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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Thanks finally to my parents, for their patient, generous and loving encouragement and to my fiance Robert for his active support.

For my parents.

Abbreviations

Ar	aromatic
aufs	absorbance units for full scale deflection
bp	boiling point
br	broad
cAMP	cyclic-adenosine-3'-5'-monophosphate
CNS	central nervous system
cpd	compound
d	doublet
def	deformation
DMSO	dimethyl sulphoxide
EC_{50}	concentration producing 50% maximal response
EI	electron impact
EtOH	absolute ethanol
FAB	fast atom bombardment
fmol	femtomole, 10^{-15} mole
g	gramme
HA	histamine
Hplc	high performance liquid chromatography
Hz	Hertz
Im	imidazol-4-yl if not specified otherwise
insol	insoluble
i.p.	intra peritoneal
IR	infra red
i.v.	intravenous
J	coupling constant
J_{av}	average coupling constant
K_i	inhibitory constant, equivalent to K_B, where B stands for biological
K_B	dissociation constant of antagonist; antagonist potency, $pA_2 = -\log_{10}K_B$
m	multiplet (NMR), medium (IR)
M	molar
m/e	mass to charge ratio
mg	milligramme, 10^{-3} gramme
ml	millilitre, cubic centimetre
mmol	millimole
min	minute

mp	melting point
µl	microlitre, 10^{-6} litre
µM	micromole
ng	nanogramme, 10^{-9} gramme
nm	nanometre
nmol	nanomole
NMR	nuclear magnetic resonance
Nuj	Nujol, paraffin oil
oopb	out of plane bending
oopd	out of plane deformation
oopv	out of plane vibration
p	level of significance, probability of incurring Type I error (i.e. when a difference is found between A and B when none actually exists)
pg	picogramme, 10^{-12} gramme
Ph	phenyl
pip	piperidine
pK_i	-log K_i
p.o.	per os, by mouth
pyr	pyridine
q	quartet
s	singlet (NMR), strong (IR)
SEM	standard error of the mean
SKF	Smith, Kline & French
sp sol	sparingly soluble
str	stretch
sol	soluble
t	triplet
THF	tetrahydrofuran
TLC	thin layer chromatography
UCL	identification tag (Universtity College London)
UV	ultraviolet
vbr	very broad
w	weak
YK	identification tag (Y. Khalaf)

Thesis compound numbers

(1) to (12)	see table 2.1.
(13) to (21)	see table 2.2.
(14/I)	4-phenyl-n-butyl methylamide
(22) to (33)	see table 2.3.
(29/I)	cyclohexylisothiourea
(34)	N-cyclohexyl-4-(imidazolyl-4-yl)-1-piperidine carbothioamide, thioperamide
(35)	imidazol-4-yl-$(CH_2)_2NHCO(CH_2)_2Ph$ (Schunack)
(36)	imidazol-4-yl-$(CH_2)_2NH$-2-pyridine, SKH130A/UCL1038
(37)	imidazol-4-yl-propenoic acid, urocanic acid
(38)	imidazol-4-yl-propenoic acid chloride
(39)	imidazol-4-yl-propenamide
(40)	imidazol-4-yl-propanamide
(41)	3-(imidazol-4-yl)propylamine
(41^{WT})	3-(imidazol-4-yl)propylamine, WT76/4396A
(42)	imidazol-4-yl-$(CH_2)_2NH(CH_2)_4Ph$ (Schunack)
(43)	1-benzyl-4-piperidone
(44)	1-benzyl-4-(2-pyridinyl)-1,2,5,6-tetrahydropyridine
(45)	1-benzyl-4(2-pyridinyl)piperidine
(46)	1-ethoxycarbonyl-4(2-pyridinyl)piperidine
(47)	4-(2-pyridinyl)piperidine
(48)	S-(2-imidazol-4-yl)ethyl)isothiourea, WT133C/UCL1095-B_2
(49)	4-(imidazol-4-yl)butylamine, WT76/457A
(50)	2(hydroxyethyl)imidazol-4-yl, WT76/3156
(51)	O-(2-pyridin-2-yl-ethyl)isourea, YK418C
(52)	O-(2-imidazol-4-yl-ethyl)isourea, YK406C

(53) 3-N,N-di-methyl-O-(2-imidazol-4-yl-ethyl)isourea,YK414A

(54) N-(N'-cyclohexyl-thiocarbamoyl-2(4-piperidin-4-ol)pyridine

(55) imidazol-4-yl-$(CH_2)_2$-NH-2(5-nitro)pyridine, SKH126B/UCL1040

(56) imidazol-4-yl-$CH_2S(CH_2)_2$-NH-2(5-nitro)pyridine, SKH156A/UCL1068

(57) imidazol-4-yl-$(CH_2)_2$-NH-2(3-nitro)pyridine, SKH117A/UCL1039

(58) imidazol-4-yl-$(CH_2)_2$-NH-CS-NH-C_6H_{11}, SKH198B/UCL1108

(59) imidazol-4-yl-$CH_2S(CH_2)_2$-NH-CS-NH-C_6H_{11}, SKH188A/UCL1109

ABSTRACT

This thesis describes various approaches to the design and synthesis of potential H_3-receptor histamine antagonists. The ultimate aim 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. Novel compounds were synthesized and submitted for testing as potential antagonists in vitro on rat cerebral cortex. The more active compounds were tested further, in vivo.The results, in terms of K_i or EC_{50} values were used as a guide in making further modifications of structures derived from compounds existing in the literature.

The published selective H_3 antagonist, N-cyclohexyl-4-(4 -imidazolyl)-1-piperidine carbothioamide (thioperamide) was used as a lead. In order to try to improve brain penetration, it was desirable to remove the imidazole group altogether, or replace it with a less hydrogen-bonding group. To this end the imidazole group was removed and the 4-piperidine position substituted with functional groups of differing polarities. The imidazole group was replaced with 2-pyridine, and the 3-(2-piperidinyl)pyridine isomer was also prepared. A significant loss of activity was observed for all these compounds.

Another approach was to open up the piperidine ring in thioperamide, thus investigating the effect of increased flexibility on the molecule. This led to a drop in activity which was restored by methylating the sulphur atom.

The potential toxicity of the thiocarbonyl group in thioperamide, was avoided by the synthesis of some esters and amides of urocanic acid. The p-nitrophenyl ester seemed to indicate a direction dependent dipolar interaction with the receptor.

A set of amines was also prepared, one following up the p-nitro effect, and two exploring the omission of the imidazole group, this time with the retention of antagonistic activity.

A number of of ethyl-imidazol-4-yl isothioureas, with a range of n-alkyl and a cyclohexyl group substituted on the isothiourea nitrogen atoms, showed a progression from an antagonist as potent as thioperamide through to an agonist. Omitting the imidazole group from the most potent isothiourea antagonist, resulted in loss of activity. S-(2-(2-Pyridinyl)ethyl)isothiourea proved to be a partial agonist, while the propyl side chain analogue was an antagonist. Some analogous isoureas were prepared.

Two novel synthetic schemes were developed, for the starting materials 2-(4-piperidinyl)pyridine and 3-(imidazol-4-yl)propylamine.

Thus, an indication of the structure activity behaviour of antagonists at the H_3 receptor has been established.

CONTENTS

CHAPTER ONE.

INTRODUCTION.

Histamine background and action

Histamine, 2-(imidazol-4-yl)ethylamine (Figure 1.1.), was first reported and synthesized by Windaus and Vogt[82] in 1907. Three years later it was shown to be produced from histidine by bacterial decarboxylation[1], to occur in ergot extracts, and to cause powerful pharmacological effects. Dale and Laidlaw[35,36] in 1910 showed that histamine was a potent stimulant of smooth muscle contraction and that it caused pronounced vascular reactions which closely resembled the effects seen after anaphylactic shock. It was later found to be a constituent of many tissues and came to be regarded as a substance liberated in response to injurous stimuli.

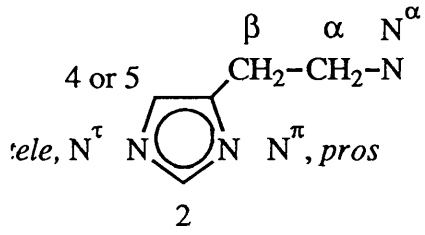


Figure 1.1. Histamine numbering according to Black and Ganellin[24].

Histamine is a chemical messenger involved in various complex biological actions and it occurs mainly in an inactive bound form in most mammalian body tissues. When released, it interacts with specific macromolecular receptors on the cell surface or within a target cell to elicit change in many different bodily functions. Receptors for histamine have not yet been isolated or identified by physical or chemical means but their presence is inferred pharmacologically by the use of synthetic agonists and antagonists. Three

types of pharmacological receptor have been described and are designated as H_1, H_2 and H_3.

Histamine acting via H_1 receptors stimulates many smooth muscles to contract such as those in the gut, the uterus and the bronchi, the latter effect being symptomatic of asthma. In some smooth muscle, such as the fine capillaries, it causes relaxation, leading to vasodilation and a fall in blood pressure. In addition, capillary wall permeability is increased resulting in more plasma constituents escaping into the tissue spaces, leading to an increase in the flow of lymph and its protein content, and the formation of oedema. These effects are typical of the redness and wheal associated with histamine release after a nettle sting for instance. It is interesting to note that the sensitivity varies between species, with the rat being relatively resistant, and man and the guinea pig being very sensitive. The presence of histamine in the body surfaces allows it to play a physiological role in the body's defence against a hostile environment. The most common situation in which histamine is liberated in man is as a result of the antibodies produced against foreign proteins. It assists the body in removing the products of cell damage during inflammation. However, under extreme circumstances such as in allergic conditions, the effects of histamine may become pathological with distressing results.

Histamine acts on the H_2 receptor to stimulate the parietal cells in the stomach to produce hydrochloric acid. Under normal circumstances, the acid controls the local bacterial population, but a pathological situation is presented by the formation of gastric or duodenal ulcers. Histamine can also stimulate the heart to beat faster or to increase its force of contraction.

The histamine H_3 receptor is relatively unexplored. It seems to regulate histamine synthesis and release in the brain, acting as an autoreceptor involved in the transmission of neuronal signals, with the possible effect of maintaining alertnesss.

Histamine biosynthesis and metabolism

Histamine is formed from the amino acid L-histidine by decarboxylation. See Figure 1.2. and Table 1.1.

No specific uptake process has yet been identified for histamine in contrast to other amine transmitters such as acetylcholine and noradrenaline whose production requires multistage biosynthetic pathways.

Neuronal histamine levels may be reduced drastically by blocking L-histidine decarboxylase with a suicidal inhibitor, α-fluoromethylhistidine. No selective and useful blockers of the main pathway of degradation of histamine, i.e. the formation of N^{τ}-methylhistamine are available. The presynaptic receptor of histamine represents an alternative way of controlling histamine levels. It is known[31] that reduced levels of histamine in the rat brain (using α-fluoromethylhistidine) result in increased learning ability. As suggested by Sir James Black at the 19th meeting of the European Histamine Research Society this year (1990), clinical testing of burimamide as an H_2-antagonist showed visual and spatial disturbances and since burimamide is an even more potent H_3-antagonist, the disturbances could be attributed to the increased histamine levels in the brain.

