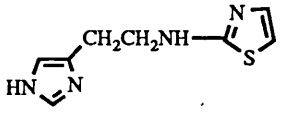
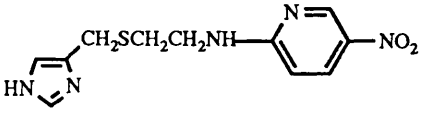
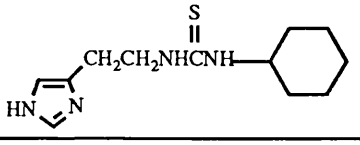
Compound No			Structure	Histamine release K_i nM
(1)	UCL1038	SKH130A		200 ±60
(2)	UCL1017	SKH91B		2100 ± 400
(3)	UCL1029	SKH103D		330 ± 100
(4)	UCL1093	SKH176A		200 ± 20
(5)	UCL1094	SKH179A		1300 ± 500
(6)	UCL1039	SKH117A		240 ± 120
(7)	UCL1040	SKH126B		29 ± 11
(8)	UCL1079	SKH135A		11000 ± 5000
(9)	UCL1090	SKH173A		340 ± 70
(10)	UCL1068	SKH156A		1300 ± 200
(11)	UCL1108	SKH198B		200 ± 100

Table 4.22: Continued.

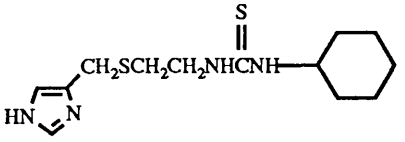
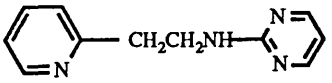
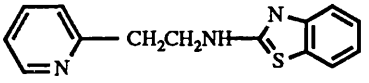
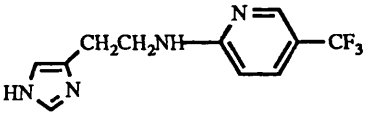
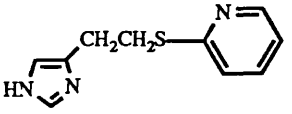
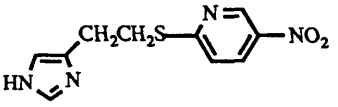
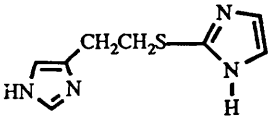
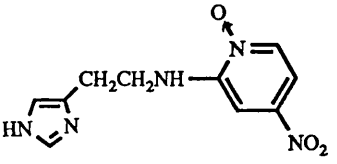
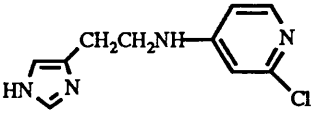
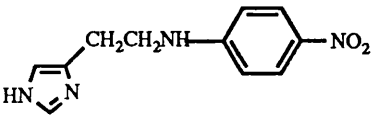
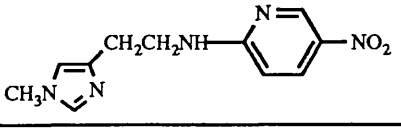
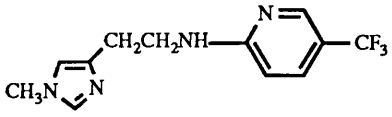
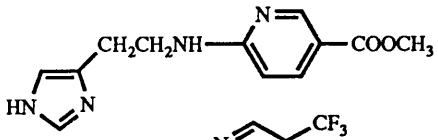
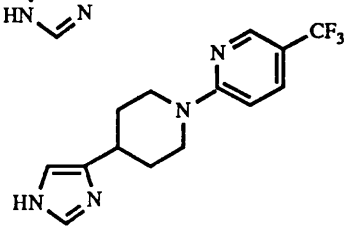
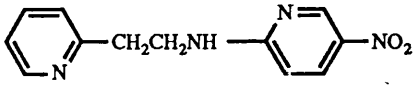
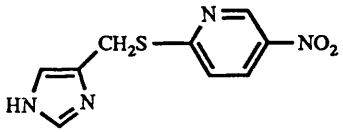
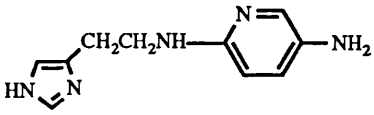
Compound No			Structure	Histamine release K_i nM
(12)	UCL1109	SKH188A		470 ± 230
(13)	UCL1030	SKH108A		>100000
(14)	UCL1031	SKH111B		3100 ± 1900
(15)	UCL1235	SKH329B		17 ± 3
(16)	UCL1162	SKH238B		92 ± 22
(17)	UCL1199	SKH298A		4.8 ± 0.9
(18)	UCL1210	SKH280A		400 ± 100
(19)	UCL1256	SKH380A		53±16
(20)	UCL1236	SKH377B		160 ± 50
(21)	UCL1205	SKH299C		23 ± 9
(22)	UCL1264	SKH418A		2500 ± 600

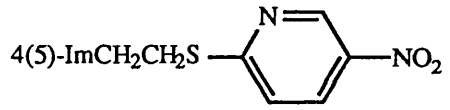
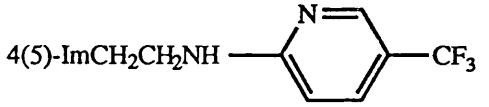
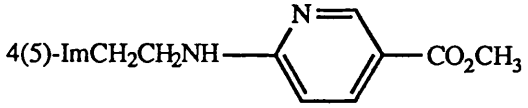
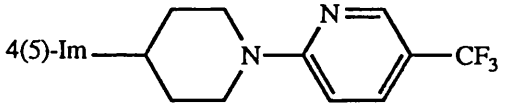
Table 4.22: Continued.

Compound No			Structure	Histamine release K_i nM
(23)	UCL1265	SKH421A		1300 ± 700
(24)	UCL1249	SKH394A		42 ± 6
(25)	UCL1283	SKH456A		42 ± 22
(26)	UCL1200	SKH307B		1000 ± 700
(27)	UCL1191	SKH277C		600 ± 400
(28)	UCL1334	SKH452A		186±37

IN VIVO RESULTS

The pharmacological testing were carried out at inserm by the courtesy of Prof. J.-C. Schwartz. Some of the antagonists displaying high *in vitro* potency, such as (17), (15) and (24)(table 4.23) were tested *in vivo* . The compounds were given orally to the mice and after 90 minutes, the animals were put down and the level of N^{τ}-methylhistamine was measured in the brain. Thioperamide was given at the same time for comparison.

Table 4.23:

Compound No		Structure	Histamine release K_i nM
UCL1199	(17)		4.8
UCL1235	(15)		17
UCL1249	(24)		42
UCL1283	(25)		42

The increase in the level of N^{τ}-methylhistamine is an index of the increase in neuronal histamine release elicited by the antagonists. The effect of some of the H_3-receptor antagonists on N^{τ}-methylhistamine levels in mouse brain is shown in table 4.24 as follows.

Table 4.24: The percentage changes of N^{τ}-methylhistamine level in the mouse brain after oral administration of the compound.

Time (hours)	Percentage changes of N^{τ}-Methylhistamine after treatment
	UCL 1199, 3mg/kg
1h 30	$+31 \pm 16^{NS}$ (6)
3h	$+33 \pm 7^{*}$ (6)
Thioperamide 10mg/kg 90min	$+11 \pm 5$ (6)

The kinetic study shows that maximal histamine release (+11± 5%) is reached by 90 minutes with thioperamide at 10mg/kg whereas with UCL1199 (17) at 3mg/kg, the histamine release by 90 minutes increases three times and the maximal release (33±7%) is reached by 3h.

In a direct comparison with thioperamide, UCL1199 at 3mg/kg after oral administration of the compound to the starved mouse, showed similar potency and duration, the increase in N^{τ}-methylhistamine lasting for 6hours.

Table 4.25 Shows the dose related activities of three of the compounds. Number of the animals sacrificed are shown in brackets.(* P< 0.05, NS= Non Significant).

Dose (mg/kg)	Percentage changes of N^{τ}-methylhistamine level 90 minutes after treatment.		
	UCL1199	UCL1235	UCL1249
0.3	$-0.2\pm 7^{NS}_{(6)}$	$+9\pm 2^{*}_{(6)}$	
1	$+28\pm 9^{*}_{(6)}$	$+43\pm 10^{*}_{(6)}$	
3	$+42\pm 10^{*}_{(11)}$	$+37\pm 9^{*}_{(6)}$	$+67\pm 13^{*}_{(6)}$
10	$+81\pm 58_{(6)}$	$+43\pm 16^{*}$ (6)	$+88\pm 6^{*}_{(6)}$
30	$+68\pm 5^{*}_{(5)}$		
Thioperamide (10mg/kg) P. O.	$+25\pm 7_{(10)}$	$+102\pm 9_{(6)}$	$+72\pm 10_{(6)}$

Recently the compound (25) was tested *in vivo* and shows a higher activity. The kinetic study of N^{τ}-methylhistamine level in mouse brain after oral administration of the compound (2mg/kg), shows a higher activity. The ED_{50} (mg/kg) value for thioperamide is 1mg/kg and for compound (25) is 0.81± 0.28mg/kg.

Conclusion:

The compound UCL1199 is equal in potency to thioperamide *in vivo*.

CONCLUSIONS

The effect of various modifications to the structure of thioperamide on histamine H_3-antagonist activity has been demonstrated, bearing in mind the need for a non-toxic brain-penetrating antagonist.

1) In the 2-aminoheterocyclic derivatives of histamine, the pK_a of the aminoheterocycles has no apparent correlation with the activity (compounds 1-4 and 9).

2) In the substitued 2-aminoheterocycles (6,7,15, 24 and 28), an electron withdrawing effect of the polar substituent may enhance activity but the position of the substituent plays a significant role. The importance of the orientational fuction of the substituent was shown in (6), (7) and (19). There is a strong possibility that dipole-assisted hydrogen bonding may be important for the antagonist -receptor interaction and the effect of these substituents on the side chain either with NH or S seems important. In these compounds, the substituents affect their ability to hydrogen bond to a potential acceptor group on the receptor.

3) A possible steric effect of the ortho substituent with respect to the side chain was demonstrated with two sets of compounds such as (6) and (7) which could imply that encouraging intramolecular hydrogen bonding reduces activity, and (4) and (5), where the introduction of an ortho methyl group reduces activity, also possibly due to steric effects.

4) Hydrophobicity is likely to be another major factor for antagonist activity, since in the substituted 2-aminoheterocyclic derivatives of histamine, the substituent with the highest contribution to partition π shows the highest activity (15).

5) The importance of retaining the strong hydrogen-bonding ability of the NH of the imidazole moiety was shown by N^{τ}-methyl substituted anlogues since N-methylation resulted in a substantial reduction of activity (22 and 23), and also in the 2-pyridyl analogues having just one acceptor hydrogen-bonding heteroatom, there is a significant reduction in activity.

6) It was shown for the first time that thioheterocycles are active at H_3-receptors. Replacing the side chain -NH- by -S- enhanced the activity, indicating that the side chain nitrogen in not necessary.

7) In the substituted 2-amino or thioheterocycle series , the ethylamine side chain was found to be the most suitable chain length, but in the open chain analogues of thioperamide, the ideal chain length was found to be a three methylene group chain.

8) The removal of the -NHC(=S)NH- served both purposes of eliminating the possible toxicity of the C=S group and reducing hydrogen-bonding ability of the molecule hence potentially improving brain penetration since the NH's of the thiourea group are hydrogen-bond donors.

9) The position of the nitrogen atom in the aminosubstituted pyridines does not seem to affect activity (1 and 20).

10) Replacing the =N- by =C- in the 2-aminoheterocycle series only encourages the lipophilicity (7 and 21) and does not affect activity, indicating that the nitrogen atom is not important for activity.

Some suggestions for further work

1) Replacing the -NH- with -S- and -O- in the 2-amino-parasubstituted phenyl derivatives of histamine.as in the analogues of 2-amino-parasubstituted pyridines, since this enhanced the activity.

2) Replacing the imidazole by thiazole thereby removing the NH of the imidazole ring.

3) Removal of the -N= of the imidazole, hence removal of a hydrogen-bonding acceptor, in order to investigate a possible contributing role of the imidazole NH of imidazole to activity.

4) To synthesise some other derivatives of 4-(4-piperidyl)imidazole or

4-(4-phenyl)imidazole since, apparently, cyclising the side chain enhances the antagonist activity.

5) Replacing the methoxy carbonyl group in compound (24) with i-propyl or cyclohexyl groups to increase the hydrophobicity.

CHAPTER 5

EXPERIMENTAL

GENERAL PROCEDURES:

The starting materials were obtained commercially, except for the 4(5)-(2-hydroxyethyl)imidazole (WT76/3156), 4(5)-hydroxymethylimidazole hydrochloride and S-{2-[4(5)-imidazolyl]ethyl}isothiourea dihydrobromide (WT133C/UCL1095-B_2), which were provided by courtesy of Mr W.Tertiuk. Melting points were determined using an Electrothermal (open capillary) melting point apparatus and are uncorrected. Thin layer chromatography was carried out on Merck Kieselgel 60 F_{254} plates, visualised at 254nm and then with either iodine vapour or KIP (potassium iodoplatinate reagent) spray. High performance liquid chromatography (HPLC, analytical and preparative) was carried out by Mr S. T. Corker or myself on a Gilson Binary Gradient System combined with Gilson 714 Software or a Hewlett Packard Integrator System for data analysis. The UV spectra were recorded on a Perkin-Elmer 554 UV-VIS Spectrophotometer, using cells of 1cm path length and methanol or water as solvent. The IR spectra were recorded on a Perkin-Elmer 983 Infrared Spectrometer, using either Nujol mulls between sodium chloride plates, or KBr discs. ^1H NMR and ^{13}C NMR were recorded on a Varian XL-200 NMR Spectrometer at 200 and 50 MHz respectively or a Varian VXR-400 NMR spectrometer at 400 and 100 MHz respectively, the higher field spectra were run by Mrs. G. Maxwell. NMR spectra are referenced to TMS or the residue from the NMR solvent used. Mass spectra (FAB and EI) were recorded by Dr. M. Mruzek on a VG 707H Double Focussing Mass Spectrometer with a Finnigan Incos data system. Microanalyses were carried out by Mr A. Stones and Mrs. J. Maxwell of the departmental microanalysis service.

This chapter is divided into two parts:

5.1) Preparation of 4-(4-piperidyl) imidazole.

5.2) Preparation of other heterocyclic derivatives of imidazole and pyridine.

5.1) Preparation of 4-(4-Piperidyl)imidazole

1) 4-Acetylpyridine oxime

(sample SKH23C)

To a stirred solution of hydroxylamine hydrochloride (99.87g, 1.24 mole) in 95 cm^3 pyridine, was added 4-acetylpyridine (58.04g, 0.48 mole), the mixture was heated under reflux for 3.5h. The reaction was poured into 300 cm^3 ice cold water, stirred for 1h, and then filtered. The crude product was washed with ice-cold water, dried at 60°C under reduced pressure for 12h., and crystallised from water to afford the pure colourless crystalline oxime 31.5g (56 % yield), mp: 163-4°C.(Lit.[47] mp: 154-7°C).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.74.

IR: KBr cm^{-1}, 3699 (w, -OH), 1600 (s, C=N), 1039 (s, N-O).

^1H NMR: (DMSO-d_6, 400MHz); δ (ppm) 2.17 (s, 3H, $-CH_3$); 7.62 (d, J = 4.58Hz, 2H, Py-3,5); 8.60 (d, J = 5.1Hz, 2H, Py-2,6); 11.750 (s, 1H, OH).

Mass spectrum: (EI); m/e 136 (M^+), 119 (loss of -OH).

Anal. Calc. for $C_7H_8N_2$: C, 61.74; H, 5.92; N, 20.57.

Found: C, 61.49; H, 5.92; N, 20.27.

The experiment was repeated using hydroxylamine hydrochloride (86.04g, 1.238 mole), and 4-Acetylpyridine (50g, 0.412 mole) in 65 cm^3 pyridine. Recrystallisation from acetone gave SKH355A (31.5g, 56 % yield)

2) Ethanone, 1-(4-pyridyl)-O-[(4-methylphenyl)sulfonyl]oxime

(sample SKH405A)

The oxime (**1**)(17g, 0.125mole) was stirred in pyridine (15 cm^3) for 2h when dissolved completely. To the bright yellow clear solution p-tosylchloride (27.18g, 0.142 mole) was added portionwise. The reaction was stirred for 24h at 21°C and then filtered. The resulting solid was washed with water (1L), dried under vacuum at 21°C and purified by crystallisation from acetone, after pretreatment with charcoal, to give the product 25.4g (70%), mp: 86°C (Lit. [47] mp. 79-81°C).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.8

IR: KBr cm^{-1} 3037 (m, C=C arom), 1594 (s, C=N, aromatic), 1381 (s, C=N, arom).

^1H NMR: ($CDCl_3$, 200MHz); δ (ppm), 2.30 (s, 3H, Py-CH_3); 2.43 (s, 3H, Ph-$\underline{CH_3}$); 7.34 (d, J = 7.8Hz, 2H, Ph); 7.41 (d , J = 5Hz, 2H, Py-3,5); 7.89 (d, J = 10Hz, 2H, Ph); 8.62 (d , 2H, Py-2,6).

Mass spectrum: (EI); m/e 291 (M^+), 155, 105.

Anal. Calc. for $C_{14}H_{14}N_2O_3S$: C,57.91; H,4.85; N,9.65; S,11.04

Found: C,57.76; H,4.69; N,9.60; S,11.14.

Some more product was prepared following the same procedure reacting the oxime (26.22g, 192 mmole) with *para* -toluenesulfonyl chloride (41g, 218 mmole) in pyridine (35 cm^3) at 0°C. The product was twice recrystallised from methanol and water to give SKH31A (35.59g, 90.7%), mp: 80°C.

3) 2,2-Dimethoxy-2-(4-pyridyl)ethylamine dihydrochloride

(sample SKH74A)

To a stirred solution of potassium methoxide [from 0.1g potassium (1.25 equimole), in 50 cm^3 MeOH], a solution of the tosyl ester (**2**)(5.90g, 0.028 mole in 20 cm^3 MeOH) was added dropwise. When the addition was complete, a gelatinous solid appeared. After 1h, the potassium tosylate was filtered off and washed with a little MeOH. The filtrate was acidified with conc HCl (16 cm^3) to pH1 and the solution became dark red. On standing, some inorganic material precipitated which was filtered off. Addition of ether afforded the product .4.64g (90%), mp: 215°C .

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.82

UV: Water; λ_{max}nm ($\log\varepsilon_{max}$): 200 (3.04); 254 (3.138).

IR: KBr cm^{-1} 1635 (m, aromatic); 1595 (s, C=N); 1068,1048 (s, C-O, acetal)

^1H NMR: (D_2O, 200MHz); δ (ppm) 2.6 [s, 6H, -$(OCH_3)_2$] ; 2.8 (s, 2H, $\underline{CH_2}NH_2$);

7.5 (d, J = 7Hz, 2H, Py-3,5); 8.23 (d, 2H, Py-2,6).

Mass spectrum: (EI); m/e 152 (M^+-CH_2NH_2), 106 (py=C=O).

Anal. Calc. for $C_9H_{16}Cl_2N_2O_2$: C, 42.36; H, 6.23; N, 10.98; Cl, 27.79

found : C, 42.23; H, 6.56; N, 10.98; Cl, 28.06.

4) 2,2-Diethoxy-2-(4-pyridyl)ethylamine dihydrochloride

(sample SKH428A)

To a cooled stirred solution of sodium ethoxide [from 0.47g Na (20 mmole) in abs. EtOH, 20 cm^3, at 0-5 °C] under N_2, a solution of tosyl ester (**2**)(6g, 0.02 mole) in abs EtOH (30

cm^3) was added dropwise within 15 min when a gelatinous white solid was formed. After the reaction mixture was stirred for 1h, sodium dried ether (200 cm^3) was added and the sodium tosylate precipitated. The prepicipitate was filtered off and HCl gas was bubbled through the filtrate for 20 min when a white solid precipitated. It was filtered off, washed with ether (50 cm^3), dried under vacuum at 21°C for 3h. The crude product was crystallised from MeOH: Acetone (50 cm^3, 2:1). The pure crystalline product was dried under reduced pressure at 21°C overnight to give SKH428A (3.86g), mp: 218-20°C. Concentrating the filtrate afforded the second crop. SKH428B (0.16g), combined yield: 63%. mp: 218-20°C (Lit.[47] mp: 121-3°C).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.65

HPLC: 99.4% at 6 minutes (reverse phase column; Lichrosorb rp select b; ion pair C_{18} $1cm^3$/min; 250x4 mm; UV 254nm; A/B (50/50) where A is water and B is MeOH. The mobile phase contain 0.1/100 cm^3 water hexanesulphonic acid + 0.5% orthophosphoric acid). A single impurity elutes at 3.55 minutes.

UV: water; λ_{max}nm ($\log\varepsilon_{max}$); 259 (3.47); 200 (3.12).

IR: KBr cm^{-1} 3424, 3223 (w, N-H str.); 1637, 1613 (s, C=N, C=C str.); 1068, 1048 (S, C-O, acetal).

^1H NMR: (D_2O, 200MHz); δ (ppm) 0.5[t, J = 7.1Hz, 6H, -($OCH_2\underline{CH_3})_2$]; 2.9[m, 7H, $(OCH_2)_2$, CH_2NH_2, partial exchange of NH_2 protons with D_2O], 7.60 (d, J = 5.2Hz, 2H, Py-3,5); 8.24 (d, J = 5.4Hz, 2H, Py-2,6).

Mass spectrum: (EI); m/e 211 (M^+), 180 (loss of CH_2NH_2), 106 (Py-C=O).

Anal. Calc. for $C_{11}H_{20}N_2O_2Cl_2$: C, 46.64; H, 7.11; N, 9.89; Cl, 25.03

found: C, 46.64; H, 7.15; N, 9.79; Cl, 24.77.

Repeating the reaction using 5 molar equiv alent of sodium ethoxide (from 3.56g Na in 60 cm^3 EtOH) and 9g of the tosyl ester and crystallisation from EtOH: ether gave SKH33C (4.5g, 71%) mp: 218-20°C (decomposition). Repeating the reaction using one molar

equivalent of potassium ethoxide (from 2.36g potassium in 50 cm^3 abs. EtOH) and 17.5g of the tosyl ester and crystallisation from abs. EtOH: ether (10:1) gave SKH72B (10.15g, 81%), mp: 219-20°C (decomposition).

The hydrochloride salt (0.52g, 1.8 mmole) was converted into the base by treating with aqueous potassium bicarbonate.(2 molar equivalent) The resulting solution was concentrated under reduced pressure and the residual moisture was removed by azeotropic distillation with i-PrOH and the solid residue was suspended in i-PrOH, heated and filtered hot. The filtrate, on evaporation, yielded an oily residue SKH36E (0.28g, 73%).

^1H NMR: (DMSO-d_6, 200MHz); δ (ppm) 1.15 (t, 6H, $-OCH_2\underline{CH_3}$); 2.86 (s, 2H, $-\underline{CH_2}NH_2$); 3.22 [m, 6H, $-(OCH_2)_2$, NH_2); 7.39 (d, 2H, Py-3,5); 8.56 (d, 2H, Py-2,6).

^{13}C NMR:(D_2O, 200MHz); δ (ppm): 157 (s, 1C, Py-4); 144 (d, 2C, Py-2,6); 128 (s, 2C, Py-3,5); 98 (s, 1C, $\underline{C}$-OEt); 61{t, 2C, $-(OCH_2)_2$ }; 46 (t, 1C, $-CH_2NH_2$); 17 {q, 2C, $-(OCH_2CH_3)_2$}.

Mass spectrum: (EI); m/e 211 (MH^+);180 ($M-CH_2NH_2$); 106 (py=C=O)

5) Attempts to hydrolyse the acetal (4) to the α-aminoketone (6)

I) To the solution of the acetal (**4**)(0.1g, 0.353 mmole) in acetone: water (1:1, 50 cm^3) Amberlyst -15 (0.02g) was added. The mixture was stirred for 20h at 20°C filtered.and then the filtrate was evaporated to dryness under reduced pressure. Unchanged starting material only was recovered. SKH310A (mp: 218- 20°C).

IR: (nujol mull) cm^{-1} 1068 (s, C-O); 1048 (s, C-O) and absence of C=O.

II) To the solution of the acetal (0.1g) in water (5 cm^3) conc H_2SO_4 (5 cm^3) was added and the solution was agitated for 3h in an ultrasonic bath. The acidic solution was

neutralised with an aqueous solution of sodium hydroxide to pH11, extracted into ether (3x10 cm^3) and then the combined extracts were dried over $MgSO_4$ overnight. Removal of the solvent under reduced pressure gave an oily residue (SKH311A).

IR: nujol mull cm^{-1} 1068 (s, C-O); 1048 (s, C-O) and absence of C=O.

III) The amine hydrochloride (4)(1g, 4.7 mmole) was dissolved in aqueous sodium hydroxide (10%, 20 cm^3) and the solution was heated under reflux for 1h and then the cooled solution was extracted with $CHCl_3$ (3x30 cm^3). The combined organic extracts were dried over $MgSO_4$ overnight. The solvent was removed under reduced pressure and the oily residue was dried under reduced pressure at 21°C for 24h. The ^1H NMR and mass spectrum spectrometry analysis corresponded to the acetal (4). The oily residue was reconverted to the dihydrochloride salt by dissolving in EtOH (5 cm^3)and treating with conc. HCl till pH1 followed by precipitation with ether. crystallisation from abs. EtOH gave SKH67A (0.7g, 70%), mp: 218-20 °C.The ^1H NMR and elemental analysis were in agreement with the corresponding starting material i.e.the acetal dihydrochlride (4).

6) 4-(2-aminoacetyl)pyridine dihydrochloride

(sample SKH441A)

A solution of the acetal **(4)**(0.5g,1.7 mmole) in conc HCl (50 cm^3) was stirred at 50°C for 3h. The solvent was removed at 40-50°C under reduced pressure and the yellow solid was dried under reduced pressure at 90°C for 8h The product was isolated as the dihydrochloride salt. 0.35g (98%), mp: 218-220°C (Lit .[68] mp: 217°C).

UV: water, λ_{max}nm ($\log\varepsilon_{max}$); 313 (2.61); 253 (3.35); 206(3.44).

HPLC: 98.07% at 3.58 minutes (reverse phase column; Lichrosorb rp select B; 1cm^3/min; 250x4 mm; UV254 nm; A/B (50/50) where A is water and B is MeOH. Mobile phase

contains 0.1/100 cm3 water hexanesulphonic acid + 0.5% orthophosphoric acid. The impurity is 1.92% at 6.02 minutes.

IR: KBr.cm^{-1} 3045, 3031 (s, N-H); 1705 (s, C=O); 1602 (m, C=N).

^{1}H NMR: (DMSO-d_6,400MHz); δ (ppm); 4.67 (broad s, 2H, CH_2NH_2); 8.15 (d, J = 5.27Hz, 2H, Py-3,5); 9.01 (d, J = 5.49Hz, 2H, Py-2,6).

^{1}H NMR: (D_2O,400MHz); δ (ppm); 3.35(s, 2H, -CH_2); 4.82 (s, -CH_2); 8.28 (dxd, J = 5.66, 5.61Hz, 2H, Py-3,5); 8.46 (dxd, J = 5.37, 5.11Hz, Py-3,5), 8.87 (dxd, J = 5.57, 2H, Py-2,6); 9.04 (dxd, J = 5.37, 5.11Hz, Py-2,6).

Mass spectrum: (EI); m/e 136 (M^+), 134 (M^+-2H), 106 (Py-COH)

Anal. Calc for $C_7H_{10}Cl_2N_2O$: C, 40.2; H, 4.82; Cl, 33.91; N, 13.40

found: C, 39.97; H, 4.63; Cl, 33.85; N, 12.94

The acetal **(3)** was hydrolysed by the same procedure as **(4)** and the product was identified qualitatively by HPLC [98.5% at 3.6 minutes under at similar condition as (4)]

7) 4-(4-pyridyl)imidazole-2-thione

(sample SKH447A)

The α-aminoketone dihydrochloride **(6)**(0.15g, 7.1 mmole) in water (20 cm^3) was treated with 2 molar equivalent of KCNS (0.139g, 1.43 mmole). The solution was heated on a steam bath for 3h and was left overnight at 21°C. The crystalline hydrochloride product was collected by filtration 0.085g (55%); mp: 254-6°C (Lit.[68] mp: 254°C decomposition).

TLC: silica (EtOAc:MeOH: NH_4OH; 5:1:1) R_f: 0.45.

The filtrate on standing afforded the second crop of the hydrochloride 0.015g. (overall yield: 65%) The product was crystallised from water (20 cm^3) mp: 248-50°C (Lit.[68] mp: 254°C)

The stirred aqueous solution of the hydrochloride salt was treated with a saturated solution of potassium bicarbonate in water and heated at 70 °C till dissolved .On standing some yellow solid deposited; this was collected, washed with water and then dried mp: 295-300°C (Lit.[56] mp: 270°C).

HPLC: 98.73% at 4.33 minutes (Lichrosorb rp select B, 250x4 mm, 1cm^3/min, UV254 nm, A/B (60/40) where A is water and B is MeOH. The mobile phase contains 0.1% trifluoroacetic acid). for the free amine.

UV: water; λ_{max}nm ($\log\varepsilon_{max}$); 305 (3.59); 253 (3.39) ; 217 (3.55).

IR: KBr cm^{-1} 3082, 3020 (w, N-H); 2090 (s, N=C=S); 1626 (s, C=N, Py).

^{1}H NMR: (DMSO-d_6, 400 MHz); δ (ppm) 8.154 (d, J = 6.40Hz, 2H, Py-3,5); 8.173 (s, 1H, Im-5); 8.76 (d, J = 4.73Hz, 2H, Py-2,6).

Mass spectrum: (EI); m/e 177 (M^+); 106 (Py-CH=NH) ; 59 (HN-C=S) .

Anal. calc. for $C_8H_7N_3S$: C, 54.21; H, 3.98; N, 23.71; S, 18.09;

found: C, 54.47; H, 4.34; N, 23.71; S, 17.99

8) An attempt to prepare the thione (7) from the acetal (4)

(sample SKH47A)

The acetal dihydrochloride (**4**)(0.25g, 0.88 mmole) and potassium thiocyanate (0.171g, 1.76 mmole, 2 molar equivalent) in water (5cm^3) were heated on a steam bath for 10 h On

standing no solid appeared . Evaporation of the solvent afforded a dark red residue which was identified by ^1H NMR and mass spectrometry as the unchanged acetal (**4**).

Mass spectrum (FAB) m/e 211 (M^+)

9) 4-(4-Pyridyl)imidazole

(sample SKH51C)

The imidazole thione free base (**7**) (0.29g, 1.6mmole) was added to water (10cm^3) containing conc HNO_3 (0.4 cm^3) and heated on a steam bath for 1h under reflux. The mixture was cooled and basified with saturated potassium bicarbonate to pH9 On standing some solid deposited; this was collected and dried 0.134g (56%), mp: 194-5^{o}C (Lit.[68] mp: 188^{o}C). Crystallisation from water afforded yellow needle crystals 0.109g; mp: 194-5^{o}C (Lit.[68] mp: 188^{o}C).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.46

^1H NMR: (Acetone- d6, 400 MHz),δ (ppm): 7.75 (s, 1H, Im-5); 7.77 (d, 2H, Py-3,5); 7.85 (s, 1H, Im-2); 8.5 (d, J = 6.23, 2H, Py-2,6).

Mas spectrum: (EI); m/e 145(M^+).

UV: water; λ_{max}nm ($\log\varepsilon_{max}$): 312 (3.26); 268 (4.12); 203 (3.91) . Lack of the sharp absorbance at 305nm ($\log\varepsilon_{max}$ 3.59), the characteristic absorbance of the thione indicates absence of the thione in the purified sample.

Anal. Calc. for $C_8H_7N_3$: C, 66.19; H, 4.80; N, 28.95.

found: C, 66; H, 4.99; N, 29.03.

10) Attempts to hydrogenate the 4-(4-pyridyl)imidazole.

I) To a solution of the pyridyl imidazole (**9**)(1g, 6.8 mmole) in 5N HCl (110 cm^3) , glacial acetic acid (20 cm^3) , Adam's catalyst (PtO_2, 1g) was added. The system was hydrogenated (under 30 atm. pressure at 21 ^oC) using Cook hydrogenation apparatus for 24h. The reaction mixture was filtered and the filtrate was basified with sodium bicarbonate. The basic solution was evaporated to dryness and the water was azeotropically removed with i-PrOH , redissolved in i-PrOH and filtered. The solvent was evaporated to half the volume and treated with ethanolic HCl. On addition of ether, a white solid precipitated which was collected. Mass spectrum and TLC (EtOAc: MeOH: NH_4OH; 5:1:1) indicated only the unchanged starting materials. .

II) The pyridyl imidazole (**9**)(1.508g, 10.33 mmole) in KOH solution (50 cm^3, 0.5 mole) and Nickel-Aluminium alloy (6.5g) were stirred at 21^oC for 4 days and finally the reaction mixture was heated at 40^oC for 7 days , filtered through celite bed and washed with water. TLC (silica; EtOAc: MeOH: NH_4OH; 5:1:1) indicated unchanged starting materials.After the addition of fresh catalyst and refluxing the aqueous solution overnight, the reaction mixture was filtered and concentrated to dryness under reduced pressure. The solid was crystallised from $CHCl_3$. mp: 189-193^oC (Lit.[68] mp. of the starting material is 188^oC).

III) The pyridyl imidazole (**9**)(2g); water (50 cm^3); conc HCl (67.5 cm^3); 5 % Rh/C (1g) were agitated under 30 atm of H_2 at 21^oC for 11 days. The catalyst was filtered off and the solvent was removed under reduced pressure. The solid was shown to be the starting material as dihydrochloride mp: 258-60^oC (comparing with the starting material[68], mp: 188^oC).

11) 4-(4-piperidyl)imidazole dihydrochloride

(sample SKH119B)

To the solution of 4-(4-pyridyl)imidazole (**9**)(0.2g, 1.37 mmole) in water (5cm^3) and conc HCl (0.75 cm^3) was added rhodium on carbon (0.1g). The mixture was stirred under hydrogen (30 atm.) for 5h at 21 ^oC. After removal of the catalyst by filtration, the evaporation of the solvent afforded the product as the dihydrochloride salt, 0.295g (97%), mp: 289-92 ^oC (Lit.[3] mp: 292-5^oC). The product was recrystallised from MeOH: Ether (70cm^3, 1:2). 0.265g mp: 289-90^oC

Solubility: sol. (water, MeOH), sp. sol. (i-PrOH), insol. ($CHCl_3$, ether).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.55

HPLC: 100% at 9.81 minutes. (Kromacil C18 5mm 250x4mm, 1cm^3/min, UV 215nm, 0.1 aufs, A/B (75/25) where A is water+ 0.1% trifluoroacetic acid with 0.05%M hexane sulphonic acid (Na salt) and B is methanol + 0.1% trifluoroacetic acid).

UV: water; λ_{max}nm ($\log\varepsilon_{max}$): 208 (3.45)

IR: KBr cm^{-1} 3082 (s, NH str.), 2961 (s, -NH- asym), 2790 (s, -NH- sym), 1607 (m, NH scissoring), 1570 , 1468, 1408 (w, C-C ring str., Im).