Histamine storage and release

Histamine is found chiefly in the mast cell and its circulating counterpart in the blood, the basophil, where it is synthesized and stored. Some tissues however, have a high capacity for synthesizing histamine but do not store it, while cells in rapidly growing tissues produce and continuously release large amounts of histamine. The existence of the tissue mast cell and blood basophil was discovered by Paul Ehrlich in 1877 and 1879 respectively, but it was Riley and West (1953)[63] who recognized the mast cell as a histamine repository. An average mast cell contains approximately 10pg of histamine,

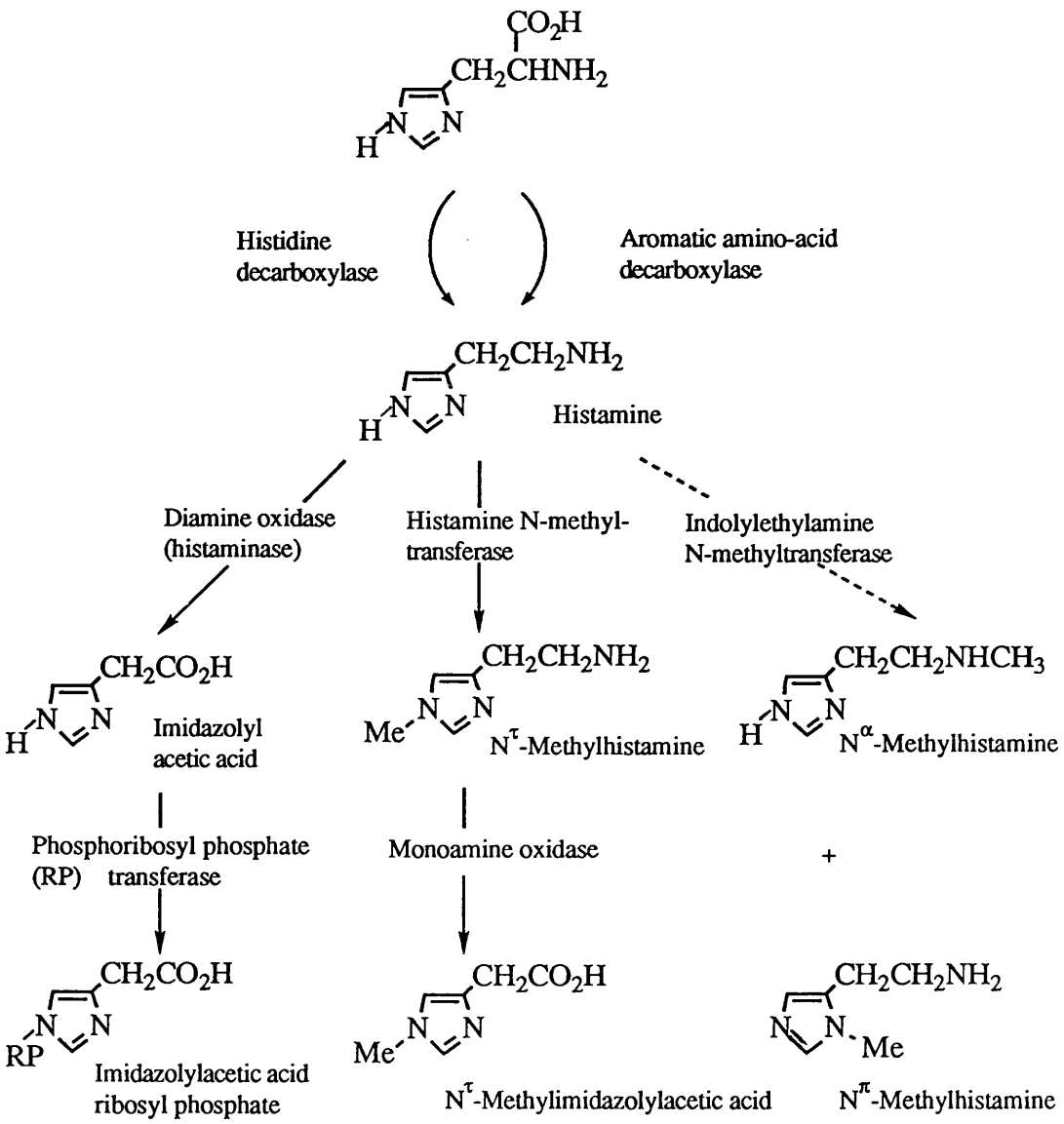
while a basophil contains about 1-1.2pg. This is released explosively, by an energy dependent mechanism in response to various small stimuli such as trauma, toxins, basic compounds and some immunological reactions. Many basic drugs containing amine, diamine, diamidine, or guanidine groups are histamine releasers, e.g. morphine, tubocurarine. Mast cells are heterogenous and respond differently to histamine-releasing agents depending on species and tissue location. Mast cells and basophils bear specific receptors on their surface for the immunoglobulin IgE, which binds IgE antibody molecules with high affinity as occurs in immediate hypersensitivity reactions. Attachment of antigen to more than one cell-bound IgE antibody (multivalent binding) triggers off a sequence of biochemical events. Thus bridging of IgE receptors on rat mast cells induces phospholipid methylation, a rise in intracellular cAMP and activation of protein kinase, mobilization of intracellular Ca^{2+} ions and an increase in Ca^{2+} flux. These responses are accompanied by rapid extrusion of granules from the mast cell. The granules then dissociate to release histamine and other mediators such as heparin and leukotrienes into the surrounding tissue and blood stream.

Synthesis and release of histamine in the brain is from a discrete set of neurones ascending the lateral hypothalamic area, and widely projecting in the telencephalon, as shown by a combination of biochemical, electrophysiological and lesion studies in rats.[12].

Some of the various cellular populations where histamine is stored and released include : immunoglobulin E receptor bearing cells (mast cells and basophils), endocrine cells, and neurones in the central and peripheral nervous system.

Response to histamine is shown by an even larger variety of cell types, including smooth muscles, neurones, endocrine glands, exocrine glands, blood cells, and cells of the immune system. The response is an increase in intracellular levels of signals.

Figure 1.2. The biosynthetic and metabolic pathways of histamine.[33]



Note

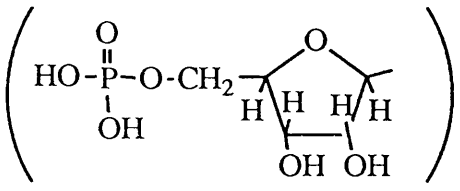
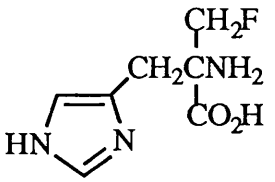
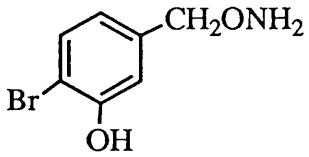
RP = 

Table 1.1. Some examples of inhibitors of enzymes involved in histamine synthesis and metabolism, and their in vitro potency.[33].

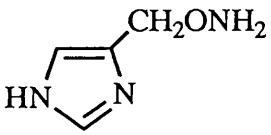
Compound	K_i(M)	Comments
Histidine decarboxylase		
α-Fluoromethyl-L-histidine	3.2×10^{-5}	Most potent and useful as a pharmacological tool in vitro and measurement of brain HA levels.
Brocresine	1.5×10^{-7}	A benzyloxyamine, non-specific, combines with pyridoxal coenzyme.
Imidazole-N-methyltransferase		
Amodiaquine	1×10^{-8}	Antimalarial.
TMQ, 2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl] quinazoline	7×10^{-9}	Folate antagonist.
Diamine oxidase (histaminase)		
Imidazol-4-ylmethoxyamine	9×10^{-9}	
Impromidine	7×10^{-9}	
Imidazoleacetate phosphoribosylphosphate transferase		
Sodium salicylate	2×10^{-4}	



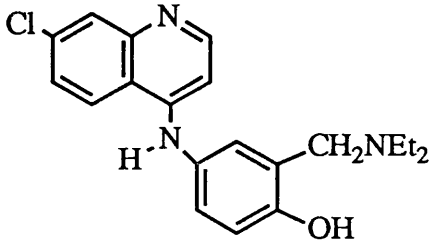
α-Fluoromethyl-L-histidine



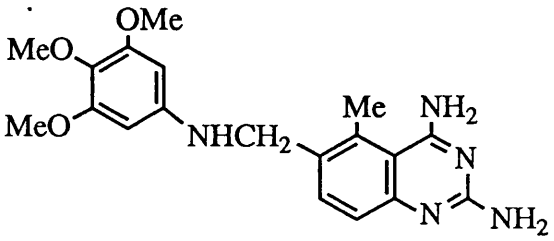
Brocresine



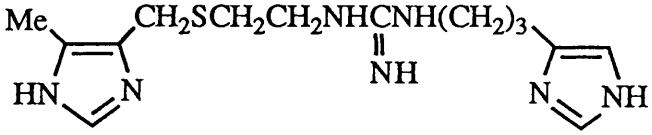
Imidazol-4-ylmethoxyamine



Amodiaquine



TMQ



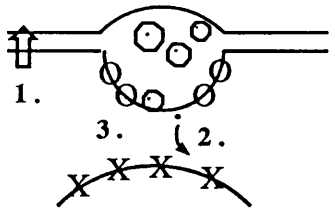
Impromidine

Histamine as a neurotransmitter

Hormones and neurotransmitters are very important endogenous compounds which transfer information. Neurotransmitters are present in the nerve endings and released upon the arrival of an action potential. The release may be controlled by several factors, such as the transmitter itself, or other mediators. The neurotransmitter is released into the synaptic cleft, subsequently may react with different receptors.

See Figure 1.3.

Figure 1.3. Neurotransmitter release and receptors at a synapse.



1. action potential
2. release of transmitter
3. synaptic cleft

O presynaptic receptors
X postsynaptic receptors

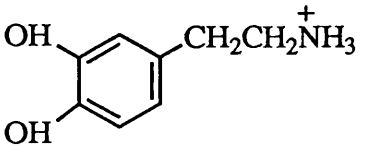
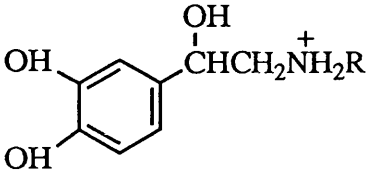
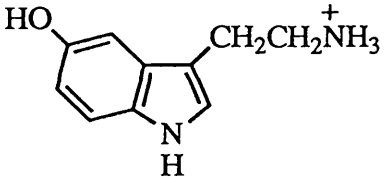
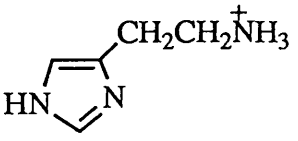
Histamine is only one of the many biogenic amines acting as chemical transmitters. They are mainly decarboxylated products of aminoacids and their derivatives. See Table 1.2.

Histamine has multiple functions as a chemical messenger in cell to cell communication, and although histaminergic neurones have not yet been histochemically visualized, it seems quite clear that histamine has a neurotransmitter role in the invertebrate and mammalian nervous systems.

It is well established that several neurotransmitters affect neuronal activity in the central nervous system through stimulation not only of postsynaptic receptors, but also of receptors located presynaptically, which often display distinct pharmacological specificity and by which they control their own release.