^{1}H NMR: (D_2O, 200 MHz); δ (ppm); 1.22 (broad m, 2H, Piperidine); 1.62 (broad d, 2H, Piperidine); 2.70 (broad t, 3H, Piperidine); 6.63 (s, 1H, Im-5); 7.96 (s, 1H, Im-2).

Mass spectrum: (EI) m/e 151 (M^+), 82 (Im).

Anal. Calc. for $C_8H_{13}N_3$, 2HCl: C, 42.86; H, 6.74; N, 18.7.

found: C, 42.30; H, 6.71; N, 18.72.

12) Treatment of the tosyl ester (2) with sodium hydroxide ;

(sample SKH430A)

To a stirred solution of the tosyl ester (2)(1g, 3.4 mmole) in water (10 cm^3), a solution of sodium hydroxide (0.27g, 6.8 mmole, 2 molar equivalent) was added dropwise. The reaction mixture was stirred at 21 °C for 24h and then heated under reflux at 80-90°C for 24h, when the reaction mixtyre turned red. The reaction mixture was extracted with $CHCl_3$ (3x50 cm^3). The extracts were combined and dried ($MgSO_4$), filtered and then the filtrate was evaporated to dryness under reduced pressure to an oily residue 0.32g (37% yield).

Mass spectrum: (EI) m/e 236 (M^+); 234; 136. The M^+ (236) corresponds to 2,5-dipyridyl-3,6-dihydropyrazine (mol. wt 236) which oxidises in air to give 2,5-dipyridyl pyrazine.(mol.wt 234); and 136 corresponds to the aminoketone (6).

IR: KBr cm^{-1}, 3331, 3189 (m, N-H str.) 1597 (s, C=N str.).

When the residual mixture was subjected to Prep. HPLC using rp select lichrosorb column eluted with A= water+ 0.1% TFA ; B= MeOH+ 0.1% TFA, the mass spectrometry analysis indicated 237 (MH^+) as the minor component but 137 (MH^+) as the major one which corresponds to the α-aminoketone (M^+ 136).

5.2) Preparation of other heterocyclic derivatives of histamine and pyridine:

General preparations of histamine base:

i) An aqueous solution of histamine dihydrochloride was treated with a saturated solution of potassium bicarbonate at pH9. The resulting solution was concentrated under reduced pressure and reesidual moisture was removed by azeotropic distillation with i-PrOH The solid residue was suspended in i-PrOH, heated and filtered hot. The filtrate, on evaporation yielded the histamine base as an oil.

ii) Histamine dihydrochloride was basified by refluxing in a solution of sodium ethoxide in ethanol (2 molar equivalent) for 1h. The mixture was then left to cool to 21°C and filtered. The solvent was removed under reduced pressure and the residue was suspended in i-PrOH, heated and filtered hot. Evaporation of the solvent afforded the histamine base as an oil.

13) 2-{2-[4(5)-imidazolyl]ethyl}aminopyridine dimaleate

(sample SKH130A/UCL1038)

A solution of histamine base (2.40g, 21.5 mmole) and 2-bromopyridine (3.41g, 2.15 mmole) in 4-methylpyridine (5 cm^3) were heated under reflux for 26h, when it turned black. The reaction mixture was treated with charcoal, filtered and the filtrate was concentrated under reduced pressure to an oily residue . The residue was chromatographed on silica eluted with MeOH (1, 5 and 20%) in $CHCl_3$ and most of the unchanged starting materials were recovered. The combined pure fractions were evaporated under reduced pressure to give an oily residue which was dissolved in EtOH and treated with an ethanolic

solution of maleic acid (2 molar equivalent). Addition of ether afforded the pure product as the dimaleate salt. The product was recrystallised from absolute ethanol. 0.56g (8%); mp: 137-8°C.

Solubility: sol. (water, abs. EtOH); insoluble (ether).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.62.; (MeOH: $CHCl_3$; 1:1) R_f: 0.58.

HPLC: 99.80% at 6.77 minutes (Lichrosorb rp select B; 250x4 mm; 1cm^3/min; UV 254nm; A/B (50/50), where A is water and B is acetonitrile. The mobile phase contains 0.1% triethylamine).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$): 304 (3.66); 237 (4.25); 211 (4.51).

IR: KBr cm^{-1} 3522, 3355 (w, N-H str.); 1657 (s, C=C-N str., Im); 1618 (s, C=N- str., Im); 1381 (C=N, ter str.); 1356 (s, C=N sec. str. arom).

^1H NMR: (D_2O, 200MHz); δ (ppm): 2.38 (t, J = 6.10Hz, 2H, $\underline{CH_2}CH_2NH$); 2.97 (t, 2H, $\underline{CH_2}$ NH); 5.52 (broad singlet, 1H, -NH-); 6.22 (m, J = 8.2, 6Hz, 2H, Py-3,4); 6.62 (broad s, 1H, Im-4(5)], 7.2 (m, J = 8.2, 6.2, 2H, Py-5,6); 7.88 (broad s, 1H, Im-2)..

Mass spectrum: (EI); m/e 188 (M^+); 107 ($Py\text{-}CH_2CH_3$); 95 ($ImCH_2CH_2$).

Anal. Calc. for $C_{10}H_{12}N_4$, 2.2 $C_4H_4O_4$: C,49.27; H,4.59; N,12.63..

found: C,49.19; H,4.76; N, 12.79.

14) 2-{2-[4(5)-imidazolyl]ethyl}aminopyrimidine hydrogen oxalate monohydrate

(sample SKH91B/UCL1017)

The solution of 2-chloropyrimidine (2.89g, 27.2 mmole) and histamine (3g, 27.2 mmole) in i-PrOH (30 cm^3) was heated under reflux for 36h. The solvent was removed under reduced pressure and the oily residue was chromatographed on silica gel eluted with

chloroform and methanol (1:1). The pure fractions were combined and evaporated under reduced pressure to give an oily residue 0.45g (27% yield). An ethanolic solution of the residue was treated with two molar equivalent of oxalic acid in abs. EtOH. Addition of ether gave the oxalate salt.SKH90A (1.13g, mp: 179-82°C). Concentrating the filtrate to 1/2 of the volume gave SKH90B (0.076g, mp: 178-82°C). Addition of more ether to the filtrate gave SKH90C (0.076g). Recrystallisation of the salt from abs. EtOH afforded the product as the hydrogen oxalate monohydrate salt. 1.13g. mp: 200- 2°C.

Solubility: sol. (water), sp. sol. (abs. EtOH, i-PrOH), insol (ether, EtOAc).

TLC: silica (EtOAc: MeOH: NH_4OH, 5:1:1) R_f: 0.57; ($CHCl_3$: MeOH) R_f: 0.64.

HPLC: 99.1% at 7.23 minutes (50x100 mm Spherisorb C18, 1 cm^3/min, UV240 nm, A/B (20/80) where A is water and B is MeOH. Mobile phase contains 0.1% triethylamine).

UV: water; λ_{max}nm ($\log\varepsilon_{max}$), 299 (3.42), 232 (4.27).

IR: KBr cm^{-1} 1671 (s, C=C-NH- str.); 1625,1604 (s, C=N str., Pym).

^1H NMR: (D_2O, TMS salt, 200 MHz); δ(ppm): 2.32 (t, 2H, $\underline{CH_2}CH_2NH$); 2.98 (t, 2H, $\underline{CH_2}NH$); 6.08 (t, 1H, Pym-5); 6.52 [s,1H, Im-4(5)]; 7.64 [d, 2H, Pym-4,6]; 7.83 [s, 1H, Im-2).

Mass spectrum: (EI); m/e: 189 (M^+); 108 ($PymNHCH_2$).

Anal Calc. for $C_9H_{11}N_5$, $C_2H_2O_4$, H_2O: C, 44.44; H, 5.08; N, 23.56.

Found: C, 44.79; H, 4.90; N, 23.53.

15) 2-{2-[4(5)- imidazolyl]ethyl}-aminobenzothiazole maleate

(sample SKH103D/UCL1029)

2-Chlorobenzothiazole (1.65g, 9.7 mmole) and histamine (1.08g, 9.7 mmole) in i-PrOH (20 cm^3) were stirred and heated under refluxed for 48h, evaporated to dryness and then the solid residue was taken up in water, stirred for 1h and filtered the insoluble solid was the pure product. 1.58g (66% yield), mp: 185-7°C.The filtrate was shown to contain some of the unchanged starting materials by TLC (EtOAc: MeOH: NH_4OH; 5:1:1). The maleate salt was prepared by treating an ethanolic solution of the product (0.37g, 1.51 mmole) with a solution of maleic acid (2 molar equivalent) in absolute ethanol. On addition of ether, the monomaleate salt of the product was obtained. Recrystallisation of the salt from absolute ethanol afforded SKH103D as a white crystalline solid. 0.42g (66% yield). mp: 137-8°C.

Solubility: sol. (water); sp.sol.(MeOH); insol. ($CHCl_3$, EtOAc).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.58.

HPLC: 99.93% at 7.72 minutes (Lichrosorb rp select B 250x4 mm, UV 254nm, A/B (40/60) where A is water and B is MeOH. Mobile phase contains 0.1% triethylamine).

UV: water; λ_{max}nm ($\log\varepsilon_{max}$): 292 (3.64); 263 (4.12); 220 (4.55).

IR: KBr cm^{-1} 3495,3349 (w, N-H str.), 1611, 1567 (s, C=N str., thiazole).

^1H NMR: (D_2O, 200 MHz); δ(ppm) 2.34 (t, 2H, $\underline{CH_2}CH_2NH$); 2.94 (br t, J = 6.35, 2H, $\underline{CH_2}NH$); 5.50 (d,1H, NH); 6.67 (m, 2H, aromatic); 6.87 (m, 2H, aromatic); 6.91 [s, 1H, Im-4(5)]; 7.81 (s, 1H, Im-2).

Mass spectrum: (EI) m/e 244 (M^+), 163 (benzothiazole-$NHCH_2$), 150 (benzothiazole-NH_2), 82 ($ImCH_2^+$).

Anal. Calc. for $C_{12}H_{12}N_4S$, $C_4H_4O_4$: C, 53.32; H, 4.47; N, 15.54.

Found: C, 53.07; H, 4.64; N, 15.22.

16) 2-{2-[4(5)-imidazolyl]ethyl}aminopyrazine oxalate

(sample SKH176A/UCL1093)

The histamine base (1g, 8.9 mmole) and 2-chloropyrazine (1.03g, 8.9 mmole) in i-PrOH (20 cm^3) were heated under reflux for 72h. The solvent was removed under reduced pressure. An attempt to purify the product by crystallisation from abs. EtOH was unsatisfactory. Purification was effected by column chromatography on silica gel using MeOH (1, 5, 10, 20%) in $CHCl_3$ as the eluent mixture. Some of the unchanged starting materials were recovered. The selected middle fractions were combined and evaporation under reduced pressure followed by extraction with i-PrOH to remove traces of silica.gel gave 0.43g (25.6%) To the solution of the product in abs. EtOH (10 cm^3) was added an ethanolic solution of oxalic acid (excess). After addition of ether until the mixture was cloudy it was kept in the fridge overnight. A white precipitate was collected and recrystallised from i-PrOH-ether (10:1). to give SKH 176A, 0.85g, mp: 201-2°C.

Solubility: sol. (water), sp. sol.(MeOH), insol. ($CHCl_3$).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.66.

HPLC: 100% at 7.99 minutes (Lichrosorb rp select b, 250x4mm, 1 cm3/min, UV254nm, A/B (80/20) wher A is water and B is acetonitrile. Mobile phase contains 0.1% trifluoroacetic acid).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$); 326 (4.39), 241 (4.89), 205 (4.37).

IR: KBr cm^{-1}; 3435, 3019 (m, N-H str.), 1634 (s, C=N, pyrazine), 1607 (m, C=N, pyrazine).

^1H NMR: (DMSO-d_6, 200MHz); δ (ppm); 2.88 (t, 2H, $ImCH_2$), 3.53 (t, J = 5.69, 2H, $\underline{CH_2}NH$), 7.26 (broad singlet, 1H, NH), 7.46 (d, 1H, Im-4(5)], 7.64 (d, 1H, pyrazine-3), 7.91 (d, J = 2.76, , 2H, pyrazine 5,6), 8.95 (s, 1H, Im-2).

Mass spectrum: (EI); m/e 189 (M^+), 108 ($PyNHCH_2^+$), 95 ($ImCH_2CH_2^+$), 82 ($ImCH_2^+$).

Anal. Calc. for $C_9H_{11}N_5$, 1.5 $C_2H_2O_4$, 0.75 H_2O: C, 42.66; H, 3.73; N, 20.73.

found: C, 42.08; H, 3.82; N, 21.42.

17) 2-{2-[4(5)-imidazolyl]ethylamino}-3,6-dimethylpyrazine dioxalate monohydrate

(sample SKH179A/UCL1094)

To the solution of histamine base (1g, 8.9mmole) and 2-chloro-3,6-dimethylpyrazine (1.28g, 8.9mmole) in i-PrOH(10 cm^3), potassium bicarbonate (one molar equivalent) was added. The reaction mixture was heated under reflux for 72h. The inorganic solid was filtered off and the filtrate was evaporated, under reduced pressure, to an oily residue. TLC (EtOAc: MeOH: NH_4OH; 5:1:1) showed some unchanged starting materials. Purification was by silica column chromatography using MeOH (1, 10, 20%) in $CHCl_3$ as the eluant mixture. Some of the unchanged starting materials were recovered and the mid fractions containing the pure product were combined and concentrated to an oily residue. This was taken into i-PrOH and filtered to remove traces of dissolved silica. The solvent was removed under reduced pressure, redissolved in abs. EtOH (10 cm^3) and treated with an ethanolic solution of oxalic acid (2 molar equivalent in 10 cm^3). Addition of ether, gave SKH178A (1.25g, 33.5%). Recrystallisation from i-PrOH (10 cm^3) and ether (100 cm^3) gave SKH179A , 1.2g, mp: 164-5°C.

Soubility: sol.(water), sp. sol.(MeOH), insol. (ether, EtOAc).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.71

HPLC: 99.51% at 8.20 minutes (Lichrosorb rp select b, 250x4mm, 1 cm^3/min, UV 254nm, 0.5aufs, A/B (80/20) where A is water + 0.1% triethylamine and B is acetonitrile + 5% water + 0.1% triethylamine). The impurity is 0.21% at 5.16 minutes.

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$), 321 (3.85), 268 (shoulder, 3.06), 238 (4.12), 210 (3.92).

IR: KBr cm^{-1} 3441 (m, N-H str.), 3372 (, N-H str.), 1624 (s, C=N, Pyrazine), 1524 (s, C=N, dimethypyrazine), 1198 (m, C-N str. Im).

^1H NMR: (DMSO-d_6, 400MHz); δ (ppm); 2.20 (s, 6H, 3,6 dimethypyrazine), 2.93 (t, J = 6.68Hz, J = 6.68, 1.57Hz, 2H, $ImCH_2$), 3.6 (poorly resolved doublet of triplet, 2H, CH_2NH), 6.49 (br. siglet, NH), 7.43 [s, 1H, Im-4(5)], 7.48 (s, 1H, pyrazine-5), 8.85 (s, 1H, Im-2).

Mass spectrum: (EI), m/e 217 (M^+), 136 (dimethylpyrazine-$NHCH_2^+$), 82 ($ImCH_2^+$).

Anal. Calc. for $C_{11}H_{15}N_5$, $C_4H_4O_8$, H_2O: C, 43.37; H, 5.09; N, 16.86.

found: C, 43.46; H, 4.97; N, 16.40.

18) 2-{2-[4(5)-imidazolyl]ethylamino}-3-nitropyridine.

(sample SKH117A/UCL1039)

Histamine base (1.135g, 102 mmole) and 2-chloro-3-nitropyridine (1.619g, 102 mmole) in i-PrOH (5 cm^3) were heated under reflux for 3h. Evaporation of the solvent under reduced pressure and purification of the solid residue by column chromatography on silica gel using a gradient of MeOH (5, 10, 20%) in $CHCl_3$ as eluate . The fractions containing the pure product were combined and the solvent was removed under reduced pressure to give SKH116A (0.61g) . The other partially impure fractions were purified once more by column chromatography on silica gel using MeOH: $CHCl_3$ (1:10) as eluate. The pure fractions were combined and the solvent was removed under reduced pressure to give SKH116B (0.925g) (Combined yield: 1.526g, 64% yield). The product was crystallised from abs EtOH: water (1:4). The orange crystalline product was collected, washed with water and dried. 1.25g (52%). mp: 177°C.

Solubility: sol ($CHCl_3$, abs.EtOH), sp. sol.(water), insol (acetone).

TLC: (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.71. ($CHCl_3$: MeOH; 1:1) R_f: 0.75.

HPLC: 99.23% at 18.69 minutes (Lichrosorb rp select B, 250x4 mm, 1 cm^3/min, UV254 nm, A= water, B= EtOH, gradient 10-25% B in 25 minutes. Mobile phase contains 0.1% phosphoric acid and 5 cm^3 of 0.1 M hexane sulphonic acid was added to every 100 cm^3 of mobile phase).

UV: methanol; λ_{max}nm ($\log\varepsilon_{max}$); 404 (3.85); 265 (3.74); 223 (4.39).

IR: KBr cm^{-1} 1540 (s, N-O str); 1338 (s, N-O str. conjugated with C=N).

^1H NMR: (DMSO-d_6, 200MHz), δ (ppm); 2.80 (poorly resolved d, 2H, Im$\underline{CH_2}$); 3.78 (q, J = 6.2, 6.7Hz, 2H, $\underline{CH_2}$NH); 6.74 (m, J = 8.5, 5.6Hz, 1H, Py-5); 6.90 (s, 1H, NH); 7.54 [s, 1H, Im-4(5)]; 8.38 (d, 1H, Py-6); 8.48 (d, 1H, Py-4); 8.68 (s, 1H, Im-2).

Mass spectrum (EI) m/e 233 (M^+); 152 (paranitropyridine-$NHCH_2$), 82.($ImCH_2^+$)

Anal. Calc. for $C_{10}H_{11}N_5O_2$: C, 51.49; H, 4.75; N, 30.03.

found: C, 51.59; H, 4.69; N, 30.04.

19) 2-{2-[4(5)-imidazolyl]ethyl}-amino-5-nitropyridine.

(sample SKH126B/UCL1040)

2-Chloro-5-nitropyridine (2.225g, 14 mmole) and a molar equivalent of histamine base (1.56g) in i-PrOH (10 cm^3) were refluxed for 48h. The reaction mixture was concentrated to dryness and the solid residue was taken into water and heated to boiling. On standing some material deposited; this was filtered away.The filtrate was concentrated to dryness and subjected to column chromatography on silica gel eluted with a gradient of MeOH (5,10 and 20%) in $CHCl_3$. The combined pure fractions were evaporated to dryness and the soild residue was crystallised from absolute ethanol.which yielded a dark yellow needle

shaped crystalline solid.0.685g (21%), mp: 162-3°C. The filtrate, on standing afforded the second crop. 0.143g (overall yield: 26%), mp: 161-2°C.

Solubility: sol (EtOH, DMSO), sp. sol (water). insol (ether).

TLC silica (EtOAc: MeOH: NH_4OH; 5: 1: 1) R_f: 0.67; ($CHCl_3$: MeOH; 1:1) R_f: 0.59.

HPLC: 100% at 9.40 minutes (Lichrosorb rp select B, 1 cm^3/min, UV 254nm, A/B (70/30), where A is water with 0.1% triethylamine and B is acetonitrilewith 0.1% triethylamine).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$nm): 357 (4.240); 218 (4.10)

IR: KBr cm^{-1} 3422, 3238 (w, N-H str), 1540 (s, N–O str. asym aromatic), 1338 (s, N-O str. aromatic), 1614 (s, C=C-NH str., Im), 1467 (s, C=N).

^1H NMR: (DMSO-d_6, 200 MHz): δ (ppm); 2.76 (m, J = 5.2Hz, 2H, Im$\underline{CH_2}$); 3.62 (m, 2H, $\underline{CH_2}$NH); 6.60 (d,1H, Py-3); 6.88 (s, 1H, NH); 7.54 [s, 1H, Im-4(5)]; 8.06 (m, 1H, Py-4); 8.22 (s, 1H, Im-2); 8.90 (d, 1H, Py-6).

Mass spectrum: (EI), m/e 233 (M^+); 152 (paranitropyridine-$NHCH_2$); 82 ($ImCH_2^+$).

Anal. Calc. for $C_{10}H_{11}N_5O_2$: C, 51.49; H, 4.75; N, 30.03

found: C, 51.63; H, 4.70; N, 30.03.

The experiment was repeated using histamine (3g, 27 mmole) and 2-chloro-5-nitropyridine (4.28g, 27 mmole) and a molar equivalent of Et_3N (2.37g). The mixture was heated under reflux at 84°C for 7h. The product was isolated by column chromatography as before.and was rcrystallised from abs. EtOH SKH149 (2.20g, 35%).

20) 2-{2-[4(5)-imidazolyl]ethylamino}-1H-phenyltetrazole

(sample SKH135A/UCL1079)

5-Chloro-1H-phenyltetrazole (0.87g, 4.82 mmole) and histamine (0.54g, 4.82 mmole) in pyridine(13 cm^3) were heated at 80°C on an oil bath for 5h. The reaction mixture was left to cool to 21°C and the crude soild was collected. On addition of ether to the filtrate, the second crop precipitated. The first and the second products were chromatographed on silica using a gradient of MeOH (0.0, 1, 10, 20, 50%).in $CHCl_3$ as eluate. The fractions containig the pure products (SKH135A) were evaporated to dryness under reduced pressure. The products were recrystallised from hot abs. EtOH.to give 0.8g (13.5%), mp: 272-3°C.The combined fractions containig the second product were evaporated to dryness and the crude product was recrystallised from hot abs. EtOH. (SKH135B): 0.16g (13%).mp: 219-20°C. The product SKH135A was fully characterised, mp: 272-3°C.

Solubility: Sol. (EtOH, MeOH), insol. (EtOAc, water, $CHCl_3$).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f:0.72, ($CHCl_3$: MeOH; 1:1) R_f: 0.84.

HPLC: 99.6% at 5.46 minutes (Lichrosorb rp select b, 250x4 mm, 1cm^3/min, UV 254nm, 0.05aufs, A/B (80/20) wher A is water with 0.1% trifluoroacetic acid and B is acetonitrile + 5% water with 0.1% trifluoroacetic acid). The impurity was 0.4% at 12.41 minutes.

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$); 254 (4.26), 206 (4.07).

IR: KBr cm^{-1} 3181 (w, C-H str.), 2982 (w, w, C-H str. arom), 1622 (s, C=N str), 1577 (s, C=N str.), 1539 (s, -NH-, Im), 1099 (s, C-N str.).

^1H NMR: (DMSO-d_6, 400 MHz), δ(ppm), 3.06 (t, J = 6.74Hz, 2H, $ImCH_2$), 4.58 (t, J = 7.23Hz, 2H, CH_2NH), 6.84 [br.s, 1H, Im-4(5)], 6.98 [t, J = 7.30Hz, 1H, phenyl-para], 7.34 (t, J = 7.62Hz, 2H, phenyl meta), 7.62 (d, 2H, phenyl ortho), 9.51 (s, 1H, Im-2).

Mass spectrum: (EI), m/e 255 (M^+), 146 (phenyl tetrazole), 82 ($ImCH_2^+$).

Anal Calc.for $C_{12}H_{13}N_7$: C, 56.45; H, 5.13; N, 38.41

found: C, 56.41; H, 4.93, N, 37.98

The second product SKH135B was shown to have the same molecular mass and The ^1H NMR (DMSO-d_6, 400 MHz) showed two triplets at δ (ppm) 2.81 and 3.51 for the $ImCH_2$ and $\underline{CH_2}NH_2$ groups respectively and indicating that the nucleophilic substitution has occured at the NH of the imidazole ring Since the compound was of no biological interest, further characterisation was not done.

Mass spectrum: (EI) m/e 255 (M^+), 82 ($ImCH_2^+$).

^1H NMR: (DMSO-d_6, 400 MHz), δ(ppm), 2.81 (t, J = 7.3Hz, 2H, $ImCH_2$); 3.51 (q, J = 7.36Hz, 2H, CH_2NH_2); 7.1 (t, J = 5.45Hz, 1H, Phenyl para); 6.8 (s, 1H, Im-4(5); 7.51-7.61 (m, J = 6.5 and 4.6Hz, 3H, Phenyl meta and para and Im-2).

21) 2-{2-[4(5)-imidazolyl]ethylamino]-thiazole dioxalate

(sample SKH173A/UCL1090)

The histamine base (1.1g, 9.86 mmole) and 2-bromothiazole (1.61g, 9.86 mmole) in i-PrOH (15 cm^3) were heated under reflux for 96h The reaction mixture was concentrated to an oily residue under reduced pressure. The oily residue was chromatographed on silica gel using a gradient of MeOH (1, 5, 20%) in $CHCl_3$ as eluant. The pure fractions were combined and concentrated under reduced pressure to an oily residue The residue was taken into abs. EtOH and was treated with an ethanolic solution of oxalic acid (2 molar equivalent). Addition of ether gave the product as the dioxalate salt. The crude product was crystallised from i-PrOH.1.15g (31% yield), mp: 198-9°C.

Solubility: sol.(MeOH, water), sp. sol.(abs. EtOH), insol (EtOAc).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.7.

HPLC: 99.445% at 5.54 minutes (Lichrosorb rp select B, 250x4 mm, 1 cm3/min, UV220 nm, A/B (80/20),where A= water +1% hexane sulfonic acid + (1%) phosphoric acid and B= MeOH+ 1% hexane sulfonic acid + 1% phosphoric acid).

UV: water, λ_{max}nm ($\log\varepsilon_{max}$); 209 (3.65), 257 (3.87).

IR: KBr cm^{-1} 3199, 2977 (m, N-H str.); 1614 (s, c=N Im); 1280 (s, C=S str.).

^1H NMR: (DMSO-d_6, 200 MHz); δ (ppm); 2.83 (t, J = 6.03Hz, 2H, $ImCH_2$), 3.44 (t, 2H, CH_2NH), 6.55 (d, J = 3.1Hz, 1H, Thiazole-4), 7 0 (d, 1H, Thiazole-5); 7.32 [s, 1H, Im-4(5)], 8.76 (s, 1H, Im-2).

Mass spectrum: (EI),m/e 194 (M^+); 113 (Thiazole-$NHCH_2$); 95 ($ImCH_2CH_2$); 82 ($Im-CH_2^+$).

Anal. Calc. for $C_8H_{10}N_4S$, $C_4H_4O_8$: C, 38.50; H, 3.76; N, 14.96

found: C, 38.28; H, 3.50; N, 14.96

22) 4-[(2-aminoethyl)thiomethyl]imidazole dihydrobromide

(sample SKH141B)

4-Hydroxymethyl imidazole hydrochloride (1g, 7.4 mmole) and one equivalent molar of 2-aminoethylthiol hydrochloride (0.74g) in aqueous HBr (48%, 15 cm^3) were heated under reflux f r 18h. After cooling the dark red solution was evaporated to dryness and the solid residue was washed with absolute ethanol-ether (30 cm^3, 1:1). 2.25g (95 %). mp: 178-9 °C. The product was recrystallised from absolute ethanol: ether (10:20 cm^3) to give 2.16g (91.5 %) of SKH141B, mp: 178-9°C.(Lit.[32]. mp: 178-9°C)

^1H NMR: ($CDCl_3$, 200MHz): δ (ppm): 0.98 (t, J = 6.3Hz, 2H, CH_2NH); 1.36 (t, 2H, SCH_2); 2.09 (s, 2H, CH_2S); 5.61 (s, 1H, Im-4); 6.85 (s, 1H, Im-2).

Mass spectrum: (EI); 158 (M^+), 128 ($ImCH_2SCH_2^+$), 114 (ImCH=S), 82 ($ImCH_2^+$).

23) 2-{[2-[4(5)-imidazolyl]methylthio]ethylamino]}-5-nitropyridine.

(sample SKH156A/UCL1068)

A solution of the amine dihydrobromide SKH141B(22)(5g, 15.6 mmole) in water (5 cm^3) was basified to pH11 with potassium carbonate (4.31g, 31.2 mmole) in water (15 cm^3). The resulting solution was concentrated under reduced pressure and the residue was azeotroped several times with i-PrOH. The solid residue was suspended in i-PrOH, heated and filtered hot. Evaporation of the filtrate under reduced pressure yielded the amine as an oil The amine (1.99g, 12.7 mmole) and 2-chloro-5-nitropyridine (2.013g, 12.7 mmole) in i-PrOH (20 cm^3) were heated under reflux for 18h. On standing, some orange solid deposited, which was collected. This solid was subjected to column chromatography on silica gel using a gradient of MeOH (1, 5, 10 and 20%) in $CHCl_3$ as eluate The combined pure fractions were concentrated to dryness under reduced pressure. Attempts to recrystallise the crude product from toluene: ethanol (10:1) containing 1% of ammonia or petroleum ether or i-PrOH: ether or acetonitrile were unsuccesful.so the crude solid (0.1g) was purified by reverse phase HPLC (Lichrosorb rp select B 10μM, UV 254nm, A/B: water/MeOH (60/40 for 15 min and a gradient of 5-95% methanol,.mobile phase contains 1% Et_3N).Evaporation of the solvent under reduced pressure to a solid residue and crystallisation from absolute ethanol yielded a pale yellow crown shape crystals 65mg (8.5% yield), mp: 145-7°C.

TLC: silica (EtOAc: MeOH: NH_4OH, 5:1:1) R_f: 0.64.

Solubility: sol (MeOH, abs.EtOH), sp. sol. ($CHCl_3$), insol. (water).

HPLC: 99.73% at 5.39 minutes, (Lichrosorb rp select B, 250x4 nm, UV254nm, A/B (60/40) where A is water and B is methanol. The mobile phase contains 0.1% triethylamine).

UV: MeOH; λ_{max} nm ($\log\varepsilon_{max}$); 357 (4.26), 216 (4.16).

IR: KBr cm^{-1} 1612 (s, C=C-NH str.), 1543 (s, N-O str.asym. arom), 1470 (s, C=N), 1336 (s, N-O str.sym.arom.).

^{1}HNMR: (DMSO-d_6, 400MHz), δ (ppm): 2.67 (s, J = 6.5Hz, 2H, S$\underline{CH_2}$); 3.57 (broad singlet, 2H, SCH_2); 3.69 (broad d,J = 6.5Hz, 2H, CH_2NH); 6.57 (d, 1H, Py-3), 6.95 (broad singlet, 1H, NH), 7.53 [s, 1H, Im-4(5)], 8.11 (d, 1H, Py-4), 8.25 (broad siglet, 1H, Im-2), 8.90 (d, 1H, Py-6).

Mass spectrum: (EI), m/e 279 (M^+), 280 (M^+), 262 (M^+-OH), 152 (5-nitropyridine-$NHCH_2$), 114 ($ImCH_2SH$), 82 ($ImCH_2^+$).

Anal. Calc. for C_{11} H_{13} N_5 O_2 S: C, 47.29; H, 4.69; N, 25.07.

found: C, 46.76; H, 4.51; N, 24.57.

24) N_1-2-[imidazol-4-yl]ethyl-N_2-cyclohexyl-thiourea

(sample SKH198B/UCL1108)

To a stirred solution of histamine (3.16g, 27 mmole) in abs. EtOH (50 cm^3), was added cyclohexylisothiocyanate (3.81g, 27 mmole) and the reaction was refluxed for 1h. The solvent was removed under reduced pressure and the white solid residue was redissolved in i-PrOH. Addition of ether afforded the product (SKH198A) as a white solid. 5.50g (80% yield); mp: 138-40°C.This was recrystallised from i-PrOH: ether (5:1) to give SKH198B as a white solid. 1.69g (3[% recrystallisation yield); mp: 138-40°C.

Solubility: sol.(MeOH); sp. sol.(water); insol. (EtOAc).

TLC: (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.65; ($CHCl_3$: MeOH; 1:1) R_f: 0.82

HPLC: 99.06% at 9.74 minutes (Lichrosorb rp select B; 250x4 mm; 0.75 cm3/min; UV 254nm; A/B (75/25) where A is water and B is acetonotrile with 5% water. Mobile phase contains 0.1% trifluoroacetic acid). The impurity is 0.63% at 5.99 min.

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$); 238 (4.19); 213 (4.12).

IR: KBr cm^{-1} 3216 (m, N-H str); 3060 (m, C-H str.; C=C); 1552 (s, -NH, Im); 1447 (m, C-N, Im); 1340 (m, C-H def.); 1280 (w, C=S str.).

^1H NMR: ($CDCl_3$; 200MHz); δ (ppm); 1.04-2.1 (m, 11H, cyclohexyl); 2.68 (t, 2H, $ImCH_2$); 3.63 (broad t, 2H, $\underline{CH_2}NH$); 6.02 (broad d, 1H, N<u>H</u>-cyclohexyl); 6.66 [s, 1H, Im-4(5)]; 7.44 (s, 1H, Im-2).

Mass spectrum: (EI); m/e 252 (M^+); 159 (cyclohexyl-$NHC(=S)NH_2)^+$; 141 (cyclohexyl-$NHCS^+$); 82 ($ImCH_2^+$).

Anal. Calc. for $C_{12}H_{20}N_4S$: C, 57.12; H, 7.99; N, 22.20; S, 12.68

found: C, 57.54; H, 7.94; N, 21.67; S, 13.02.

<u>25) N_1-2-[Imidazol-4-yl-(thiomethyl]ethyl-N_2-cyclohexyl thiourea monooxalate.</u>

(sample SKH188A/UCL1109)

I) Conversion of **(19)**(SKH141B) to base:

The aqueous solution of 4-[(2-aminoethyl) methylthio] imidazole dihydrobromide **(19)**(2.50g, 7.8 mmole in 5cm^3 water) was treated with a saturated aqueous solution of $NaHCO_3$ till pH9 and the solvent was removed under reduced pressure and the residual moisture was removed by azeotropic distillation with i-prOH. The residue was taken up in hot i-PrOH, filtered and the filtrate was evaporated under reduced pressure to give SKH187A as an oil. 1.18g (96% yield).

II) To a stirred solution of the amine (1.18g, 7.5 mmole) in abs. EtOH (20 cm^3) was added cyclohexyl isothiocyanate (1.06g, 7.5 mmole) and the reaction mixture was refluxed for 45 minutes. The solvent was removed under reduced pressure and the crude hygroscopic solid

residue (1.98g, 89 % yield) was redissolved in i-PrOH (20 cm^3) and converted to the oxalate salt by treatment with a solution of oxalic acid (excess) i.-PrOH followed by addition of ether (100 cm^3) The crude oxalate salt was recrystallised three times from i-PrOH: ether (5:1) to give SKH188A as a white solid.2.30g (81 % yield); mp: 148-50°C.

Solubilty: sol. (MeOH); sp. sol. (water).

TLC: (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.74.

HPLC: 100% at 16.90 minutes (Lichrosorb rp selsct B; 250x4 mm; 1 cm^3/min; UV 254nm, A/B (75/25) where A is water and B is acetonitrile with 5% water. The mobile phase contains 0.1% trifluoroacetic acid).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$) 241 (4.15), 212 (4.26).