H_1 and H_2 receptors are postsynaptic receptors. H_1 receptors are linked to a phosphatidylinositol pathway and mobilization of intracellular Ca^{2+}, while H_2-receptors mediate via adenylcyclase activation, the production of cAMP. For both H_1 and H_2 receptors, histamine is the endogenous agonist, while the antagonist belongs to rather different chemical classes of compounds.

Table 1.2. Histamine, one of the biogenic amines.

BIOGENIC AMINES	RECEPTORS	STRUCTURE
ACETYL CHOLINE	NICOTINIC MUSCARINIC M_1, M_2, M_3, M_4, M_5	$CH_3CO.O.CH_2CH_2\overset{+}{N}Me_3$
DOPAMINE	D_1, D_2, D_3	
NORADRENALINE (R = H)	α_1, α_2, β_1,β_2,β_3	
SEROTONIN (5-HYDROXYTRYPTAMINE)	$5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$, $5HT_{1D}$, $5HT_2$, $5HT_3$, $5HT_4$	
HISTAMINE	H_1, H_2, H_3	
GABA γ-AMINOBUTYRIC ACID	$GABA_A$, $GABA_B$	$^-O_2CCH_2CH_2CH_2\overset{+}{N}H_3$

Evidence for the existence of H_3 receptors has come from a series of experiments by Arrang et al.[12]. In the experiments, slices of rat cerebral cortex were labelled by preincubation with [^{3}H]-L-histidine (i.e. tritiated hydrogen atoms at the 2- and 5-positions of the imidazole ring). Depolarization with 30nm K^+ caused the release of 15% [^{3}H]-Histamine, which was reversibly inhibited (up to 60%) when exogenous histamine was administered. These characteristics of saturability and reversibilty of inhibition are

indicative of a receptor mediated process. Stimulation of presynaptic receptors influences the release of agonist, and the receptor is known an autoreceptor. Such autoreceptors have been demonstrated in the case of noradrenaline (α_2), dopamine, serotonin, acetylcholine, and γ-amino butyric acid (GABA) neurones. The negative feedback system controls the release of neurotransmitter at higher concentrations, while the postsynaptic control seems to be responsible for fine regulation of neurotransmission. H_3 receptors are presynaptic autoreceptors, i.e. histamine inhibits its own release. The signal transfer process is Ca^{2+} dependent.

It has been reported[12.,74.] that the release of histamine could be blocked to a maximal degree of 60% using the K^+ depolarization technique to induce release, while a 100% blockade could be achieved using electrical stimuli as induction of release (possibly due to different Ca^{2+} gating mechanisms being involved). Furthermore, a strong effect of the frequency of stimulation on the inhibitory effect of histamine is observed, indicating the possible presence of spare receptors.

Histamine seems to be the natural agonist of the H_3 receptor, although the possible importance of N^{α}-methyl or N^{α}, N^{α}-dimethyl histamine may have a physiological role, since these methylated histamine analogues have been found to be present in body fluids.[74.]

Agonists and antagonists at the H_1 and H_2 histamine receptors

H_1 agonists: Typical examples include 2- methylhistamine, 2- thiazolylethylamine, 2-pyridinylethylamine. They are used as research tools only, while a potent selective compound has not been available. Recently, the most potent H_1 agonist available was presented by Schunack and his colleagues[84.], as part of a series of 2-substituted histamine derivatives, 2-(3-fluoro)phenylhistamine with a relative potency to histamine of 87%.

H_1 antagonists: Typical examples include chlorpheniramine, diphenhydramine, promethazine, mepyramine. H_1 antagonists are being applied mainly in allergic conditions. The classical antihistamines were found to induce strong CNS effects of which sedatory effects found a use, for instance as sleeping aids. Indeed, some classes of CNS agents, e.g. antidepressants, have originally been found as antihistamines. The newer compounds such as terfenadine, astemizole and loratidine have the advantage that they have much less tendency to cause sedation.

H_2 agonists: Typical examples include 4-methylhistamine, dimaprit, impromidine, clonidine, tolazoline. H_2 agonists have been suggested[20] as cardiac stimulants (e.g. impromidine) though gastric acid production could constitute a problem.

H_2 antagonists: Typical examples include burimamide, metiamide, cimetidine, ranitidine, famotidine, tiotidine, oxmetidine. H_2 antagonists have become important as peptic ulcer healing agents.

Note Burimamide and impromidine are also active H_3 antagonists.

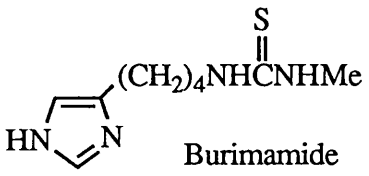
See Table 1.3. below for structures and activities of most of these compounds.

H_3 agonists and antagonists in relation to H_1 and H_2 active compounds

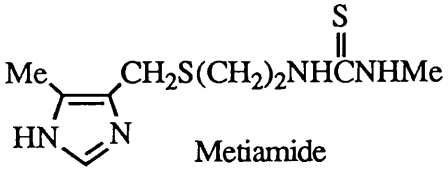
The structure activity relationship (SAR) of histamine analogues is different for the three histamine mediated activities. If a receptor system exists there should be a possibility to block the activity. Table 1.3. shows that some, but not all H_2 ligands, agonists and antagonists, are H_3 receptor antagonists. It is notable that the weak H_2 antagonist burimamide is a potent H_3 antagonist, whereas some more active H_2 antagonists, including cimetidine, ranitidine, metiamide, and tiotidine, are much weaker H_3 antagonists. Impromidine (a partial H_2 agonist) shows a very potent antagonistic effect at the H_3 receptor.

Table 1.3 [7.,12.] Comparison of potencies of histaminergic agents on the inhibition of rat cortical ^{3}H-histamine release (H_3 receptors), guinea pig ileum contraction (H_1 receptors), and atrial rate (H_2 receptors). Antagonists were assayed against histamine.

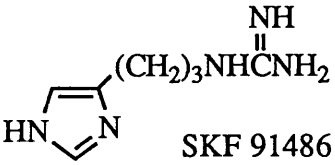
Agent	H_1	H_2	H_3
	Relative agonist potency		
Histamine	100	100	100
N^{τ}-methylhistamine	0.42	<0.1	<4
N^{π}-methylhistamine	<0.01	<0.1	<4
N^{α}-methylhistamine	72	74	270
N^{α}, N^{α}-dimethylhistamine	44	51	170
2-Methylhistamine	16.5	4.4	<0.08
2-Thiazolylethylamine	26	2.2	<0.008
4-Methylhistamine	0.23	43	<0.008
Dimaprit	<0.0001	71	<0.008
Impromidine	<0.001	4,810	<0.03
	Antagonist activity (K_i, M)		
Mepyramine	4.4×10^{-10}		$>5.8 \times 10^{-8}$
Cyclizine	1.3×10^{-8}		$>5.8 \times 10^{-7}$
D-Chlorpheniramine	5×10^{-10}		$>5.8 \times 10^{-8}$
L-Chlorpheniramine	1.5×10^{-8}		$>5.8 \times 10^{-8}$
Metiamide	$>10^{-3}$	9.2×10^{-7}	2.5×10^{-6}
Cimetidine	4.5×10^{-3}	7.9×10^{-7}	3.3×10^{-5}
Thioperamide	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-5}$	4.3×10^{-9}
Burimamide		7.8×10^{-6}	7×10^{-8}
Ranitidine		6.3×10^{-8}	$>1.2 \times 10^{-6}$
Tiotidine		1.5×10^{-8}	$>1.2 \times 10^{-5}$
Impromidine		4.8×10^{-7}	6.5×10^{-8}
SKF 91486		2.2×10^{-5}	8.8×10^{-8}



Burimamide



Metiamide



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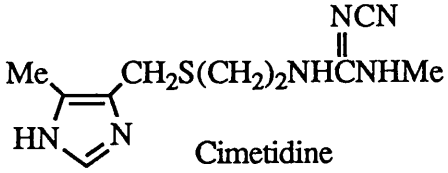
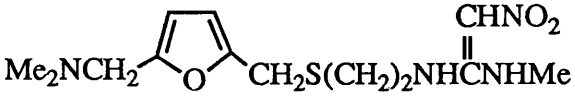
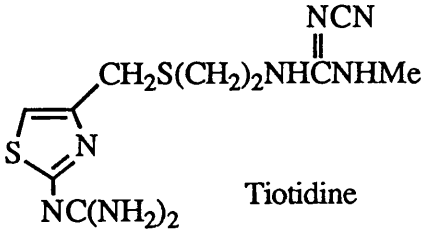


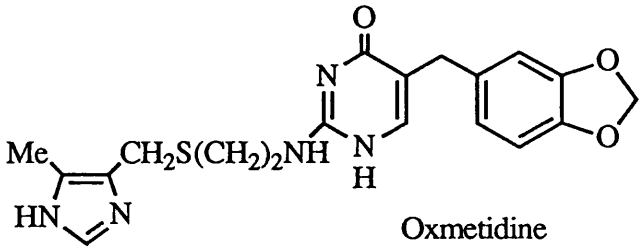
Cimetidine



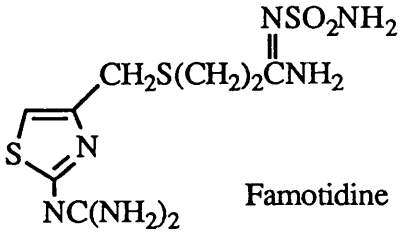
Ranitidine



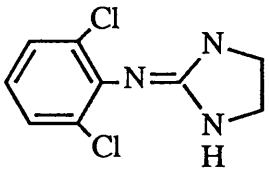
Tiotidine



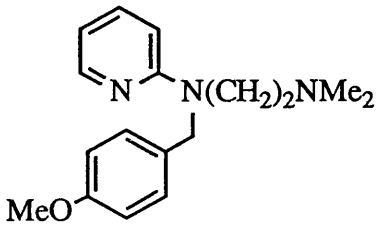
Oxmetidine



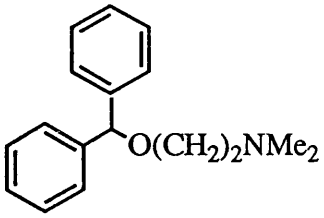
Famotidine



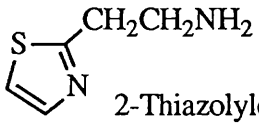
Clonidine



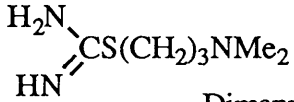
Mepyramine



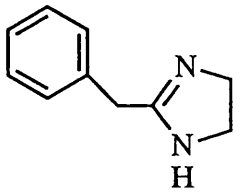
Diphenhydramine



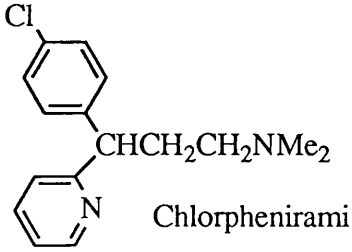
2-Thiazolylethylamine



Dimaprit

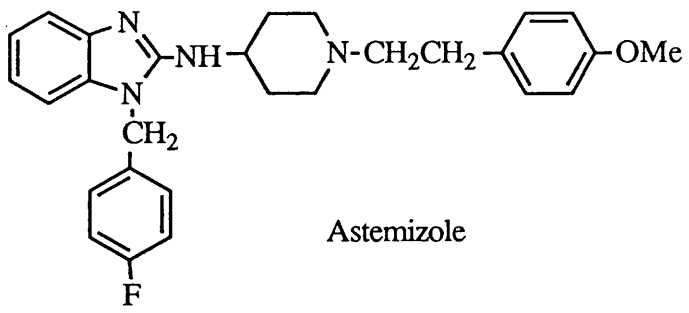


Tolazoline

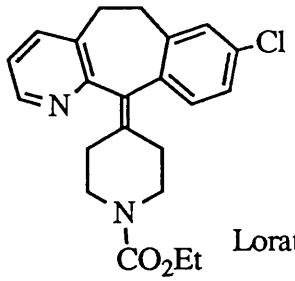


Chlorpheniramine

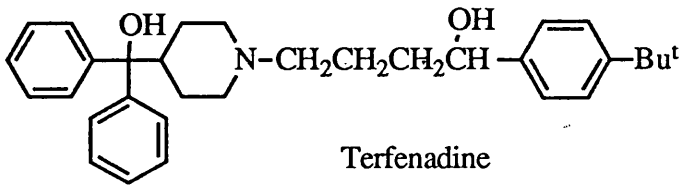
Promethazine



Astemizole



Loratidine



Terfenadine

Note K_i values for H_3 antagonists were calculated[12.,32.] assuming competitive antagonism, and neglecting the influence of endogenous histamine.

EC_{50} values for H_3 agonists are defined as the concentration resulting in a half-maximal inhibition of K^+-evoked [^{3}H]histamine release.

Relative potency was calculated as the ratio (EC_{50} value of histamine/EC_{50} value of agonist) x 100.

K_i was calculated according to the equation[32.] : $IC_{50}/(1 + S/EC_{50})$

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.

The K_i values obtained from Inserm are mean values ($\pm$ SEM). The Student-t-test manipulation of the results ensures that $p < 0.05$, i.e. the results are considered statistically significant if there is more than 95% probability that the difference observed is an actual difference and not a fluctuation in the sample population.

The thiourea-containing burimamide and the guanidino moiety containing impromidine are equipotent H_3 antagonists. In the H_2 antagonistic series these two groups played a crucial role.

The imidazole ring is not absolutely necessary for H_3 antagonistic activity, since the weak H_1 agonist betahistine[10.] (a pyridine derivative) and phencyclidine[5.] (a phenyl compound) are also reported to have some activity, although they are quite weak.

See Table 1.4.

The highly selective, potent and competitive H_3 antagonist, thioperamide **(34)**, (structure shown on page 35) first reported by Arrang et al[12.] can be considered to be a burimamide analogue (structure shown on page 23), in which the side chain has been cyclized into a piperidine ring, and a cyclohexyl group substituted on the terminal thioureido N atom in place of methyl. Thioperamide was selected from a series of histamine analogues with reduced side-chain flexibility, but no data (at Inserm) on this series has been published so far. The compound is very weakly active at the H_1 and H_2 receptors.

Incidentally, the effect of thioperamide at receptors other than H_3 receptors is reported[30.] to show only very weak affinity in the relevant in vitro tests at noradrenaline α_2 (pK_B = 5.2) and $GABA_A$ (pK_B = 5.2) receptors, and slightly higher affinity for 5-HT_3 (pK_i = 6.2) and dopamine D_1 (pK_i = 6.0) receptors. Thioperamide is therefore at least 150 times more potent at H_3 receptors than at any other receptor tested by Butler, Burridge and Kilpatrick.[30.]

The position of imidazole substitution in the piperidine ring appears to be important since the 3-isomer of thioperamide is reported to be about 10 times less active (see Table 1.4.).

The urea analogue of thioperamide is reported[8.,33.] to be about eight times less potent.

Amide derivatives of histamine (based on ~~the endogenous ligand~~ N^{α}-acetylhistamine, K_i = 1.4 x10^{-6}M) have also been reported[56.] to be H_3 receptor antagonists , as shown in Table 1.4. The compounds shown are the best of a series, optimizing the chain length

(ranging from 2 to 4 methylene groups and also incorporating ether and thioether linkages) between the amino nitrogen and the lipophilic group (including phenyl, cyclohexyl, more hydrophilic moieties such as imidazol-4-yl and 2-pyridine, and the bulky diphenyl group).

The amidic type of functionality does not seem to be absolutely necessary, since the phenoxyethylpiperidine analogue of thioperamide was also reported[8.,33.] to have activity, as had N^{α}-phenylalkyl-histamines[56.] with the propyl and butyl chains being optimal (see Table 1.4., further modifications not shown include replacing a methylene in the ethyl chain with O or S, and substituting the phenyl ring with a p-chloro group, resulting in the same range of activity), although it appears likely that amines are generally less active than amides.

The urea and thiourea compounds[71.] shown in Table 1.4. do not show increased activity.

Efforts to exchange the histamine part for related structures[71.] seem to result in loss of receptor affinity. Molecules incorporating betahistine, a partial cimetidine structure, or R-(-)-α-methylhistamine were not able to exert H_3 antagonistic activity (see Table 1.4).

It is to be noted that H_3 autoreceptors appear to be chemically stereoselective[17.], with structural requirements different from those of H_1 and H_2 receptors. The H_3 agonists α,N^{α}-dimethyl histamine, and N^{α}-methyl-α–(chloromethyl) histamine showed pronounced stereoselectivity, (+) isomers being highly preferred at H_3 receptors. The (-) isomers were more potent at H_2 receptors, while H_1 receptors showed no preference. However, an impromidine derivative, sopromidine and its S(+) enantiomer, both acted as H_3 antagonists with similar potencies. The (-) isomer acted as an H_2 agonist, while the (+) isomer acted as an H_2 antagonist. See Table 1.5. below.

Table 1.4. Some more compounds reported to be H_3 receptor histamine antagonists.[5.,8.,10.,56.,71.]

Compound	K_i (nM)
Betahistine, 2-Pyr-$(CH_2)_2NHCH_3$	6900
Phencyclidine, Cyclohexyl-1-(N-piperidine)-1-phenyl	25000
N-cyclohexyl-3-(imidazol-4-yl)-1-piperidinecarbothioamide	40
$ImCH_2CH_2NHCO(CH_2)_3Ph$	80
$ImCH_2CH_2NHCO(CH_2)_3C_6H_{11}$	50
Im-4-piperidine-N-CH_2CH_2OPh	67
$ImCH_2CH_2NH(CH_2)_mPh$, m = 2	3200
m = 3	670
m = 4	700
m = 5	2200
$ImCH_2CH_2NHCONH(CH_2)_2Ph$	240
$ImCH_2CH_2NHCSNH(CH_2)_2Ph$	350
2-Pyr-$CH_2CH_2(NCH_3)CO(CH_2)_3C_6H_{11}$	>10000
5-Methyl-imidazol-4-yl-$CH_2CH_2NHCO(CH_2)_3C_6H_{11}$	>10000
Im-$CH_2CH(CH_3)NHCO(CH_2)_3Ph$	1100

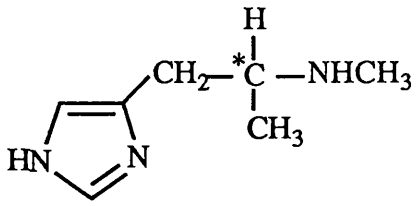
N^{α}-methyl analogues of histamine as H_3 agonists are very active, see Table 1.3. So far the most active H_3 agonist was (R)-α-methylhistamine, showing a stereoselectivity much more pronounced at the H_3 receptor than at either the H_1 or the H_2 receptor. It is intriguing that both α-methyl and N^{α}-methylhistamine are potent H_3 agonists, whereas the combination of both substitutions (α,N^{α}-substituted histamine derivatives) leads to weak compounds. Recently, the potency of several α/β-mono- and dialkylated side chain branched histamine derivatives was investigated[55.] resulting with the emergence of the most active compound αR,βS-dimethylhistamine (1800% relative to histamine)

which is very stereoselective at the H_3 receptor and highly receptor specific (H_3 vs. H_2/H_1).

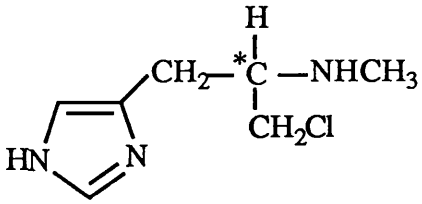
Table 1.5. Potencies of chiral histamine derivatives for the inhibition of rat cortical [^{3}H]histamine release (H_3 receptors), the stimulation of guinea-pig ileum contraction (H_1 receptors) and the stimulation of atrial rate (H_2 receptors).[17.,7.]

Agents	H_3	H_1	H_2
Agonists at the H_3 autoreceptor (relative potencies, RP)			
Histamine	100	100	100
S(-)α,N$^{\alpha}$-dimethylhistamine	0.13	0.7	1
R(+)α,N$^{\alpha}$-dimethylhistamine	4.1	0.7	0.4
R(-)N$^{\alpha}$-methyl-α-chloromethylhistamine	0.006	0.3	51
S(+)N$^{\alpha}$-methyl-α-chloromethylhistamine	1.1	0.3	7
(R)-α-methylhistamine	1550	0.49	1.02
(S)-α-methylhistamine	13	0.49	1.74
Antagonists at the H_3 autoreceptor (K_i values)			
Sopromidine (R(-)enantiomer)	6×10^{-8}M		(RP =740)
S(+)enantiomer	4×10^{-8}M		2×10^{-6}M

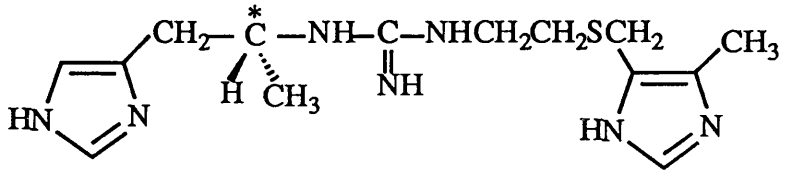
α,N^{α}-dimethylhistamine



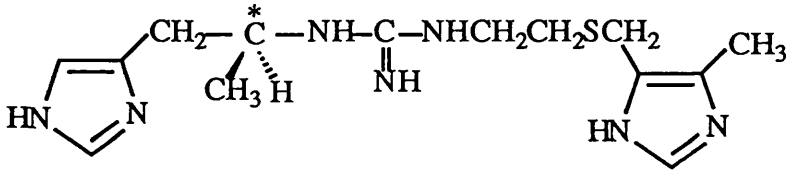
N^{α}-methyl-α-chloromethylhistamine



Sopromidine [R-(-)]



S-(+)-enantiomer of sopromidine



H_3 histamine receptor localization and possible functions

H_3 histamine receptors were first reported[12] in 1983 to be found in rat cerebral cortex. Five years later, human cerebral cortex (removed during neurosurgery) subjected to equivalent experiments[6] showed that H_3 receptors also control histamine release in the human brain since the potencies of the agonist histamine and the H_3 receptor antagonists impromidine and thioperamide were similar to the results obtained with rat cerebral cortex. This suggests that there may well be therapeutic applications arising in the future.