IR: KBr cm^{-1}; 3097 (m, N-H str; -NH); 3043 (m, C-H str. C=C-H); 1621 (s, C-N str., arom); 1543 (s, -NH, Im); 1221 (m, C=S str.).

^{1}H NMR: (DMSO-d_6, 200 MHz), δ (ppm); 0.94- 1.90 (m, 11H, cyclohexyl); 2.50 (t, 2H, SCH_2); 3.50 (t, 2H, $\underline{CH_2}NH$); 3.80 (s, 2H, CH_2S); 7.48 [s, 1H, Im-4(5)]; 8.84 (s, 1H, Im-2).

Mass spectrum: (EI); m/e 299 (MH^+); 217 ($ImCH_2SCH_2CH_2NH$); 141 ($ImCH_2SCH_2CH_2$); 82 ($ImCH_2^+$).

Anal. Calc. for $C_{13}H_{22}N_4S_2$; 0.9 $C_2H_2O_4$: C, 46.83; H, 6.32; N, 14.76; S, 16.89

found: C, 47.31; H, 6.44; N, 14.76; S, 16.89

26) 2-{2-[2-pyridyl]ethyl}aminopyrimidine maleate

(sample SKH108A/UCL1030)

2-Pyridylethylamine (1.89g,16.5 mmole) and 2-chloropyrimidine (2.02g, 16.5 mmloe) in i-PrOH (30 cm^3) was stirred and heated under reflux for 19h when it turned black. The solvent was evaporated under reduced pressure and the oily residue was chromatographed

on a column of silica gel using a gradient of MeOH (5, 10 and 20%) in $CHCl_3$ as an eluate. Some of the unchanged starting materials were recovered. The combined fractions containig the pure product were concentrated to an oily residue which on standing at 21°C gave SKH106A as crown shaped crystals. 1.053g (32%).mp: 121-3°C. The monomaleate salt of the product was prepared by treating an ethanolic solution of the product (0.59g, 2.9 mmole) with a solution of maleic acid in absolute ethanol (0.336g, 1 molar equivalent).On addition of ether, a white solid precipitated, filtered and washed with ether. 0.727g (79.2%). The salt was recrystallised from i-PrOH. 0.54g (59%), mp: 132-3°C.

Solubility: sol (water, i-PrOH), sp sol (abs. EtOH, MeOH), insol ($CHCl_3$).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.74.

HPLC: 99.9% at 8.37 minutes.(Lichrosorb rp select B, 5μM, 250x4 mm, 0.5 cm^3/min, UV 254nm, A/B (50/50), where A is water with 0.1% triethylamine and B is MeOH with 0.1% triethylamine).

UV: water; λ_{max}nm ($\log\varepsilon_{max}$): 298 (3.40); 263 (3.66); 258 (3.77); 233 (4.30).

IR: KBr cm^{-1} 3510, 3388 (m, N-H); 1609 (s, C=N, Pym); 1583 (m, C=N, Pym); 1547 (s, C=N, Pyridine).

^1H NMR: ($CDCl_3$, 200 MHz), δ (ppm) 3.08 (t, J = 8Hz, 2H, $\underline{CH_2}CH_2NH$); 3.84 (q, J = 8 and 6Hz, 2H, $\underline{CH_2}NH$); 5.88 (Broad singlet, 1H, -NH-); 6.48 (t, J = 8 and 4Hz, 1H, Pym-5); 7.16 (m, J = 8Hz, 2H, Pyridyl-4,5); 7.58 (dxt, J = 8 and 6Hz, 1H, Pyridyl-3); 8.25 (d, J = 4Hz, 2H, Pym-4,6); 8.54 (d, J = 6Hz, 1H, Pyridyl-6).

Mass spectrum: (EI) m/e 200 (M^+), 106 ($PyCH_2CH_2$); 93 (Pym NH).

Anal Calc.for $C_{11}H_{12}N_4$, $C_4H_4O_4$: C,56.95; H,5.09: N,17.71.

found: C,57.07; H,5.06; N,17.78.

27) 2{2-[(2-pyridyl)ethyllamino}-benzothiazole dimaleate

(sample SKH111B/UCL1031)

A solution of 2-chlorobenzothiazole (1.48g, 8.7 mmole) and 2-pyridylethylamine (1g, 8.7 mmole) in i-PrOH (30cm^3) were heated under reflux for 30h. The solvent was removed under reduced pressure and the product was purified by column chromatography on silica gel eluted with a gradient of MeOH (5,10 and 20%) in $CHCl_3$. The fractions containing the pure product were combined and then concentrated under reduced pressure to an oily residue.1.076g (46%). Addition of ether to the mixture of an ethanolic solution of the product (0.926g, 3.6 mmole) and a solution of maleic acid (2 molar equivalent) in abs.EtOH , afforded the pure product as dimaleate salt 1.31g (98.5%). mp: 114-6°C. The salt was recrystallised from i-PrOH.

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.8.

HPLC: 100% at 6.18 minutes (Lichrosorb rp select B, 250x4 mm, 1cm^3/min UV 254nm, A/B (30/70) where A is water with 0.1% triethylamine and B is MeOH with o.1% triethylamine).

UV: water; λ_{max}nm ($\log\varepsilon_{max}$): 293 (3.57), 264 (4.14), 221 (4.52).

IR: KBr cm^{-1} 3381, 3320 (m, N-H str.); 1613,1580 (s, C=N str.thiazole).

^{1}H NMR: (D_2O, 200 MHz) δ (ppm), 2.75 (t, J = 6Hz, 2H, $\underline{CH_2}NH$); 3.2 (t, 2H, $PyCH_2$); 5.47 (s, 4H, -NH-), 6.57 (m, 1H, Py-3); 6.69 (s, 2H, aromatic); 6.84 (d, J = 8Hz, 1H, aromatic); 7.18 (t, J = 8Hz, 1H, Py-4); 7.27 (d, J = 8Hz, 1H, aromatic); 7.77 (t, J = 8Hz, 1H, Py-5); 7.95 (d, J = 6Hz, 1H, Py-6).

Mass spectrum: (EI), m/e 255 (M^+), 163 (benzothiazole-$NHCH_2$), 150.(benzothiazole-NH_2).

Anal.Calc.for $C_{14}H_{13}N_3S$, $C_8H_8O_8$: C, 54.2; H, 4.34; N, 8.61; S, 6.57.

found: C, 54.15; H, 4.33; N, 8.53; S, 6.50.

28) 2-{2-[4(5)-imidazolyl]ethylamino}-5-trifluoromethylpyridine dioxalate

(sample SKH329B/UCL1235)

A stirred mixture of histamine base (1g, 8.9 mmole) and 2-chloro-5-trifluoromethylpyridine (1.63g, 8.9 mmole) and potassium bicarbonate in i-PrOH (5 cm^3) was refluxed for 3h. The cooled reaction mixture was evaporated to dryness under reduced pressure and the solid residue was chromatographed on a column of silica gel using $CHCl_3$ and MeOH (10:1).as eluant. Some of the unchanged starting materials were recovered. The pure fractions were combined and the removal of the solvent under reduced pressure gave a white very hygrocopic solid SKH328B(1.25g, 55% yield). Attempts to crystallise the white fine solid were unsuccessful. A solution of the product (SKH329B, 0.4g, 1.5 mmole) in EtOH (5 cm^3) was treated with a solution of oxalic acid (2 molar equivalent) in abs. EtOH followed by addition of ether gave the product as dioxalate salt. Recrystallisation of the salt from i-PrOH and ether (10: 1).gave SKH329B 0.5g, mp: 174-5°C, (46% yield).

Solubility: sol (water, abs. EtOH), sp. sol. (i-PrOH, MeOH), insol.(ether).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.6; ($CHCl_3$: MeOH; 5:1) R_f: 0.22

HPLC: 100% at 17.89 minutes (Lichrosorb rp select B, 250x4mm, 1cm^3/min, UV 240nm, A/B (75/25) where A is water and B is MeOH The mobile phase contains 0.1% trifluoroacetic acid).

UV: water; λ_{max}nm ($\log\varepsilon_{max}$), 296 (3.45); 249 (4.15).

IR: KBr cm^{-1} 3131 (m, N-H str.), 3022 (w, C-H arom.); 1623 (s, C=N str.Py); 1327 (s, C-F str.).

^{1}H NMR: (DMSO-d_6, 200 MHz) δ (ppm); 2.90 (t, J = 7.0Hz, 2H, $ImCH_2$); 3.58 (m, J = 6.14Hz, 2H, $\underline{CH_2}NH$); 6.57 (d, J = 9.2Hz, 1H, Py-3); 7.37 [s, 1H, Im-4(5)]; 7.51 (broad t, 1H, $CH_2\underline{NH}$); 7.62 (dxd, 1H, Py-4); 8.28 (s, 1H, Im-2); 8.84 (s, J = 10.6Hz, 1H, Py-6).

Mass spectrum: (EI) m/e 256 (M^+), 154 (loss of CF_3), 81 ($ImCH_2$).

Anal. Calc. for $C_{11}H_{11}N_4F_3$, 2 $C_2H_2O_4$, 0.5 H_2O: C, 40.25; H, 3.62; N, 12.58

found: C, 40.25; H, 3.59; N, 12.41

29) 2-{2-[4(5)-imidazol-4-yl]-ethylthio}pyridine dihydrobromide monohydrate

(sample SKH238B/UCL1162)

A solution of 4(5)-[2-hydroxyethyl]imidazole (sample WT76/3156)(0.5g, 4.4 mmole) and 2-mercaptopyridine (0.49g, 4.4 mmole) in aqueous HBr (47%, 5 cm^3) were heated under reflux for 24h. The solvent was removed under reduced pressure by azeotropic distillation with i-PrOH. The oily residue was redissolved in hot boiling i-PrOH and on cooling, some of the product crystallised out, filtered, washed with i-PrOH. SKH238A (0.72g, 43 %). Recrystallisation of the product form i-PrOH (10 cm^3) gave SKH238B (0.56g), mp: 189-90°C.

Solubility: sol. (MeOH, water), sp. sol. (i-PrOH), insol. (ether, EtOAc).

TLC: silica (EtOAc: MeOH: NH_4OH; 5: 1: 1) R_f: 0.71; (MeOH: $CHCl_3$; 1:1) R_f: 0.71

HPLC: 99.38% at 13.3 minutes (Lichrosorb rp select b, 250x4mm, 0.75 cm^3/min, UV 254nm, A/B (75/25) where A is water + 0.1% triethylamine and B is MeOH + 0.1% triethylamine).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$), 285 (3.74), 243 (4.06), 208 (3.94).

IR: KBr cm^{-1} 3290 (s, C=N str Py), 2956 (m, S=C-N, Py), 1646 (s, C=N, arom. ring vibrations), 1624 (s, S=C-N, arom.ring vibrations), 1542 (s, C=N, arom.).

^1H NMR: (DMSO-d_6, 200MHz); δ (ppm); 3.10 (t, J = 8.2Hz, 2H, $ImCH_2$), 3.58 (t, 2H, CH_2S), 6.94 (broad singlet, 1H, ImNH), 7.34 (t, J = 6Hz, 1H, Py-3), 7.54 (s, 1H, Im-4,5), 7.59 (d, J = 6.0Hz, 1H, Py-3), 7.89 (t, J = 6, 6.2Hz, 1H, Py-4), 8.56 (d, J = 6.2Hz, 1H, Py-6), 9.18 (s, 1H, Im-2).

N.B.: On addition of a few drops of D_2O to the NMR sample, the peak at 6.94ppm disappeared due to proton exchange of the imidazole NH with D_2O and the Imidazole-4(5) proton peak was shifted downfield and the pyridine-3 proton peak shifted upfield.

Mass spectrum: (EI), m/e 205 (M^+), 95 ($ImCH_2CH_2^+$), 81 ($ImCH_2$).

Anal. Calc. for $C_{10}H_{11}N_3S$, 2HBr, H_20: C, 31.18; H, 3.92; N, 10.91; S, 8.32.

found: C, 31.59; H, 3.87; N, 10.96; S, 8.65

30) 2-{2-[4(5)-imidazolyl]ethylthio}-5-nitropyridine

(sample SKH298A/UCL1199)

To a stirred solution of S-{2-[4(5)-imidazolyl]ethyl}isothiourea dihydrobromide WT133C/UCL1095-B_2 (0.2g, 1.1 mmole) in water (2 cm^3), a hot solution of 2-chloro-5-nitropyridine (0.209g, 1.32 mmole) in absolute ethanol (5 cm^3) was added. The resulting suspension was stirred under nitrogen for 30 minutes The reaction mixture was cooled to 0-5°C and a solution of sodium hydroxide (four molar equivalent, 0.16g) in water (2 cm^3) was added dropwise under N_2. The reaction mixture was stirred for 1h at the same temperature and for 3h at 21°C and the filtered.TLC (EtOAc: MeOH: NH4OH; 5:1:1) indicated the product and traces of the starting materials in the precipitate and the filtrate. The filtrate and the precipitate were combined and the solvent was evaporated under reduced pressure. The residue was subjected to silica column chromatography eluted with a mixture of MeOH(1-10%) in $CHCl_3$ Some of the unchanged 2-chloro 5-nitropyridine was recovered.The combined pure fractions were filtered through fluted filter paper and then taken into $CHCl_3$. After 1h, the deposited silica was filtered off the solvent was removed under reduced pressure. Attempts to recrystallise the produce from hot i-PrOH or i-PrOH-ether were unsuccessful. Finally the solvent was removed.and the pale yellow solid was dried under vacuum at 100°C for 8h to give SKH298A (75mg, 47%), mp: 147-8°C.

Solubility: sol. ((MeOH); sp. sol. (i-PrOH, water); insol. (EtOAc).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.67; (MeOH: $CHCl_3$; 1:1)R_f: 0.73

HPLC: 99.97% at 12.85 minutes (Lichrosorb rp select B, 250x4 mm, 0.75 cm^3/min, UV 254nm, A/B (75/25) where A is water + 0.1% trifluoroacetic acid and B is MeOH + 0.1% trifluoroacetic acid).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$): 331 (4.18), 212 (4.05).

IR: KBr; cm^{-1} 3405, 3077 (w, N-H str.), 1583 (s, C=N rin str.), 1565 (s, N-O str.), 1343 (s, N-O str.), 1102 (s, S-C=N).

^1H NMR: (DMSO-d_6, 200 MHz); δ (ppm); 2.19 (t, J = 7.8Hz, 2H, Im$\underline{CH_2}$); 3.50 (t, 2H, Im$CH_2\underline{CH_2}$); 6.87 [s, 1H, Im-4(5)]; 7.54 (d, 1H, Py-3); 7.59 (s, 1H, Im-2); 8.37 (dxd, J = 7.8Hz, 1H, Py-4); 9.25 (d, J = 2.8Hz, 1H, Py-6).

Mass spectrum: (FAB+ NaI) m/e 251 (MH^+), 137 ($PySCH_2CH^+$), 95.($ImCH_2CH_2$).

Anal. Calc. for $C_{10}H_{10}N_4O_2S$, 0.25 H_2O: C, 47.13; H, 4.15; N, 21.99; S, 12.58.

found: C, 47.45; H, 3.86; N, 21.46; S, 12.58

31) 4(5)-[2-Chloroethyl]imidazole hydrochloride

(sample SKH312A)

4-(2-Hydroxyethyl)imidazole (2g, 17.8 mmole) was heated under reflux in conc. HCl (5 cm^3) for 1h, then the solvent was removed under reduced pressure and the oily residue was dried under vacuum for 8h. To the stirred suspension of this hydrochloride salt in CCl_4 (20 cm^3) under N_2, a solution of freshly distilled thionyl chloride (50 cm^3) in CCl_4 (80 cm^3) was added dropwise.over 3h at 21°C. After the addition of benzene (50 cm^3) to the reaction mixture the solid product was collected by decanting off the solvent. The pale yellow solid was dried under vacuum. SKH312A (2.82g, 95%), mp: 121-3°C.

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.51.

^1H NMR: (DMSO-d_6, 200MHz); δ (ppm); 3.13 (t, 2H, $ImCH_2$), 3.94 (t, 2H, CH_2Cl), 7.52 (s, 1H, Im-4(5), 9.07 (s, 1H, Im-2).

Mass spectrum: (EI), m/e, 130 (M+), 95 ($ImCH_2CH_2$), 81 ($ImCH_2$).

32) 2-{2-[4(5)-imidazolyl]ethylthio}imidazole oxalate

(sample SKH280A/UCL1210)

To a solution of 4(5)-[2-chloroethyl)imidazole hydrochloride SKH312A (183mg, 1.1 mmole) and 2-mercaptoimidazole (110mg, 1 mmole) in i-PrOH was added solid potassium hydroxide (200mg, 3.62mmole, 3.3 molar equivalent). The reaction mixture was heated under reflux for 4h, then left to cool and filtered. The filtrate was concentrated under reduced pressure to a semi solid residue. An attempt to isolate the pure product from the reaction mixture by crystallisation from i-PrOH was unsatisfactory. Purification was effected by silica gel column chromatography using $CHCl_3$: MeOH (5:1) as eluant. The pure fractions were combined and the solvent was evaporated under reduced pressure to give SKH279A (88mg, 42%). This was converted to oxalate salt by treating a solution of the solid in ethanol (5 cm^3) with an ethanolic solution of oxalic acid (2 molar equivalent) and then precipitation by ether gave SKH279B (0.135g, 87% salt), mp: 224-6°C. The product SKH279B was recrystallised from EtOH: ether (10:1) to give SKH280A (0.12g, mp: 224-6°C .

Solubility: sol. (water), sp. sol.(EtOH, MeOH), insol. (ether).

TLC: silica (EtOAc: MeOH: NH4OH; 5:1:1) R_f: 0.46, (MeOH: $CHCl_3$; 1:5) R_f: 0.37.