Histamine systems are postulated[7] to be involved in the control of states of sleep and wakefulness, cerebral circulation, energy metabolism and hypothalamic hormone secretion.

It has been suggested that facilitation of histaminergic neurotransmission via blockade of H_3 receptors may have a waking effect, promote increased blood flow and energy metabolism and modify the secretion of pituitary hormones, whereas stimulation of H_3 receptors could cause sedation, have anti-convulsant effects and diminish microvessel permeability. H_3 receptor antagonist and agonists therefore present tremendous therapeutic potential.[65.,67]

The presence of H_3 receptors in peripheral tissues has also been reported, where they also appear to be involved with the nervous system. For instance, histamine depresses sympathetic neurotransmission in the guinea pig mesenteric artery by interacting with H_3 receptors on the perivascular nerve terminals[51], which suggests that histamine may control the release of other neurotransmitters.[73] The H_3 receptors found in the guinea pig ileum appear to modify the amount of histamine-mediated contraction rather than affecting histamine release.[77] The discovery of H_3 receptors in the guinea pig lung[7] raises the question of whether they control histamine release in anaphylaxis and whether they may be manipulated to provide therapy in asthma. It has been suggested[18] that H_3 receptors are present on airway nerves and that (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 completely blocked by thioperamide. H_3 receptors are further said to modulate non-adrenergic non-cholinergic neural bronchoconstriction and neurogenic microvascular leak. They are also said to play a modulatory role in allergen-induced histamine release in the lung, since thioperamide markedly potentiates the bronchoconstrictor effect of i.v. allergen in sensitized guinea pigs, which is largely due to histamine release, suggesting that there are inhibitory H_3 receptors on pulmonary mast cells.

It has been shown[15] that (R)-α-methylhistamine in cat gastro-intestinal (GI) tract reduces secretions and motility, and in the respiratory tract reduces histamine synthesis and inhibits bronchoconstrictions mediated by release of cholinergic and peptidergic transmitters. Due to its claimed[15] very low animal toxicity, (R)-α-methylhistamine is under going clinical trials. It is well tolerated by humans and it is hoped that it will help to define H_3 receptor involvement in various diseases.

H_3 histamine receptor visualization

H_3 histamine receptors have been labelled using rat brain and guinea pig lung.[7] In binding assays with [^{3}H]-(R)-α-methylhistamine K_i values have been found which were 5-10 times lower than the EC_{50} values from functional studies, a possible explanation having been shown recently[16], i.e. the H_3 receptor exists in two different affinity states. Like many 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 guanyl nucleotides and Ca^{2+}. A potent radiolabelled antagonist would constitute a 'safer' probe, since it cannot be excluded that [^{3}H] (R)-α-methylhistamine labels more than a single H_3 receptor subtype.
Alternative binding conditions have been suggested[53] resulting in improved specific binding >90% of total binding of [^{3}H] (R)-α-methylhistamine.

Using [^{3}H] (R)-α-methylhistamine[7] the label was found mainly in the rat cerebral cortex, with a low H_3 receptor density (30 ± 3 fmol/mg of protein), much lower than for H_1 and H_2. See Photograph 1.1.

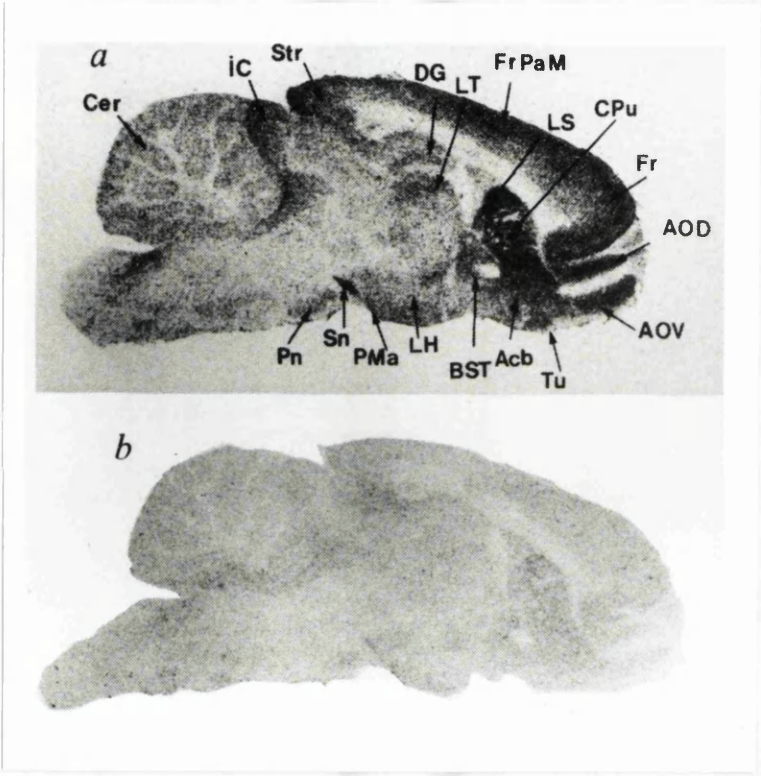
The telencephalic areas show the highest grain densities, the cerebellum shows fainter labelling and the hypothalamus shows a thin band of dense labelling.

The receptor density is (5 ± 2 fmol/mg of protein) in guinea pig lung tissue.

Photograph 1.1.7. Autoradiographic visualization of H_3-receptors in rat brain using [^{3}H](R)α-MeHA (sagittal sections).

a, Total binding in the presence or 1nM [^{3}H](R)α-MeHA.

b, Non-specific binding obtained by addition of 1 μM thioperamide in the incubation medium.



Note the low and rather uniform distribution of autoradiographic grains in *b*. Abbreviations: Acb, Accumbens nucleus; AOD, anterior olfactory nucleus, dorsal; AOV, anterior olfactory nucleus, ventral; BST, bed nucleus of stria terminalis; Cer, Cerebellum; CPu, caudate putamen; DG, dentate gyrus; Fr, frontal cortex; FrPaM, frontoparietal motor cortex; IC, inferior colliculus; LH, lateral hypothalamic area; LS, lateral septum; LT, lateral thalamus; PMa, perimammillary area; Pn, pontine nuclei; Sn, substantia nigra; Str, striate cortex; Tu, olfactory tubercule.

The search for potent H_3 antagonists

Not much is published in the literature about H_3 receptors and its active ligands. A total of twenty four references[5.,6.,7.,8.,9.,10.,11.,12.,13.,14.,15.,16.,17.,18.,30.,49.,51.,53.,55.,56.,66.,71.,74.,80.] which include seven posters were found since the original publication by Schwartz and his group in 1983.
The other publishing groups involved in the synthesis of potential H_3 active compounds are those of Schunack in Berlin and Robba in Caen who collaborate with Schwartz in Paris, and Timmerman in Amsterdam who have their own testing system.

Generally, when searching for an active drug, it is important to be aware of the forces involved in drug-receptor interaction.[4.] These include : electrostatic attraction (involving ions and dipoles), hydrogen bond formation (involving proton-donating and -accepting groups), van der Waals dispersion forces (involving non-polar molecules in close proximity) and hydrophobic binding forces (based on the escape of hydrophobic parts of the molecule from the highly polar water medium under formation of aggregates with other hydrophobic groups such that the biological medium is a positive factor with respect to the binding energy gained from the hydrophobic bond formation). The spatial arrangement of the molecule may also be important, with factors such as location, size, volume and charge of particular groups (e.g. bulky substituents causing steric hindrance, or a lone pair of electrons affecting orientation of the molecule).

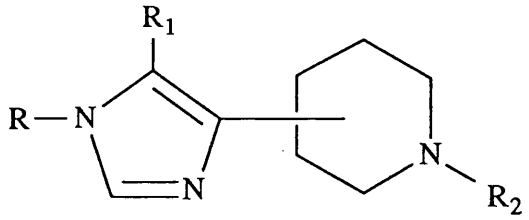
In an effort to find potent H_3-antagonists, two types of chemical starting points have been used in the past : known H_1 or H_2 receptor ligands, and the histamine molecule.

There are various problems associated with using known H_1 or H_2 receptor ligands as chemical starting points for suitable antagonists of histamine at cerebral H_3 receptors able to cross the blood-brain barrier. The lipophilic H_1 receptor antagonists tested showed negligible affinity. Several agonists at H_1 (e.g. betahistine, agonist potency relative to histamine for guinea pig ileum contraction is 8%) or at H_2 (e.g. impromidine), as well as

H_2 antagonists (e.g. burimamide), displayed significant antagonist activity, see Tables 1.3. and 1.4. However, these are generally highly hydrophilic and/or positively charged molecules with little ability to enter the brain from the circulation.[83.]

Another approach[7.], entails using the histamine molecule as the chemical starting point. The action of histamine self-inhibiting release from depolarized rat cerebral cortex is mimicked closely by related imidazole derivatives (such as R-(α)-methylhistamine, H_3 agonist, 15 times more potent than histamine itself), and antagonized in a competitive manner by a series of compounds.[13.]

Analogues of histamine have been synthesized by Schwartz and his associates, in which the ethylamine side chain was extended and its nitrogen atom included in a piperidine ring. This resulted in a loss of agonist activity. The affinity of compounds for H_3 receptors was progressively optimized by varying the nature of the piperidine substituting group. The general formula of the over 70 potential H_3 receptor antagonists synthesized[8.] is :



where R_1 = Me, Et, H

R = H, R_2

R_2 = alkyl groups, piperonyl, (benzimidazolyl-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, $CH-C_6H_5-R_3$

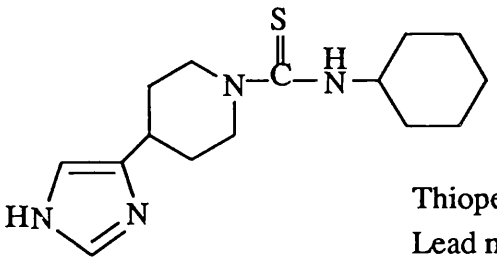
R_3 = H, Me, halogen, CN, CF_3, COR_4

R_4 = alkyl, cycloalkyl, phenyl (Me, F substituted)

Z = O, S, NH, NH-Me, N-CN

R_5 = alkyl, cycloalkyl, (phenyl substituted, alkyl substituted), phenyl (Me, halogen or CF_3 substituted), phenyl-alkyl, napthyl, adamantyl, p-toluenesulphonyl.

The most potent H_3 antagonist designed was N-cyclohexyl-4-(imidazol-4-yl)-1-piperidine carbothioamide, whose structure is shown below :



Thioperamide **(34)**
Lead molecule, $K_i = 4.3 \times 10^{-9}$ M

Thioperamide is inadequate for in vivo and human use for two main reasons.

Firstly, it is said to have a toxic effect (shown during in vivo testing at Inserm), presumably because of the thiourea group. When metiamide, the H_2 antagonist, caused reversible granulocytopenia (reduction of the number of circulating white cells in the blood) in a small number of patients[43], it was considered that this effect could be related to the presence of the thiourea group in metiamide.

Secondly, brain penetration of thioperamide is presumably inadequate. It was observed at Inserm that very high doses were needed for an in vivo effect from this compound which is very potent in vitro. This is probably because of its high polarity. Brain penetration should be improved by reducing overall hydrogen bonding ability. This is based on the physicochemical model for brain penetration as has been applied by Ganellin et al[83] to the design of centrally acting H_2 receptor histamine antagonists. The

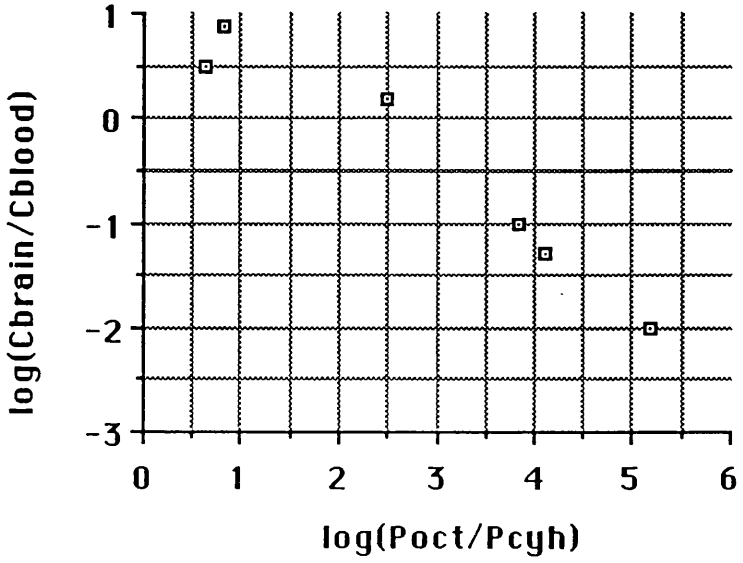
model shows a good correlation between the logarithms of the equilibrium brain/blood concentration ratios in the rat, and the partition parameter Δ log P, defined as log P (1 - octanol/water) - log P (cyclohexane/water). Δ log P is related to the overall H-bonding ability of a compound by the following equation :

$$\Delta \log P = \log P_{oct} - \log P_{cyh} = \Sigma I_H - b$$

where I_H denotes the additive increment to H-bonding by a molecular fragment and b is a constant.

See Figure 1.4., which shows that brain penetration will be increased by lowering the overall hydrogen bonding ability of a compound and is in accordance with Overton's rules relating to the effects of strong H-bonding groups on the ability of compounds to cross cell membranes.

Fig. 1.4.[83] Relationship between brain penetration and Δ logP for three H_2 antagonists and three compounds that readily cross the blood-brain barrier, e.g. mepyramine.



It should be possible to reduce hydrogen bonding ability either by removing polar groups not essential for antagonist activity or by reducing the polarity of groups considered necessary for antagonist activity. The latter might be accomplished by encouraging intramolecular hydrogen bonding, shielding with non-polar groups, making less polar labile derivatives (i.e. prodrugs) of an active polar compound, or making analogues having inherently lower hydrogen-bonding ability.

My work involves continuing the optimization of thioperamide. Thus, different series of compounds were made incorporating specific types of structural changes, usually based on attempts that would lead to reduction of toxicity and/or improved brain penetration.

It is clear that there is much to be explored in examining structure activity relationships for H_3 receptor antagonists, although it remains to be seen whether or when a drug will become established for a therapeutic application.

CHAPTER TWO.

SELECTION OF COMPOUNDS.

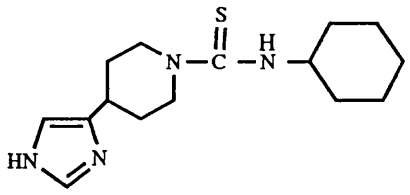
Thirty three compounds were synthesized and submitted for testing as potential H_3 receptor antagonists. Twenty four of these are novel. A further three were synthesized but not submitted for testing so far due to severe purification difficulties. The syntheses called for the development of two novel multi-step synthetic schemes to obtain starting material.

The leads used in this work, as a guide in the selection of compounds include H_3 antagonists (mainly the most potent lead, thioperamide **(34)**, but also weaker antagonists such as 1-(imidazol-4-yl-ethyl)-3-(phenyl)-propanamide **(35)**, N-4-butylphenyl-2(imidazol-4-yl)ethylamine **(42)**, and betahistine [N-methyl-2(pyridin-2-yl)ethylamine]) and a very potent agonist S-(2-imidazol-4-yl)ethyl)isothiourea **(48)**.

Searching for a non-toxic, brain-penetrating, active H_3 receptor antagonist, entailed structurally modifying the leads, and also the 'follow-up' molecules obtained in many ways, including : removing the heterocycle, replacing the heterocycle with an alternative heterocycle or other groups, making the molecule more flexible or rigid, changing the chain length, adding new substituents, replacing functional groups with other ones, reversing the polarity of the molecule, increasing lipophilicity, adding bulky substituents, i.e. changing the physical and chemical characteristics of the molecule. The changes were selected with the aim of exploring the relationship of activity, affinity and binding of the H_3 receptor antagonists and agonists, in order to obtain information on the structural requirements for an antagonist at the H_3 histamine receptor.

Three tables of the compounds synthesized for testing are shown below.

See Tables 2.1., 2.2. and 2.3.



Thioperamide

(34)

Using thioperamide (structure **(34)** shown above) as the direct lead (K_i = 4.3 x 10^{-9}M), the importance of the imidazole group was investigated by removing it, and substituting the 4-piperidine position with a range of substituents of different polarities. This modification was desirable since reducing the hydrogen-bonding centres in the molecule should improve brain penetration[83], which is a problem with thioperamide (as indicated by the relatively high in vivo dose required, despite the high in vitro potency, see Chapter Four) . The following seven compounds were made in this series :

N-(N'-cyclohexyl-thiocarbamoyl)piperidine (1);

N-(N'-cyclohexyl-thiocarbamoyl)morpholine (2);

N-(N'-cyclohexyl-thiocarbamoyl)piperid-4-one (3);

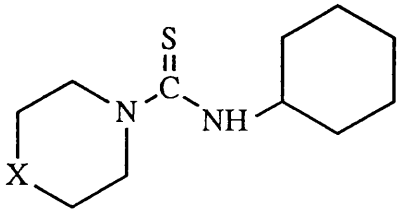
N-(N'-cyclohexyl-thiocarbamoyl)piperidine-4-ethanol (4);

N-(N'-cyclohexyl-thiocarbamoyl)piperidine-4-carboxylic acid hydrate(5);

N,N'-di(cyclohexyl-thiocarbamoyl)piperazine hydrate (6);

N-(N'-cyclohexyl-thiocarbamoyl)-4-(benzaldiminomethyl)-piperidine (7).

General structure of compounds **(1)** to **(7)** :



where X is CH_2, O, C=O, CH-CH_2CH_2OH, CH-COOH, N-C=S-NH-C_6H_{11} and CH-CH_2-N=CH-Ph, for compounds **(1)** to **(7)** respectively.

Table 2.1. Shows compounds (1) to (12).

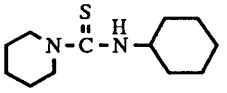
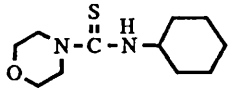
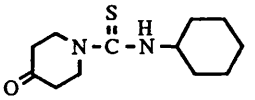
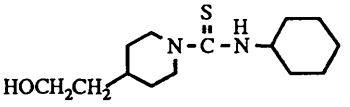
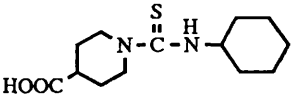
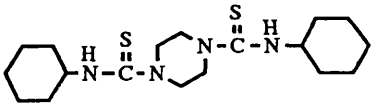
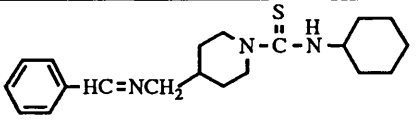
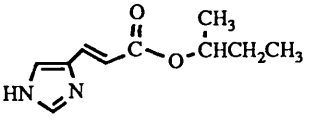
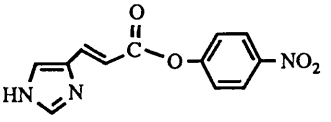
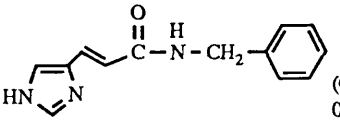
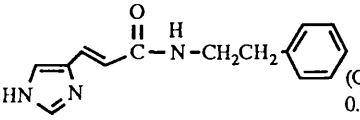
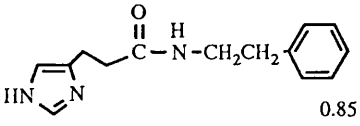
Cpd No.	COMPOUND		New	STRUCTURE
(1)	UCL1010	YK41C		
(2)	UCL1011	YK45A		
(3)	UCL1013	YK69B	*	
(4)	UCL1022	YK57D	*	
(5)	UCL1023	YK89B	*	
(6)	UCL1012	YK49C	*	
(7)	UCL1014	YK73C	*	
(8)	UCL1008	YK34D		 1.1 HCl 0.25 H_2O
(9)	UCL1009	YK37E	*	 HCl
(10)	UCL1028	YK115C	*	 $(CHCOOH)_2$ 0.25 H_2O
(11)	UCL1027	YK99I	*	 $(CHCOOH)_2$ 0.4 H_2O
(12)	UCL1063	YK218B	*	 0.85 $(COOH)_2$

Table 2.2. Shows compounds **(13)** to **(21)**.

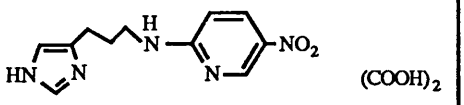
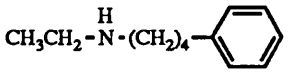
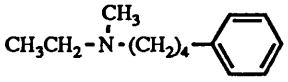
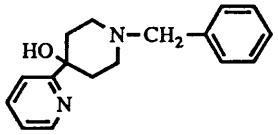
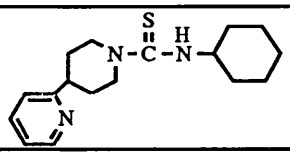
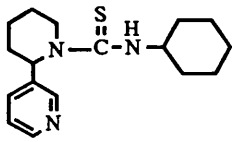
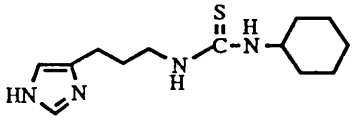
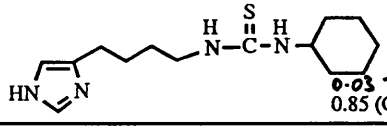
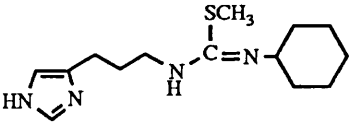
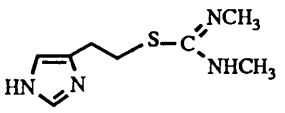
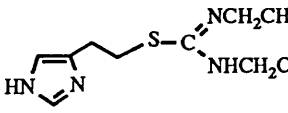
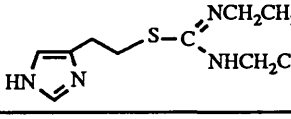
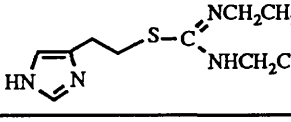
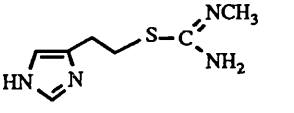
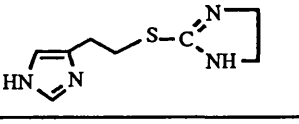
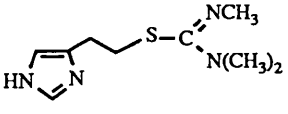
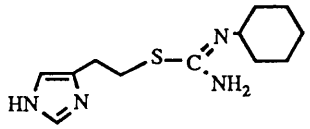
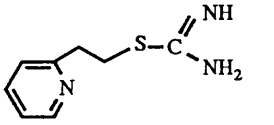
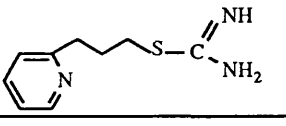
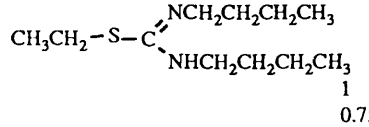
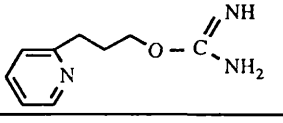
Cpd. No	COMPOUND		New	STRUCTURE
(13)	UCL1073-J	YK215C	*	 $(COOH)_2$
(14)	UCL1221-A	FS19C		 HCl
(15)	UCL1229-J	FS37C	*	 $(COOH)_2$
(16)	UCL1033	YK111F	*	 2 HCl
(17)	UCL1196	YK411B	*	 1.2 CF_3CO_2H 0.15 ^iPrOH
(18)	UCL1220-J	YK427B	*	 1.5 CF_3CO_2H 0.5 ^iPrOH
(19)	UCL1053	YK192B	*	
(20)	UCL1088	YK251F	*	 0.03 ^iPrOH 0.85 $(COOH)_2$
(21)	UCL1134-C_2	YK336C	*	 2 HI 0.5 H_2O