HPLC: 100% at 3.15 minutes (Lichrosorb rp select b, 7μM, 1cm3/min., UV240nm, A/B (90/10) where A is water+ 0.1% trifluoroacetic acid and B is MeOH+ 0.1% trifluoroacetic acid).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$), 240 (3.76), 214 (4.06).

IR: KBr cm^{-1} 3117(m, N-H str.), 2923 (s, N-H str.), 1585 (s, C=C-N str.), 1439 (m, C=N), 1248 (m, C-S Im).

^1H NMR: (D_2O, 200MHz), δ (ppm); 2.41 (t, J = 8Hz, 2H, $ImCH_2$), 2.75 (t, 2H, CH_2S), 6.55 (s, 1H, 4(5)-$\underline{Im}CH_2$), 6.72 [s, 2H, 4,5-(2-mercaptoimidazole)], 7.85 (s, 1H, Im-2).

Mass spectrum: (EI), m/e, 194 (M^+), 100 (ImSH), 95 ($ImCH_2CH_2$), 81 ($ImCH_2$).

Anal. Calc. for $C_8H_{10}N_4S$, 1.61 $C_2H_2O_4$: C, 39.62; H, 3.91; N, 16.38; S, 9.37.

Found: C, 39.53; H, 3.83; N, 16.46; S, 9.52.

33) 2-Chloro-4-nitropyridine-N-oxide

(sample SKH375A)

I) To a stirred solution of 2-chloropyridine-N-oxide hydrochloride (1.94 g, 11 mmole) in conc. H_2SO_4 (2 cm^3) was added a mixture of conc. H_2SO_4 and fuming HNO_3 (6 cm^3, 2:1) The solution was heated at 100°C for 4h on an oil bath. The cooled acidic solution was neutralised with sodium carbonate The reaction mixture was diluted with water to twice the volume and was basified to pH11 using more sodium carbonate. The aqueous solution was extracted with benzene (3x20 cm^3). The combined extracts were dried ($MgSO_4$) overnight and then filtered. The filtrate was evaporated under reduced pressure to a solid residue which was crystallised form abs. EtOH. yield 1.85g (90%); mp: 152-3°C.(Lit. [21] mp: 152-3°C).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:!) R_f: 0.9.

IR: KBr cm^{-1} 1523 (s, N-O str.conjugated nitro), 1369 (s, N-oxide), 1354 (s, N-O, str. conjugated nitro), 1155 (s, N-oxide).

Mass spectrum: (EI), m/e 174 (M^+), 158 (loss of oxygen), 112 (Loss of NO_2).

II) To the stirred solution of 2-chloropyridine-N-oxide hydrochloride (0.7g, 4.2 mmole) in conc. H_2SO_4 (2 cm^3) was added a mixture of conc. sulphuric acid and nitric acid (sp. gr. 1.42); 2:1 (6 cm3) was added. The solution was heated at 100°C for 4h over an oil bath. A brown gas was evolved. The reaction mixture was allowed to cool and was then treated with an aqueous soln of sodium carbonate till pH11. The aqueous solution was extracted with benzene and the combined extracts was dried ($MgSO_4$), filtered and the filtrate was concentrated to dryness under reduced pressure to give 0.71g The crude soid was recrystallised from absolute ethanol (10 cm^3). yield: 0.65g (89%). mp: 152-3°C.

TLC (EtOAC: MeOH: NH4OH) R_f: 0.91.

Mass spectrum: m/e 174 (M^+), 158 (loss of oxygen).

34) 2- {2-[4(5)-imidazolyl]-ethylamino}-4-nitropyridine-N-oxide.

(sample SKH380A/UCL1256)

To a solution of 2-chloro-4-nitropyridine-N-oxide (**33**)(60 mg, 0.34 mmole) in i-PrOH (5 cm^3), potassium bicarbonate (0.4g) was added and the mixture was stirred for 10 minutes To this solution a solution of histamine base (42 mg, 0.38 mmole, 1.1 molar equivalent) was added and stirred at 21°C for 3 days. The inorganic material was filtered off and the filtrate was concentrated under reduced pressure to dryness. Purification was by silica gel column chromatography using a mixture of $CHCl_3$: MeOH (10:1) as eluate. Some of the unchanged starting materials were recovered and the middle fractions containing the product and traces of other impurities were combined and evaporated under reduced pressure to an oily residue. The residue was purified further by semi preparative HPLC (Lichrosorb rp select b, 250x10mm column, UV 254nm detector, 1cm^3/min flow, A/B=90/10, A is water+ 0.1% trifluoroacetic acid and B is acetonitrile + 5% water+ 0.1% trifluoroacetic acid) On removal of the solvent under vacuum, the product was obtained as

a dark yellow solid. The hygroscopic product was recrystallised form i-PrOH-ether (10:1) to give SKH380A (25mg, 15%), mp: 193-4°C.

Solubility: sol. (MeOH, i-PrOH, water); insol. ($CHCl_3$).

TLC: (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.52; (MeOH: $CHCl_3$; 1:4): R_f: 0.34.

HPLC: 99.26% at 12.36 minutes (Lichrosorb rp select B; 250x4 mm; 1 cm3/min; UV 254nm; A/B (50/50) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 0.1% trifluoroacetic acid + 5% water). The impuriry is 0.21% at 4.38 minutes.

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$), 268 (3.89), 234 (3.98), 208 (4.09).

IR: KBr cm^{-1} 3106 (m, N-H str.); 1543 (s, N-O conjugated); 1353 (s, N-O conjugated); 1307 (s, N-O str., aromatic N-oxide).

^1H NMR: (DMSO-d_6+ D_2O, 200MHz); δ (ppm); 2.96 (t, 2H, $ImCH_2$), 3.67 (t, J = 4.06Hz, 2H, CH_2NH), 7.43 (dxd, J = 4.11Hz, 2H, Py-5,6), 7.54 (s, 1H, Im-4(5), 8.33 (d, J = 7.17Hz, 1H, Py-3), 8.84 (broad singlet, 1H, Im-2). The peaks at 1.17 and 4.04 indicates the presence of i-PrOH in the sample.

Mass spectrum: (FAB, MNOBA matrix); m/e 250 (M^+); 233 (cleavage of N-O); 186 (cleavage of NO_2); 82 ($ImCH_2^+$).

Anal. Calc. for: $C_{10}H_{11}N_5O_3$, 1.45 CF_3COOH, 0.1 C_3H_8O: C, 37.69; H, 3.17; N, 16.65

found: C, 37.90; H, 3.02; N, 17.01

35) 4-{2-[4(5)-imidazolyl]-ethylamino}-2-chloropyridine

(sample SKH377B/UCL1236)

To a stirred solution of 2-chloro-4-nitropyridine-N-oxide SKH375A(**33**)(55mg, 0.31 mmole) in i-PrOH (15 cm^3) potassium bicarbonate (0.5g) and a solution of histamine base (38mg, 1.1 equimole, 0.34 mole) was added. The reaction mixture was stirred at 21°C for 3 days, and then filtered. The filtrate was evaporated to give a solid residue and was

chromatographed over silica gel using $CHCl_3$ and MeOH (10:1) as eluent. The combined pure fractions were evaporated under reduced pressure to yield a solid residue. The crude product was crystallised from i-PrOH: Ether (1:1), to give SKH377B (61mg 38% yield), mp: 164-5°C. (Traces of the compound UCL-1256/SKH380A/34 were isolated and mass spectra and NMR were corresponding to compound 34).

Solubility: sol. (MeOH, i-PrOH, aq. HCl), sp. sol. (ether), insol.(water).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.56; (MeOH: $CHCl_3$; 1:5) R_f: 0.25.

HPLC: 100% at 4.57 minutes (Lichrosorb rp select B, 250x4 mm, 5µM, 1 cm^3/minute, UV 240nm, A/B (98/2) where A is water with 0.1% trifluoroacetic acid and B is MeOH with 0.1% trifluoroacetic acid.

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$); 252 (4.26), 210 (4.23).

IR: KBr disc cm^{-1} 3439 (m, N-H str.), 3131 (m, N-H str.), 3089 (m, C-H str.arom), 1623 (s, C=N str.py), 1337 (m, C=C-NH, Im), 1143 (s, C-Cl), 836, 722 (m C-H out of plane bending).

^1H NMR: (DMSO-d6, 200MHz), δ (ppm); 2.56 (t, J = 7Hz, 2H, $ImCH_2$), 3.23 (m, J = 7 and 5.1Hz, 2H, CH_2NH), 6.44 (d, 2H, Py-3,5), 6.82 (s, 1H, Im-4(5), 6.98 (t, J = 8 and 4Hz, 1H, $CH_2\underline{NH}$), 7.51 (s, 1H, Im-2), 7.76 (d, J = 4Hz, 1H, Py-6).

Mass spectrum: (EI); m/e 223 (M^+), 225 (M^++2), 154 (2-chloropy-NHCH=CH), 81 ($ImCH_2$).

Anal. Calc. for $C_{10}H_{11}N_4Cl$, 0.2 H_2O: C; 53.07; H, 5.07; N, 24.76.

found: C, 53.31; H, 4.84; N, 24.71.

36) 1-{2-[4(5)-imidazolyl]-ethylamino}-4-nitrobenzene oxalate

(sample SKH299C/UCL1205)

To a stirred solution of histamine (1.46g, 13.1 mmole) and a molar equivalent of 1-chloro-4-nitrobenzene (2.06g) in i-PrOH (10 cm^3), potassium bicarbonate was added. The

reaction mixture was heated under reflux for 3 days. The solvent was removed and The solid residue was chromatographed over silica gel eluted with $CHCl_3$ and MeOH (1:10%). Some of the unchanged starting materials were recovered. The pure fractions were combined and evaporation under reduced pressure gave a solid which was redissolved in minimum amount of i-PrOH (10 cm^3). On standing some silica deposited which was filtered off and the filtrate was evaporated under reduced pressure to a solid residue 0.85g (28% yield); mp: 165-8°C. Several attempts to crystalise the product form abs. EtOH: ether; i-PrOH: ether were unsuccessful and finally the product was isolated as oxalate salt after treating an ethanolic solution of the product with a solution of oxalic acid (excess) in abs. EtOH followed by addition of ether and decanting the ethereal solution several times to remove the excess of oxalic acid. Crystallisation from a mixture of abs. EtOH and $CHCl_3$ (10:1, 20 cm^3) yielded yellow needle shaped crystalline product 65mg (67% oxalate yield); mp: 185-6°C

solubility: sol. (abs. EtOH, MeOH, water); insol.($CHCl_3$, ether).

TLC: silica (EtOAc: MeOH: NH_4OH) R_f:0.56; ($CHCl_3$: MeOH) R_f: 0.24.

HPLC: 99.51% at 7.73% (Lichrosorb rp select B, 250x4 mm; 1 cm^3/min.; UV 254nm; A/B (60/40) where A is water and B is MeOH. The mobile phase contains 0.1% trifluoroacetic acid).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$); 380 (4.25); 211 (3.98).

^1H NMR: (DMSO-d_6 + D_2O, 200 MHz); δ (ppm): 2.97 (t, 2H, $ImCH_2$); 3.53 (t, 2H, CH_2NH); 6.54 (d, J = 7Hz, 2H, Ph); 7.32 (s, 1H, Im-4(5)); 8.07 (d, J = 7Hz, 2H, Ph); 8.66 (s, 1H, Im-2).

IR: KBr cm^{-1} 3439, 3403 (w, N-H str.); 1601 (s, N-O str.); 1317 (s, N-O str.); 1298, 1279 (s, Im).

Mass spectrum: (EI); m/e, 232 (M^+); 151 (nitrobenzene$NHCH_2^+$), 105.(benzene$NHCH_2^+$).

Anal. Calc. for $C_{11}H_{12}N_4O_2$; 1.25 $C_2H_2O_4$: C, 47.0; H, 4.23; N, 16.24.

found: C, 46.39; H, 4.02; N, 16.36

37) 5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidine

(sample SKH410A)

Histamine base (14.92g, 0.134 mole) obtained, by treatment of the dihydrochloride salt with sodium ethoxide in abs. EtOH; and carbonyldiimidazole (43.67g, 0.269 mole) were heated together with stirring to 100-105°C over 1.5h, when the mixture turned brown and then heated at 125-30°C for 45 minutes. After cooling to 21°C, the solid base was ground to a fine powder under EtOH. The powder was filtered and collected. The solid was crystallised from hot EtOH to give a shiny white crystalline solid which was dried under vacuum to give SKH 410A (11.55g), mp: 221-2°C. Evaporation of the filtrate to 1/3 of the volume gave a second crop, SKH410B (2.50g), mp: 222-3°C, (lit.[33]. mp: 221-2°C). Combined yield: 76.87%.

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:) R_f: 0.63.

IR: KBr cm^{-1} 3137 (s, N-H str.), 1748 (s, N-C(=O)-N).

^1H NMR: (DMSO-d_6, 200MHz), δ (ppm); 2.87 (t, 2H, $ImCH_2$), 3.35 (t, 2H, CH_2NH), 6.81 (s, 1H, Im-4(5), 8.0 (s, 1H, Im-2), 8.18 (broad siglet, 1H, Im-NH-).

Mass spectrum: (EI), m/e, 137 (M^+), 81 ($ImCH_2$).

In a second preparation, histamine base (5g, 44.9 mmole) (generated from the dihydrochloride salt by passing through a column of DOWEX IRA-400 OH^-) form and carbonyldiimidazole (11.30g, 69.7 mmole) in chloroform (30 cm^3) were refluxed for 6h. The solvent was removed under reduced pressure and the white solid residue was taken into abs. EtOH. The mixture was stirred for 1/2h and then filtered. The white solid was filtered and dried to give SKH333A (4.12g, 67%), mp: 212°C.(lit.[53]. mp: 215-220°C).

38) 2-Methyl-5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidinium iodide

(sample SKH412A)

A solution of the cyclic ureaSKH410A (37)(5.27g, 38 mmole) and MeI (32.21g, 227 mmole, 6 molar equivalent) in freshly distilled and dried DMF (20 cm^3) were stirred at 21°C overnight. On addition of ether, the product precipitated, this was filtered off and washed with cold MeOH to give 5.1g of a soild (mp: 225-7°C, lit. mp: 225-7°C). Recrystallisation of the product from cold MeOH -ether (10: 1) gave a white crystalline solid SKH412A (4.91g, 85%), mp: 225-8°C.(lit. [33]mp: 225-27°C).

TLC: silica (EtOAc: MeOH: NH4OH; 5:1:1) R_f: 0.4.

IR: KBr cm^{-1} 1749 (s, NH-C(=O)-NH).

^1H NMR: (DMSO-d_6, 200MHz), 3.0 (t, 2H, $ImCH_2$); 3.44 (m, J = 6.34, 2H, CH_2NH); 3.85 (s, 3H, N^{τ}-Me); 7.6 (s, 1H, Im-4(5); 9.02 (broad singlet, 1H, -NH-); 9.67 (s, 1H, Im-2). Two drops of D_2O was added to the NMR sample and the broad singlet at δ 9.02 (-NH-) disapeared due to proton exchange with D_2O. ^1H NMR: (200MHz), δ (ppm); 2.98 (t, 2H, $ImCH_2$), 3.44 (m, 2H, CH_2NH), .3.85 (s, N^{τ}-Me), 7.48 (s, 1H, Im-4(5), 9.56 (s, 1H, Im-2).

Mass spectrum: (EI), m/e, 151 (M^+-H), 95 ($ImCH_2CH_2$) and HI (128).

The synthesis was repeated on a larger scale using SKH410A, 410B and 333A (8.5g, 62 mmole) and MeI (9.67g, 68 mmole, 1.1 molar equivalent) in freshly distilled and dried DMF (100 cm^3). The solvent was evaporated under reduced pressure and the residue was taken into cold MeOH(50 cm^3), stirred till dissolved and ether(10 cm3) was added and the milky solution was kept in fridge overnight. The white solid precipitate was filtered off and dried to give SKH412C (12.5g, 72%), mp: 235-8°C. Recrystallisation of SKH412C from cold MeOH: ether two successive times gave shiny white crystalline solid SKH412D (8.5g, 49%), mp: 225-7°C.

39) N^{τ}-methylhistamine dioxalate.

(sample SKH414B)

A solution of 2-Methyl-5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidinium iodide **(38)** SKH412A (4.50g, 16 mmole) in conc. HCl (12M, 20 cm^3) was heated under reflux for 24h under argon and was neutralised with aqueous NaOH. The reaction mixture was evaporated to dryness under reduced pressure. Trituration of the dried reaction mixture with $CHCl_3$ (200 cm^3) afforded pure N^{τ}-methyl histamine SKH414A (1.95g, 92%) as an oil.

IR: Neat cm^{-1} 3349 (s, N-H str.), 2917 (s, N-H str.), 1508 (s, C=N, Im).

^1H NMR: ($CDCl_3$, 200MHz), δ (ppm); 2.68 (t, J = 6.3Hz, 2H, $ImCH_2$); 2.97 (t, 2H, CH_2NH_2); 3.61 (s, 3H, N^{τ}-methyl); 6.69 (s, 1H, Im-5), 7.36 (s, obscured by the $CDCl_3$ peak, 1H, Im-2). The spectrum shows a peak at δ 3.66ppm for absorbed water.

The product was very hygroscopic therefore the base was converted to the dioxalate salt by treating an ethanolic solution of the base (1.80g, 14 mmole) with a solution of oxalic acid (2 molar equivalent) in abs. EtOH.Addition of ether afforded the product as the dioxalate salt SKH414B (3.5g, 82% salt), mp: 173-5°C.

^1H NMR: (D_2O, 200MHz), δ (ppm), 2.36 (t, 2H, $ImCH_2$), 2.56 (t, 2H, CH_2NH), 3.12(s, 3H, N^{τ}-methyl), 6.62(s, 1H, Im-5), 7.88(s, 1H, Im-2).

Mass spectrum: (EI), m/e, 126 (MH^+), 96 (N^{τ}-methylimidazole-CH_3), 81 (N^{τ}methylIm)

The preparation was repeated by refluxing the methiodide SKH 412D (8.5g, 30 mmole) in conc HCl (12M, 50 cm^3) under argon followed by neutralisation (NaOH) and trituration of the dried reaction mixture with $CHCl_3$(500 cm^3) afforded SKH 415A (3.3g, 87%) as an oil. the mass spectrum and ^1H NMR were in agreement with the first preparation.

40) 2-{2-[1-methyl-4-imidazolyl]ethylamino}-5-nitropyridine

(sample 418A/UCL1264)

To a solution of N^{τ}-methylhistamine SKH415A (0.4g, 3.19 mmole) and 2-chloro-5-nitropyridine (0.55g, 3.51 mmole, 1.1 molar equivalent) in abs. EtOH (5 cm^3), was added potassium bicarbonate (0.6g) and the reaction mixture was heated under reflux at 50°C for 3h.and at 80°C for 1h The reaction mixture was left to cool and the product crystallised out of the solution. The supernatant solution was decanted and the solid was washed with the minimum amount of abs. EtOH and dried under vacuum at 90°C for 3h to give SKH417A (0.438g, mp: 191-2°C). Concentrating the filtrate to 1/2 the volume afforded SKH418B (0.105g, mp: 188-90°C). Recrystallisation of the product SKH417A from hot i-PrOH (200 cm^3) gave SKH418A which was dried under reduced pressure for 6h at 90°C (0.425g, mp: 189-90°C). The filtrate from SKH418A, after concentrating under reduced pressure to a small volume afforded SKH418C (0.040g),mp: 189-90°C . Combined yield: 72%. The sample SKH418A was characterised

Solubility: sol. (MeOH, EtOH), sp. sol. (i-PrOH).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.62; (MeOH: $CHCl_3$) R_f: 0.58.

HPLC: 99.94% at 13.93 minutes (LiLichrosorb rp select b, 0.1aufs, 7mm, 1 cm^3/min., UV 254nm, A/B (a gradient of B from 0-50% in 20 miutes) where A is water +0.1% trifluoroacetic acid and B is acetonitrile + 0.1% trifluoroacetic acid). The impurity is 0.05% at 17.66 minutes.

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$), 359 (4.25), 220 (4.07).

IR: KBr cm^{-1}, 3451 (w, N-H str.), 3243 (w, N-H str.), 1611 (s, -C=C-NH- str.), 1588 (m, N-O asym. arom.), 1328, 1288 (s, N^{τ}-methylimidazole C-N).

^{1}H NMR: ($CDCl_3$, 200MHz); δ (ppm), 2.79 (t, J = 5.4Hz, 2H, Im$\underline{CH_2}$), 3.54 (s, 3H, N^{τ}-Methyl); 3.61 (q, 2H, $\underline{CH_2}$NH); 6.29 (d, J = 8.2Hz, 1H, Py-3); 6.36 (broad siglet, 1H, NH); 6.68 (s, 1H, Im-4(5), 7.30 (s, 1H, Im-2); 8.05 (m, J = 8.1Hz, 1H, Py-4); 8.92 (d, 1H, Py-6).

Mass spectrum: (EI), m/e 247 (M^+), 152 (paranitroPy-$NHCH_2$), 96 (N^τ-methyimidazole-CH_3), 81 (N^τ-methylimidazole).

Anal. Calc. for $C_{11}H_{13}N_5O_2$: C, 53.43; H, 5.29; N, 28.32.

found: C, 53.29; H, 5.23, N, 27.93

41)2-{2-[1-methy-4-imidazolyl]ethylamino}-5-trifluoromethylpyridine dioxalate.

(sample SKH421A/UCL1265)

To a stirred solution of N^τ-methylhistamine (0.4g, 3.19 mmole) and 2-chloro-5-trifluoromethylpyridine (0.58g, 3.19 mmole) in abs. EtOH, was added potassium bicarbonate (0.32g). The reaction mixture was heated under reflux for 24h at 80°C. Removal of the solvent under reduced pressure gave a dark red oily residue Purification was by silica gel column chromatography using MeOH: $CHCl_3$ (1: 5) as eluate. The fractions containing the pure product were combined and the solvent was removed under reduced pressure to give an oily residue which was redissolved in $CHCl_3$ (10 cm^3). This was left overnight in the fridge and filtered through a fluted filter paper to remove dissolved silica gel. The filtrate was evaporated under vacuum to give an oily residue SKH420A (0.25g). The other partially impure fractions were combined and were subjected to column chromatography on silica gel and evaporating the combined pure fractions under reduced gave SKH421A (0.145g). Combined yield: 0.395g (46%) Some of the unchanged N^τ-methylhistamine (0.15g) was recovered. The oily residue SKH420A (0.25g) was converted to the oxalate salt by treating a solution of the product in abs. EtOH (5cm^3) with an ethanolic solution of oxalic acid (excess, in 5 cm^3 abs. EtOH) followed by addition of ether (10 cm^3). The solution was decanted and more ether was added to remove excess of oxalic acid. Recrystallisation of the product from abs. EtOH afforded SKH421A as the dioxalate

salt, yield: 80 mg, mp: 179-80°C. Concentrating the filtrate under vacuum gave the second crop SKH421B (50mg, mp: 179-81°C).

Solubility: sol. (water, EtOH, MeOH), insol.(EtOAc).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.78; (MeOH: $CHCl_3$; 1:5) R_f: 0.59.

HPLC: 100% at 10.32 minutes (Lichrosorb rp selevct b, 7μM, 250x4mm, 1 cm^3/min., UV 254nm, 0.1aufs, A/B (90/10), where A is water +0.1 trifluoroacetic acid and B is acetonitrile + 0.1% trifluoroacetic acid).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$), 296 (3.53), 250 (4.32), 209 (4.01).

IR: KBr cm^{-1}, 3441 (s, N-H str.), 1621 (s, C=N Py), 1327 (s, C-F str.).

^{1}H NMR: (DMSO-d_6, 200MHz), δ (ppm), 2.81 (t, J = 7Hz, 2H, $ImCH_2$), 3.54 (poorly resolved q, 2H, $\underline{CH_2}NH$), 5.15 (broad singlet, oxalic acid), 6.54 (d, J = 9.2Hz, 1H, Py-3); 7.30 (s, 1H, Im-4(5); 7.46 (poorly resolved triplet, 1H, NH); 7.59 (dxd, 1H, Py-4); 8.27 (s, 1H, Im-2); 8.6 (s, 1H, Py-6).

N.B. On addition of D_2O to the NMR sample, the broad singlet from the oxalic acid disappears and also the poorly resolved triplet at 7.46 ppm from the side chain. This is due to proton exchange with the solvent.

Mass spectrum: (EI), m/e 270 (M^+), 146 (trifluoromethylPy$^+$), 108 (trifluoro methyPyNH_2), 96 (N^τ-methylimidazoleCH_3), 81 (N^τ-methylimidazole).

Anal. Calc. for $C_{12}H_{13}F_3N_4$, $2C_2H_2O_4$: C, 42.67; H, 3.81; N, 12.44

found: C, 43.08; H, 3.80; N, 12.45

42) 6-chloro-3-carbomethoxy pyridine

(sample SKH391A)

Diazomethane was prepared by the method described by J. De Boer et al[31]. In a distiliing flask was placed a solution of potassium hydroxide (2.92g) dissolved in water (5 cm^3) and

EtOH (20 cm^3) and a Teflon coated magnetic stirrer bar. A dropping funnel was attached and adjusted so that the stem was just above the surface of the solution in the distilling flask. there was placed a solution of p-toluenesulphonylmethylnitrosamide (11.186g, 0.052 mole in ether (70 cm^3). The distilling flask was heated in a water bath at 70-80°C. The stirrer was started and the nitrosamide solution was added at a regular rate during 20-30 minutes. As soon as the addition was complete, ether was added On addition of the ethereal solution of diazomethane to a solution of 6-chloronicotinic acid (1.74g, 99 mmole) in THF (freshly distilled, 50 cm^3), the yellow colour solution was cooled to 0-5°C for 3h. To the solution 5 drops of acetic acid were added to destroy the excess of diazomethane, followed by silica gel till no effervescence was observed. The silica was filtered off and the filtrate was concentrated to dryness SKH391A (1.80g, 95 %). mp: 80-85°C (lit.[61]. mp: 85-90°C).

TLC: silica (EtOAc: MeOH: NH4OH; 5:1:1) R_f: 0.99

IR: KBr cm^{-1} 3034 (s, C-H ring str py), 1760 and 1724 (s, -CO-O-, ester).

^1H NMR: ($CDCl_3$, 60MHz), δ (ppm), 3.73 (s, 3H, methylester); 7.1 (d, 1H, Py-3); 8.1 (dxd, 1H, Py-4); 8.80 (d, Py-6).

Mass spectrum: (EI), m/e 171 (M^+), 140 ($M-OCH_3$).

43) 2-{2-[4(5)-imidazolyllethylamino}-5-carbomethoxypyridine dioxalate

(sample SKH394A/UCL1249)

To a solution of histamine base (0.5g, 4.5 mmole) and 6-chloro-3-methylcarboxypyridine (0.77g, 4.5 mmole) in THF (freshly distilled, 20 cm^3) was added potassium bicarbonate (2 molar equivalent). The reaction mixture was stirred at 21 °C for 24h and was refluxed for 24h. The inorganic precipitate was filtered off and the solvent was removed under reduced pressure to give a white solid which was purified by silica gel column chromatography using MeOH: $CHCl_3$ (1:4) as eluate Some of the methyl ester and the histamine base was

recovered. The pure mid fractions were combined and evaporated under reduced pressure to give SKH393A (0.26g) as an oily residue. The other partially impure fractions were purified by column chromatography on silica gel using the same solvent mixture as eluent and evaporation of the pure fractions under vacuum to an oily reidue gave SKH393B(0.1g). Combined yield: 32%. The oily residue was dissolved in MeOH (5 cm^3), and a solution of oxalic acid (2 molar equivalent) in MeOH (5 cm^3) was added. Precipitation by ether (20 cm^3) gave a white gelatinous solid. The solvent was evaporated under reduced pressure and the white semi solid residue was triturated with ether several times and evaporated under vacuum to dryness. The white hygroscopic solid residue was taken into ether and the ethereal solution was decanted to remove excess of oxalic acid and the white solid was recrystallised from MeOH: ether (1: 1).to give SKH394A (0.28g), mp: 209-10 °C.

Solubility: sol. (water, MeOH), insol.(ether, EtOAc).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.52, (MeOH: $CHCl_3$; 1:4) R_f: 0.38

HPLC: 98.65% at 11.85 minutes (Lichrosorb rp select b, 5 mM, 1 cm^3/min., UV 254nm, A/B (75/25) where A is water and B is acetonitrile. The mobile phase contains 0.1% trifluoroacetic acid). The impurity is 1.32% at 15.32 minutes.

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$), 279 (4.32), 210 (4.07).

IR: KBr cm^{-1}, 3443 (m, N-H str), 1760, 1724 (s, -CO-O-, arom ester), 1427 (s, C=N Im).

^1H NMR: (DMSO-d_6, 200MHz); δ (ppm): 2.84 (t, J = 6Hz, 2H, $ImCH_2$); 3.55 (broad q, 2H, CH_2NH); 6.42 (d, J = 8Hz, 1H, Py-3); 7.30 (s, 1H, Im-4(5); 7.48 (broad siglet, 1H, NH); 7.71 (d, J = 8Hz, 1H, Py-4); 8.48 (s, 1H, Im-2); 8.76 (s, 1H, Py-6).

Mass spectrum: (EI), m/e 246 (M^+), 165 (carbomethoxyPyNHCH$_2$), 82 ($ImCH_2^+$).

Anal. Calc for $C_{12}H_{14}N_4O_2$, 1.7 $C_2H_2O_4$: C, 46.31; H, 4.39; N, 14.03.

found: C, 46.35; H, 4.65; N, 13.97

44) 2-{4- [4(5)-imidazolyl]-piperidyl}-N-5-trifluoromethylpyridine

(sample SKH456A/UCL1283)

I) A solution of 4-(4-piperidyl)imidazole dihydrochloride SKH119B(**11**)(1g, 4.4 mmole) in water (5 cm^3) was basified to pH9 with potassium bicarbonate (0.45g) in water.(5 cm^3). The resulting solution was concentrated under reduced pressure and residual moisture was removed by azeotropic distillation with i-PrOH. The solid residue was suspended in i-PrOH, heated and filtered hot. Evaporation of the filtrate under reduced pressure yielded the amine as a white solid (0.58g),mp: 185-6°C,(86% yield).

II) To a stirred solution of 4-(4-piperidyl)imidazole (0.5g, 3.3 mmole) with potassium bicarbonate (0.6g) in i-PrOH (50 cm^3) was added 2-chloro-5-trifluoromethylpyridine (0.66g, 3.63 mmole), when the colourless reaction mixture turned bright yellow. The reaction mixture was heated under reflux for 12h and the inorganic residue was filtered off. The filtrate was concentrated under reduced pressure to an oily residue. This residue was purified by silica gel column chromatography using $CHCl_3$: MeOH (5: 1) as eluent. Some of the unchanged starting materials were recovered. The pure fractions of the product were combined and the solvent was removed under reduced pressure to give a white hygroscopic solid. The product was recrystallised from ether: $CHCl_3$ to give SKH456A (0.56g),57% yield ; mp: 154-5°C.

Solubility: sol. (MeOH); sp.sol. (ether); insol. (EtOAc, water).

TLC: silica (EtOAc: MeOH; NH_4OH; 5:1:1) R_f: 0.76; ($CHCl_3$: MeOH; 5: 1) R_f: 0.58.

HPLC: 100% at 16.29 minutes (Lichrosorb rp select B, 250x4mm, 1cm^3/min., UV 254nm, A/B (80/20), where A is water and B is MeOH. The mobile phase contains 0.1% trifluoroacetic acid).

UV: MeOH; λ_{max} nm (log ε_{max}): 310 (3.54); 261 (4.33).

IR: KBr cm^{-1}; 3105 (w, N-H str.); 2894(m, C-H arom), 1604 (s, C-N str. Py); 1329 (s, C-F str.).

^{1}H NMR: ($CDCl_3$, 200 MHz); δ (ppm); 1.47 (m, J = 15.6Hz, 2H, $ImCH(CH_2)_2$, axial); 1.95 (d, J = 15.2Hz, 2H, $ImCH(CH_2)_2$, equatorial); 2.81 (m, 1H, ImCH); 2.95 (t, J = 12Hz, 2H, CHNCH, axial); 3.44 (br. s, 1H, CHN), 4.44 (d, J = 13.4Hz, 2H, CHNCH, equatorial); 6.73 (s, 1H, Im-5); 6.95 (d, J = 8.9Hz, 1H, Py-3); 7.49 (s, 1H, Im-2); 7.74 (dxd, J = 6.8Hz, 1H, Py-4); 8.37 (s, 1H, Py-6).

Mass spectrum: (EI), m/e 297 (M^+); 147 (trifluoromethyPyNH$^+$), 108 ($ImCH_2CH_2CH_2^+$), 95 ($ImCH_2CH_2^+$).

Anal. Calc. for $C_{14}H_{16}F_3N_4$: C, 56.55; H, 5.42; N, 18.84.

found: C, 56.49; H, 5.42; N, 18.92.

45) 2-[2-(2-pyridyl)ethylamino]-5-nitropyridine

(sample SKH307B/UCL1200)

2-Pyridylethylamine (1.615g, 12.6 mmole) and 2-chloro-5-nitropyridine (2g, 12.6 mmole) in i-PrOH (5 cm^3) were stirred for 2h at 50°C under N_2, the mixture turned dark. The solvent was removed under reduced pressure and the residue was chromatographed over silica gel eluted with $CHCi_3$: MeOH (5:1). Some of the unchanged 2-pyridylethylamine was recovered The combined fractions containing the product and the chloro compound were chromatographed again Over a column of silica gel eluted with chloroform. The fractions containing the pure product were combined and concentrated to dryness under reduced pressure. The product was recrystallised from i-PrOH. 1.35g (44%), mp: 143-4°C.

Solubility: sol. (MeOH), sp. sol. (water, i-PrOH), insol. (EtOAc).

TLC: silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.75; (MeOH: $CHCl_3$) R_f: 0.76.

HPLC: 99.67% at 11.06 minutes (Lichrosorb rp select B, 250x4 mm; 1 cm^3/min; UV 240nm; A/B (75/250 where A is water with 0.1% trifluoroacetic acid and B is MeOH with 0.1% trifluoroacetic acid The impurity is 0.13% at 15.79 min).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$); 355 (4.25), 257 (3.70), 218 (4.06).

IR: nujol mull cm^{-1} 3241 (w, N-H str.); 1610 (s, C=C str., C=N str.); 1586 (s, N-O str.); 1323 (s, N-O str.).

^{1}H NMR: ($CDCl_3$, 200MHz): δ (ppm): 3.11 (t, 2H, $PyCH_2$); 3.88 (q, J = 5.0, 5.2Hz, 1H, $\underline{CH_2}NH$); 6.37 (d, J = 10.4Hz, 1H, nitropyridine-3); 6.48 (br.s, $CH_2\underline{NH}$); 7.20 (m, J = 7.8Hz, 2H, py-4,5); 7.65 (t, 1H, py-3); 8.11 (dxd, J = 7.8Hz, 1H, nitropyridine-4); 8.57 (d, 1H, py-6); 9.02 (d, J = 2.6Hz, 1H, nitropyridine-6).

Mass spectrum: (EI); m/e 244 (M^+); 152 (paranitroPyNH$^+$), 92.($PyCH^+$).