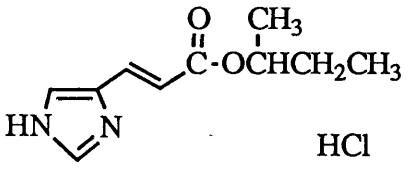
Table 2.3. Shows compounds (22) to (33).

Cpd. No.	COMPOUND		New	STRUCTURE
(22)	UCL1124	YK302C		 2 HBr
(23)	UCL1211-J_2	YK365E		 3 $(CO_2H)_2$ 0.03 ^{i}PrOH
(24)	UCL1192-J_2	YK403D	*	 2.25 $(CO_2H)_2$
(25)	UCL1176-J_2	YK369C	*	 2 $(CO_2H)_2$
(26)	UCL1123	YK298D		 2 HBr
(27)	UCL1133	YK314F		 2.4 HBr
(28)	UCL1140-B_2	YK332F	*	 2 HBr
(29)	UCL1209-J_2	YK392A	*	 2.5 CF_3CO_2H 0.2 ^{i}PrOH
(30)	UCL1151	YK373C		 2 HBr
(31)	UCL1152	YK377C	*	 2 HBr
(32)	UCL1213-J	YO16A	*	 1 $(CO_2H)_2$ 0.75 H_2O
(33)	UCL1208-A	YK423B	*	 2 HCl

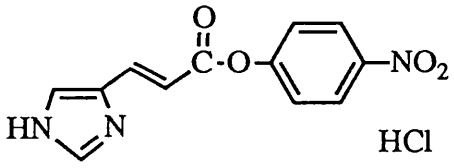
The semi-rigid piperidine ring of thioperamide (capable of boat-chair interconversions) was then replaced by a rigid trans double bond, and the cyclohexyl thiourea group was replaced with an alkyl (sec-butyl) and an aromatic (p-nitro-phenyl) ester function which would remove the toxicity effect of the C=S group observed by Schwartz et al at Inserm with thioperamide. This is presumably due to liver toxicity. Toxicity of a thiourea group containing compound has been observed previously with metiamide, which caused reversible granulocytopaenia.[43]. Replacing C=S by C=O is reasonable since sulphur is directly below oxygen in group VI of the Periodic Table; it has similar chemical properties, but is larger and more polarizable.

The following two compounds were made :

1-(1-methylpropyl)-3-(imidazol-4-yl)-2-trans-propenoate hydrochloride (8); p-nitrophenyl-3-(imidazol-4-yl)-2-trans-propenoate hydrochloride (9). The structures are shown below.



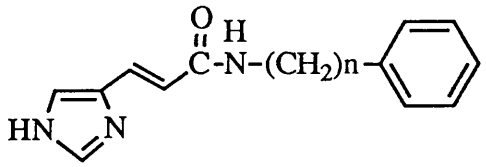
(8)



(9)

The effect of an amide instead of an ester function was investigated by making a benzyl and an ethyl phenyl amide :

N-benzyl-3-(imidazol-4-yl)-2-trans-propenamide maleate hydrate (10) and **N-(2-phenylethyl)-3-(imidazol-4-yl)-2-trans-propenamide maleate hydrate (11)**.



x $(CHCOOH)_2$
y H_2O

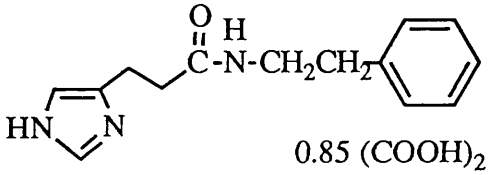
	n	x	y
(10)	1	1	0.25
(11)	2	1	0.40

N-(2-Phenylethyl)-3-(imidazol-4-yl)propanamide oxalate (12) was synthesized to follow up a question which arose when the following two compounds were compared :

Im CH = CH CO NH CH_2CH_2Ph **(11)**

Im CH_2 CH_2 NH CO CH_2CH_2Ph **(35)**

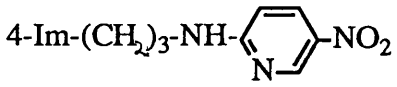
Although the compounds are isosteres, compound **(35)** (prepared by Schunack et al[56].) is considerably more active than compound **(11)**. See Chapter Four for activity results. It could be postulated that either the reverse amide nature or/and the ability of the compound to adopt the gauche form, accounts for its increased activity. In order to determine this, Im-CH_2CH_2-CO-NH-CH_2CH_2-Ph **(12)** was synthesized.



0.85 $(COOH)_2$ **(12)**

2-(3-(Imidazol-4-yl)propyl)-amino-5-nitro-pyridine oxalate (13) (shown below), may be considered an isostere of (**9**), in the sense that the relative position of the

nitro group to the imidazole ring is similar, even though the side chain in **(13)** is flexible and the ester function is replaced by an amino group.

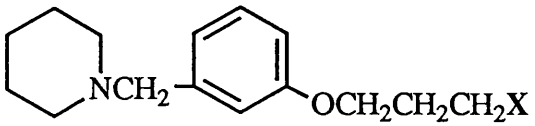


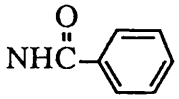
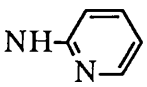
$.(CO_2H)_2$ **(13)**

At the time, **(13)** was synthesized because the work of my colleague Mr. S.K. Hosseini (who is carrying out research in the same field with Prof. C.R. Ganellin) on aminoheterocycles showed promising results, as shown by the example **(36)** below.

4-Im-$(CH_2)_2$-NH-(2-pyridine) **(36)** $K_i = 2.0 \pm 0.6 \times 10^{-7}M$

A precedent in replacing the amide function with an amino heterocycle (**(12)** to **(13)**) is represented by a stage in the course of development of H_2 receptor antagonists by Ganellin and co-workers.[83] When comparing the benzamide derivative to the aminopyridine derivative brain penetration increases remarkably, as shown below, and as predicted by their physicochemical model for brain pentration.



X	H_2 receptor antagonism (guinea pig atrium), K_B (M)	C_{brain}/C_{blood}
	1.62×10^{-8}	0.57
	8.13×10^{-7}	4.90

The amide moiety can act as both a strong hydrogen-bond donor and acceptor. Replacing this with an aminoheterocycle retains activity by keeping a hydrogen-bond-donor function required for activity in antagonists of this type, while minimizing overall hydrogen-bonding ability and thus increasing brain penetration.

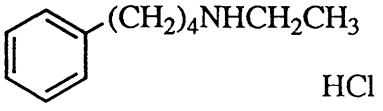
(13) could be compared to **(55)** and **(56)** , prepared by Mr. S.K. Hosseini.

Im-$(CH_2)_2$-NH-2(5-nitro)pyridine	**(55)**	$K_i = 2.9 \pm 1.1 \times 10^{-8}M$
Im-$CH_2S(CH_2)_2$-NH-2(5-nitro)pyridine	**(56)**	$K_i = 1.3 \pm 0.2 \times 10^{-6}M$

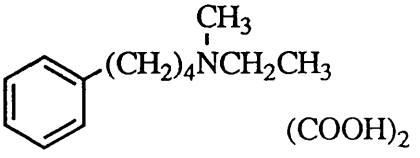
It was expected that extension of the methylene side chain would result in increased activity (a trend shown in the past in the development of the H_2 antagonist cimetidine[46]. and also more recently in H_3 antagonist work, see Chapter Four.

3(Imidazol-4-yl)propylamine (41) was required as a starting material, and a small quantity of this was obtained by courtesy of Mr. W. Tertiuk (**41**^{WT}), but it it was chosen to develop an independent synthetic route for the starting material, see Scheme 3.1. in Chapter Three. The novel scheme started with urocanic acid (**37**) which was converted to the propenamide (**39**) via the acyl chloride (**38**); the trans double bond was reduced to give the propanamide (**40**) which was then reduced to give the product amine (**41**).

Two more amines **N-ethyl-4-phenylbutylamine hydrochloride (14)**, and **N,N-ethylmethyl-4-phenylbutylamine oxalate (15)**



(14)



(15)

were examined, and compared directly with compound **(42)** which was prepared by Schunack et al[56]., since the structure in one case simply omitted the imidazole group, and

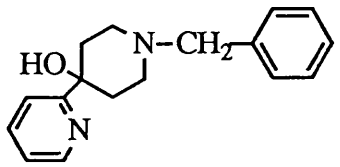
in the other case the amine was also methylated. These were made by a project student (UCL 1990), Miss F. Siddiqi.

$ImCH_2CH_2NH(CH_2)_4Ph$ **(42)** $K_i = 6.3 \times 10^{-7}M$

The effect of replacing the imidazole group in thioperamide **(34)** with 2-pyridine was considered worth investigating. It is known (as H_2 studies with cimetidine and analogues thereof have shown)[83] that imidazole can be replaced by other heterocycles often with retention of activity, provided that a pyridine-type N is present in a position ortho to the side chain. See Table 4.5. in Chapter Four for examples of H_3 affinity ratios for different pairs of 4-Imidazole and 2-Pyridine compounds.

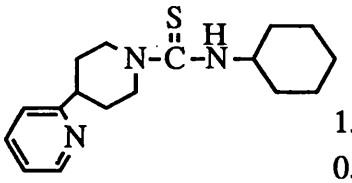
Pyridine itself is a useful replacement, as it provides only a H-bond acceptor, without the additional H-bond donor of the imidazole group, while also allowing antagonist activity to be retained. It is therefore hoped that replacing imidazole with pyridine will improve brain penetration. Furthermore, it is known[10] that betahistine acts as a histamine H_1 agonist (on guinea pig ileum), a weak partial H_2 agonist[25], and most importantly as an H_3 antagonist (K_i=6.9x10^{-6}M).