Anal Calc. for : $C_{12}H_{12}N_4O_2$: C, 59.0; H, 4.95; N, 22.93.

found: C, 59.04; H, 4.93; N, 22.83.

46) 4(5)-Chloromethylimidazole hydrochloride

(sample SKH262A).

To a stirred suspension of 4(5)- hydroxymethylimidazole hydrochloride (1g, 7.4 mmole) in CCl_4 (20 cm^3) was added a solution of freshly distilled thionyl chloride (50 cm^3) in CCl_4 (100 cm^3) dropwise under N_2 and the mixture was heated under reflux for 8h. Addition of benzene (500 cm^3, dried over $CaCl_2$) to the cooled reaction mixture, afforded the product as hydrochloride salt. The solid was dried under vacuum over P_2O_5 over 3 days to give SKH262A (1.10g, 97% yield); mp: 148-50°C (lit.[78].mp: 138-41°C).

^{1}H NMR: (DMSO-d_6; 200 MHz); 4.68 (s, 2H, CH_2Cl); 7.82 (s, 1H, Im-4(5)]; 9.13 (s, 1H, Im-2).

Mass spectrum: (EI); m/e 116 (M^+); 81 ($ImCH_2$).

47) 2-[{4(5)-imidazolyl}methylthio]-5-nitropyridine

(sample SKH277C/UCL1191)

A mixture of 4(5)-chloromethylimidazole hydrochloride SKH262A (**46**)(0.6g, 3.92 mmole) and thiourea (0.3g, 1 molar equivalent) in ethanol (10 cm^3) was heated under reflux for 30 minutes. To this mixture was added ethanol (5 cm^3), water (20 cm^3) and 2-chloro-5-nitropyridine (0.74g, 1.2 molar equivalent) The resultant solution was cooled to 0-10°C and a solution of sodium hydroxide (0.49g, 012.5 mmole) in water (10 cm^3) was added dropwise under nitrogen at 0-10°C followed by stirring for 1h at the same temperature and for an additional 3h at 21°C. The precipitate was collected and washed with water to afford 0.54g, of the crude product. The crude product was purified by silica gel column chromatography eluted with a gradient of MeOH (1, 5 and 20%) in $CHCl_3$. Some of the starting materials were recovered. The combined pure fractions were evaporated to dryness under reduced pressure . The residue was crystallised from i-PrOH to give SKH277C (0.36g, 40%), mp: 155-6°C.

Solubility: sol. (MeOH, EtOH); sp. sol. ($CHCl_3$); insol. (EtOAc).

TLC: silica, (EtOAc: MeOH: NH4OH; 5:1:1) R_f: 0.8.

HPLC: 100% at 26.03 minutes (Lichrosorb rp select B; 250x4 mm; 1 cm^3/min; UV 254nm; A/B (75/25) where A is water with 0.1% triethylamine and B is MeOH with 0.1% triethyamine).

UV: MeOH; λ_{max}nm ($\log\varepsilon_{max}$); 328 (4.18), 212 (4.09).

IR: KBr cm^{-1}, 1580 (s, N-O arom.), 1558 (s, C=N), 1338 (s, N-O arom.), 1102 (s, S-C=N).

^{1}H NMR: (DMSO-d_6; 200MHz); δ (ppm); 4.39 (s, 2H, CH_2); 7.05 (broad siglet, 1H, Im-4(5)]; 7.52 (poorly resolved d, J = 10Hz, 1H, Py-3); 7.58 (singlet obscured by the Py-3 doublet, 1H, Im-2); 9.21 (poorly resolved doublet, J = 2Hz, 1H, py-5).

Mass spectrum: (FAB, MNOBA matrix + NaI)); m/e 237 (M^+); 113 ($ImCH_2S^+$); 81 ($ImCH_2$).

Anal. Calc. for $C_9H_8N_4O_2S$: C, 45.75; H, 3.41; N, 23.71; S, 13.57

Found: C, 45.78; H, 3.23; N, 23.53; S, 13.12

48) Attempts to prepare 2-{2-[4(5)-imidazolyl]ethyl}-amino-5-aminopyridine

I) The nitro compound SKH126B (**19**)(100mg, 0.42 mmole), hydrazine hydrate (21mg, 0.42 mmole), palladium on charcoal ((10%, 10mg) in abs.EtOH (20 cm^3) were stirred for 3h at 21°C and for 3h at 40-50°C. The charcoal was filtered off and the filtrate was evaporated to an oily residue when the residue turned dark red due to oxidation. A small quantity of the residue (20mg) was redissolved in abs. EtOH (5 cm^3) and was treated with ethanolic HCl (5 cm^3). On addition of ether, a dark red solid precipitated. The crude product was shown to be a mixture of many impurities. An attempt to isolate and identify the impurities by silica gel column chromatography using a mixture of MeOH (1, 5 and 10%) in $CHCl_3$ was unsatisfactory.

II) The reaction described in part I was repeated using the nitro compound (**19**)(100 mg, 0.43 mmole) and hydrazine hydrate (21 mg, o.43 mmole) in freshly distilled THF (5 cm^3). The reaction mixture was stirred at 21°C for 2h and at 80°C for 3h. The solvent was removed under reduced pressure to give an oily residue.The residue was shown to be a mixture of many impurities by TLC (EtOAc: MeOH: NH_4OH; 5:1:1).

49) 2-{2-[4(5)-imidazolyl]ethyl}-amino-5-aminopyridine

dioxalate monohydrate

(sample SKH452A/UCL1334)

The 2-{2-[4(5)-imidazolyl]ethyl}-amino-5-nitropyridine was prepared by the same procedure described for SKH126B/UCL1040 (**19**) to give 35% yield.

The nitro compound (1.54g, 6.60 mmole) was dissolved in abs. EtOH (100 cm^3). To the solution, palladium on charcoal catalyst (10%, 0.75g) and a few drops of acetic acid were added. The mixture was agitated under hydrogen (1.5 atm) at 21°C for 4h in a Cook hydrogenation apparatus. The catalyst was filtered off and the filtrate was reduced to a small volume (10 cm^3) and the unstable solution of the amine was treated with an ethanolic solution of oxalic acid (2 molar equivalent in 15 cm^3). Addition of ether gave the crude product as the dioxalate salt. Recrystallisation of the salt from abs. EtOH: ether (10:1) two times gave the product as the dioxalate monohydrate salt.SKH452A (1.27g),95% mp: 178-9°C.

Solubility: Sol. (MeOH, water); sp. sol. (EtOH); insol. ($CHCl_3$, ether).

TLC: silica, (EtOAc: MeOH; NH_4OH; 5:1:1) R_f: 0.9; ($CHCl_3$: MeOH; 1:1) R_f: 0.29.

HPLC: 98.45% at 6.68% minutes (Lichrosorb rp select b, 1cm^3/min., UV 215nm, A/B (99/1) where A is water with 0.1% trifluoroacetic acid and B is MeOH with 0.1% trifluoroacetic acid).

UV: Water; λ_{max}nm ($\log\varepsilon_{max}$): 334 (3.71); 245 (4.15); 213 (4.07).

IR: KBr cm^{-1} 3450-3390 (broad, primary aromatic amine), 2978, 2851(m, N-H str.), 1627 (s, C-N aromatic deformation), 1578 (s, C-N aromatic), 1242 (m, C=N-N, Im).

^1H NMR: (DMSO-d_6+ D_2O, 400 MHz), δ (ppm): 2.96 (t, J = 6.83Hz, 2H, Im$\underline{CH_2}$), 3.54 (t, J = 6.86Hz, 2H, $\underline{CH_2}$NH), 6.86 (d, 1H, Py-6), 7.37 (dxd, J = 9.88Hz, 1H, Py-3), 7.39 [s, 1H, Im-4(5)], 7.47 (dxd, J = 9.3Hz, 1H, Py-4), 8.79 (s, 1H, Im-2).

Mass spectrum: (EI), m/e 203 (M^+), 122 ($H_2NpyNHCH_2^+$), 82 ($ImCH_2^+$).

Anal. calc. for $C_{10}H_{13}N_5$, 2 $C_2H_2O_4$, H_2O: C, 41.90; H, 4.77; N, 17.45;

found: C, 41.62; H, 4.57; N, 17.84.

50) 4(5)-[2-Bromoethyl]imidazole hydrobromide:

(sample SKH231C)

4(5)-[2-Hydroxyethyl)imidazole (0.57g, 5mmole) in aq. HBr (47%, 30 cm^3) was heated under reflux for 10h. The solvent was removed under reduced pressure and the residual moisture was removed by azeotropic distillation with i-PrOH. The residue was washed with ether several times to afford the product as the hydrobromide salt. 1.24g (92%), mp: 155-8 °C (lit [16]mp: 156-8°C).

^1H NMR: (DMSO-d_6, 200 MHz); δ (ppm): 3.14 (t, 2H, $ImCH_2$); 3.58 (t, J = 2.84Hz, 2H, CH_2Br); 4.75 (broad s, HBr salt); 7.42 [s, 1H, Im-4(5)]; 9.06 (s, J = 1.27Hz, 1H, Im-2).

N.B: On addition of one drop of D_2O to the solution, the peak at 4.75 disappears.

Mass spectrum: (EI), m/e 175 (M^+), 81 ($ImCH_2$).

51) 2-{2-[4(5)-imidazolyl]ethylthio}pyrimidine hydrobromide

(sample SKH231A)

4(5)-(2-Bromoethyl)imidazole hydrobromide (0.97g, 3.7 mmole) and 2-mercaptopyrimidine (0.415g, 3.7 mmole) in a mixture of abs. EtOH (50 cm^3) and aqueous HBr (47%, 10 cm^3) were heated under reflux for 5 days. The solvent was removed under reduced pressure and the oily residue was chromatographed on a column of silica gel using

a mixture of MeOH (1; 5; 20%) in $CHCl_3$ as eluent.The pure fractions were combined and concentrated under reduced pressure to give an oily residue After taking up the oily residue into ether, a white solid was deposited and filtered off to give SKH230A (30 mg; mp: 108-10°C). The filtrate was discarded. The other partially impure fractions were concentrated under reduced pressure to an oily residue and after treament with ether and standing, the product deposited, filtered and dried to give SKH230B (0.58g, 93% crude yield), mp: 110-112°C. The product was recrystallised from ab. EtOH and ether (1:1, 20 cm^3) and was dried under vacuum over P_2O_5 for several days. 0.21g (17.6), mp: 108-110°C.

Solubility: sol. (water, MeOH, abs. EtOH), insol. (EtOAc, ether).

TLC: Silica (EtOAc: MeOH: NH_4OH; 5:1:1) R_f: 0.69.

HPLC: 98.53% at 13.31 minutes (Lichrosorb rp select B, 250x4 mm, 0.75 cm^3/min, UV 254nm, A/B (60/40) where A is water and B is MeOH with 5% water. The mobile phase contains 0.1% triethylamine). The impurities are 0.53% and 0.62% at 2.07 and 2.20 minutes respectively.

IR: KBr cm^{-1}, 3290 (s, C=N str.), 2956 (m, S=C=N str.), 1646 (s, -C=C-NH-, str.), 1624 (s, S=C-N, str.), 1542 (s, -C=N-, Im).

^1H NMR: (DMSO-d_6, 200MHz), δ (ppm), 2.87 (t, J = 7Hz, 2H, $ImCH_2$), 3.34 (t, 2H, CH_2S), 6.84 [s, 1H, Im-4(5)], 7.18 (t, J = 8Hz, 1H, Pym-5), 7.56 (s, 1H, Im-2), 8.63 (d, J = 4.8Hz, 2H, Pym-4,6).

Mass Spectrum: (EI), m/e 206 (M^+), 127 ($ImCH_2CH_2S$), 81 ($ImCH_2$).

Anal. Calc. for $C_9H_{10}N_4S$, HBr: C, 37.62; H, 4.73; N, 17.55·

found: C, 38.44; H, 4.48; N, 17.58.

The sample was purified further by semi preparative column chromatography but elemental analysis was unsatisfactory.

51) 6-{2-(5-nitro)pyridyl-5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidine

(sample SKH167A)

To a stirred solution of 5-oxo-5,6,7,8-tetrahydroimidazol[1,5-c]pyrimidine SKH157 (37)(1.5g, 10.9 mmole) and 2-chloro-5-nitropyridine (2.073g, 13 mmole, 1.2 molar equivalent), in DMF (freshly distilled and dried, 10 cm^3) under N_2 was added NaH (0.314g, 30 mmole, 3 molar equivalent). The Reaction mixture was stirred at 21°C for 5h. The solvent was removed under reduced pressure and the residue was subjected to silica gel column chromatography using a gradient of MeOH (1 and 10%) in $CHCl_3$.as eluate. The pure fractions were combined and evaporated to dryness under vacuum. The product was crystallised from i-PrOH: ether (1:1, 10 cm^3) to give SKH167A (1.58g, 57%), mp: 180-1°C.

IR: KBr cm^{-1} 1708 (s, N-CO-N), 1511 (s, N-O str. arom), 1332 (s, N-O str. arom).

^1H NMR: (DMSO-d_6, 200MHz), 3.06 (t, J = 6.20Hz, 2H, $ImCH_2$), 4.34 (t, 2H, $\underline{CH_2}NH$), 6.86 (s, 1H, Im-4), 8.09 (d, J = 9.2Hz, 1H, Py-3), 8.27 (s, 1H, Im-2), 8.64 (dxd, J = 6.45, 9.20Hz, 1H, Py-4), 9.27 (d, J = 2.79Hz, 1H, Py-6).

Mass spectrum: (EI), m/e 259 (M^+), 94 ($ImCH_2CH_2$).

CHAPTER 6

PHARMACOLOGICAL TESTING

Pharmacological testing for H_3-histamine receoptor antagonism was carried out at Centre Paul Broca de l'Inserm, Paris. All the compounds were tested *in vitro* on rat cerebral cortex and those that showed particular activity were tested further *in vivo* using mice (see chapter 4). 20-50 mg of material were submitted for testing. *In vitro* testing results were in terms of a K_i value. *In vivo* testing results were in terms of N^{τ}-methylhistamine concentration with time after dosage.

In vitro testing

The bioassay developed by Prof. Schwartz and his group[5,7] is described as follows: The rat cerebral cortex slices are incubated with [^{3}H]histidine, leading to endogenous synthesis of [^{3}H]histamine within the histaminergic neurones. The preparation is well washed and then exposed to K^+, which evokes an increase in histamine efflux. The presence of additional unlabelled histamine (or other agonists) in the bathing medium depresses the evoked release of histamine (measured as [^{3}H]histamine) without affecting basal efflux. Antagonists block the depressive action of histamine. The different technique of superfusion using electrically evoked release has been established by Prof. Timmerman and his group.[119]

Slices (0.3mm thick) form cerebral cortex of male Wistar rats (180-200g)(IFFA-CREDO, France) were prepared with a McIlwain tissue chopper and resuspended in modified Krebs-Ringer bicarbonate medium (mM): 120 NaCl, 0.8 KCl, 2.6 $CaCl_2$, 0.67 $MgSO_4$, 1.2 KH_2PO_4, 27.5 $NaHCO_3$, 10 glucose, pH 7.4 gassed with O_2/CO_2 (95:5).

The slices (about 12mg protein/cm^3) were preincubated for 30 minutes at 37°C in the presence of 0.4 mM [^{3}H]L-histidine to ensure [^{3}H]histamine synthesis. After 30 minutes, slices were transferred to an open plastic cylinder with a nylon mesh fitted to the bottom as a small basket and washed to remove excess [^{3}H]histidine and to obtain a constant spontaneous [^{3}H]histamine efflux. Hence the basket was succesively transferred to seven beakers containig fresh Krebs-Ringer solution at 37°C in O_2/CO_2 (95:5) in which it was kept for periods of 4 minutes (first four washings) and 2 minutes (last three washings). After the last washing period, 250 cm^3 aliquots of the various drugs to be tested were added. Five minutes later, 250 cm^3 of a modified Krebs-Ringer solution were added to give a final concentration of either 2mM or 30 mM K+. Exogenous unlabelled histamine (10^{-6}M) was added, when required, together with 30mM K^+. Incubations were stopped 2 minutes later by rapid centrifugation, the pellets were homogenised in 200 cm^3 0.01M HCl and [^{3}H]histamine present in the pellet and supernatant solution was measured after isolation by ion-exchange chromatography on amberlite CG 50 Columns. [^{3}H]Histamine recoveries from the columns in the isolation procedure were generally around 84 ±1% and [^{3}H]histidine contaminations were generally less than 0.01% and the data were corrected accordingly.

Concentration-response curves as well as inhibition curves were analysed for determination of IC_{50} values of antagonists by fitting the data with an iterative computer least squares method. The apparent dissociation constants (K_i values) of antagonists were calculated from their IC_{50} values, assuming competitive antagonism, according to the equation of Cheng and Prussof:[120]

$$K_i = IC_{50}/ (1+ S/EC_{50})$$

where S represents the concentration of exogenous histamine (10^{-6} M) and EC_{50} the amine concentration eliciting a half maximal inhibitory effect on K^+ -evoked release of [^{3}H]histamine.

Statistical evaluation of the results was done with Student's T-test. Proteins were determined by the Folin procedure[115] with bovine serum albumin as standard.

IN VIVO testing

The radioimmunoassays have been developed by Garbarg et al [116] for sensitve evaluation of the levels of histamine and N^{τ}-Methylhistamine. Since transmethylation is the sole metabolic pathway in the brain, measurement of rates of accumulation of N^{τ}-methylhistamine following inactivation of monoamine oxidase is a useful index of HA turnover, hence the effect of the drug on histamine levels in the brain can be measured.

Simple radioimmunoassays developed by Garbarg er al.[116] showed a marked difference in both histamine and N^{τ}-methylhistamine immunoreactivities.The hypothalamus was found contain the highest level of the amine. The regional differences were less marked for N^{τ}-methylhistamine.

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