To synthesize **N-cyclohexyl-4-(2-pyridinyl)-1-piperidine-carbothioamide trifluoroacetate (17)** it was necessary to make the starting material **2-(4-piperidinyl)pyridine (47)**, and this resulted in the development of a novel multi-step synthetic scheme, see Scheme 3.2. in Chapter Three. The starting point was 1-benzyl-4-piperidone **(43)**, which was converted into **1-benzyl-4-(2-pyridinyl)-piperidin-4-ol hydrochloride (16)**. This intermediate was submitted for testing, just for interest.The tertiary alcohol was reduced **(44)**, the double bond hydrogenated **(45)**, and the debenzylation effected via the the 1-ethyl formate **(46)** to give the product amine **(47)**.



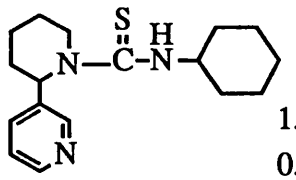
2 HCl

(16)



1.2 CF_3CO_2H
0.15 $^{i}PrOH$

(17)

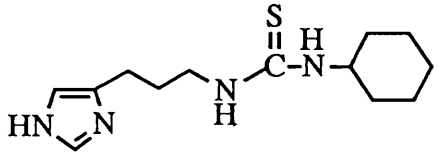


1.5 CF_3CO_2H
0.5 $^{i}PrOH$

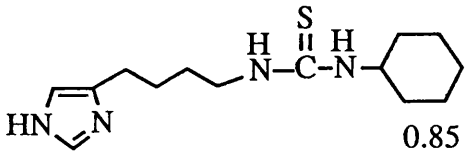
(18)

Another isomer of thioperamide **N-cyclohexyl-2-(3-pyridinyl)-1-piperidine carbothioamide trifluoroacetate (18)** was made, since 3-(2-piperidine)pyridine (anabasine) was commercially available. It may be noted that N-cyclohexyl-4-(pyridin-2-yl)-1-piperazine carbothioamide (UCL1002) synthesized by D. Gellert (UCL 3rd Year Project, 1986) was inactive as an H_3 antagonist (K_i >>10^{-5}M).

Replacing the piperidine group of thioperamide with propylamine and butylamine, resulted in two compounds : **N-(N'-cyclohexyl-thiocarbamoyl)-3-(imidazol-4-yl)propylamine (19), and N-(N'-cyclohexyl-thiocarbamoyl)-4-(imidazol-4-yl)butylamine oxalate (20).**



(19)



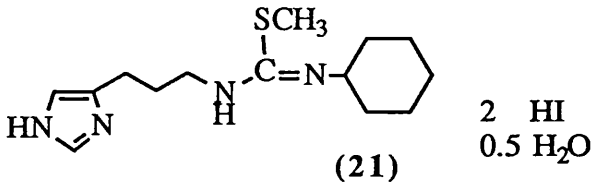
0.85 $(COOH)_2$

(20)

The chain of three methylene groups in **(19)** represents the same number of C-C bonds as in the piperidine ring of thioperamide **(34)** (although a longer distance in real space),

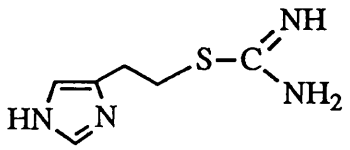
while the tertiary amine group has been replaced by a secondary one, and the semi-rigidity of the piperidine ring (capable of boat chair interconversions) has become more flexible.

The S in the propylamine analogue above **(19)** was methylated to give **S-methyl-N-cyclohexyl-N'-(3-imidazol-4-yl)propyl)isothiourea dihydroiodide hemihydrate (21).**



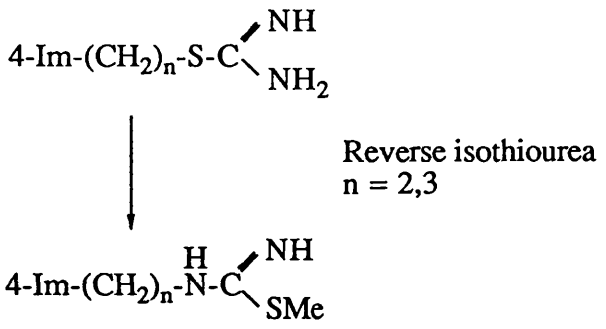
(21)

The reasoning behind this was as follows : S-(2-(imidazol-4-yl)ethyl) isothiourea dihydrobromide **(48)**, made by Mr. W. Tertiuk (sample WT133C/UCL1095-B_2) shown below,



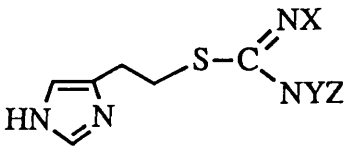
(48)

was found to be a very potent H_3 agonist, or 'superagonist' with EC_{50} = 1 x 10^{-9} M, i.e. 5-10 x potency of R-(α)-methyl histamine or 75-150 x potency of histamine itself. On the way to discovering cimetidine[46], this same super H_3 agonist and its propyl equivalent were tested for H_2 antagonism. Reversal of the isothiourea (side chain N instead of S) shown below,



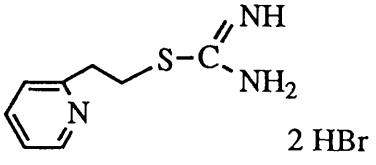
caused decrease for n = 2, and increase for n = 3 in H_2 antagonist activity. It was therefore considered interesting to synthesize and test a reverse isothiourea, this being compound **(21)**.

Following up the 'superagonist' **(48)**, a group of eight S-ethyl-imidazol-4-yl isothioureas was made. The NH sites of the isothiourea group were blocked off in an effort to determine which were necessary for binding to the receptor.The importance of the N groups in binding was investigated by varying the substituents in size and lipophilicity. A range of n-alkyl groups, an ethyl bridge and a cyclohexyl group were used. By increasing the size of the alkyl groups, the importance of the hydrophobic effect on binding at the receptor could be investigated. The compounds made were : **N,N'-dimethyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dihydrobromide (22); N,N'-diethyl-S-(2-(imidazol-4-yl)ethyl)isothiourea trioxalate (23); N,N'-di-n-propyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dioxalate (24); N,N'-di-n-butyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dioxalate (25); N-methyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dihydrobromide (26); 2-(2-(imidazol-4-yl)ethyl)-thioimidazoline dihydrobromide (27); N,N,N'-trimethyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dihydrobromide (28) and N-cyclohexyl-S-(2-(imidazol-4-yl)ethyl)isothiourea ditrifluoroacetate (29).**

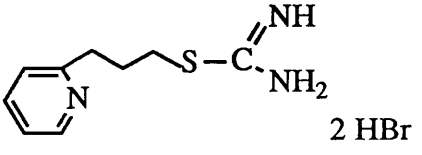


Compound	X	Y	Z	Salt
(22)	Me	H	Me	2 HBr
(23)	Et	H	Et	3 $(COOH)_2$, 0.03 ^{i}PrOH
(24)	n-Pr	H	n-Pr	2.25 $(COOH)_2$
(25)	n-Bu	H	n-Bu	2 $(COOH)_2$
(26)	Me	H	H	2 HBr
(27)	CH_2	H	CH_2	2.4 HBr
(28)	Me	Me	Me	2 HBr
(29)	C_6H_{11}	H	H	2.5 CF_3COOH, 0.2 ^{i}PrOH

S-(2-(2-Pyridinyl)ethyl)isothiourea dihydrobromide (30), and **S-(3-(2-pyridinyl)propyl)isothiourea dihydrobromide (31),** were prepared to investigate the effect of replacing 4-imidazole with 2-pyridine on the same principle as discussed earlier, and in addition extending the length of the methylene chain in **(48)**.

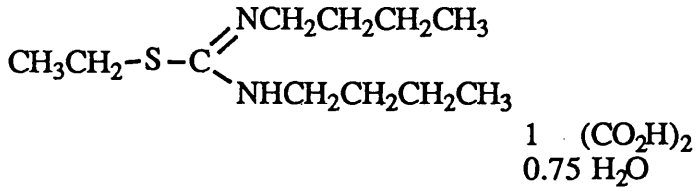


(30)



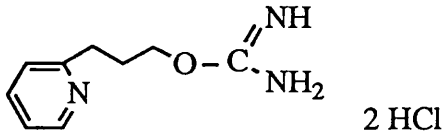
(31)

The effect of removing the imidazole group from the most potent isothiourea antagonist synthesized was investigated, by synthesizing **N,N'-di-n-butyl-S-ethyl isothiourea oxalate (32)**. This was made by a project student (UCL, 1990), Miss Y. Ohtake.

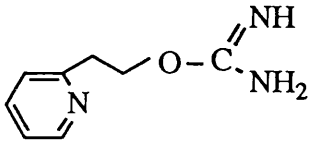


(32)

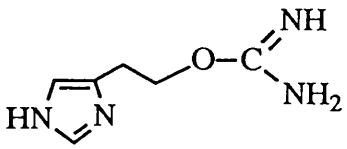
Removing the potentially toxic S group from the isothioureas, and replacing it by the similar sized, but presumably more innocuous O group made the synthesis of some analogous isoureas desirable. Four compounds were made : **O-(3-pyridin-2-yl-propyl)isourea dihydrochloride (33), O-(2-pyridin-2-yl-ethyl)isourea (51)** (sample YK418C), **O-(2-imidazol-4-yl-ethyl)isourea (52)** (sample YK406C), **N,N-di-methyl-O-(2-imidazol-4-yl-ethyl)isourea (53)** (sample YK414A), but only the first of these could be purified to such a degree as to allow testing. Isoureas are unstable to base, and in some cases, to prolonged standing at room temperature. This made adequate purification very difficult. The isourea structures are shown below.



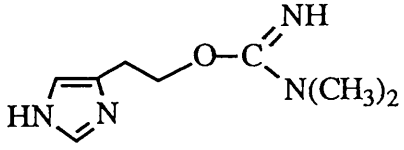
(33)



(51)



(52)



(53)

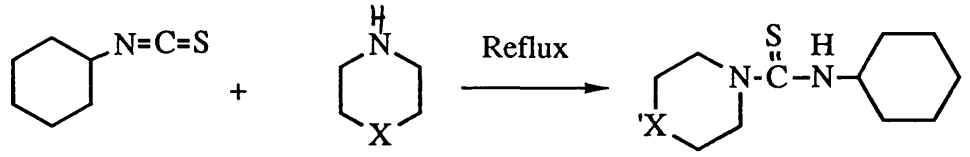
CHAPTER THREE.

SYNTHESIS.

Syntheses can be divided into two types: those leading directly to a compound submitted for testing, and those yielding an intermediate to such a compound, in particular the two major novel schemes for 4-(2-pyridinyl)piperidine and 3-(imidazol-4-yl)propylamine.

The compounds were identified by the standard spectroscopic (UV, IR, NMR, MS) and chromatographic (TLC, usually HPLC), and physical (melting point when solid, and elemental analysis) methods. Details are given in Chapter Five.

Synthesis of **N-(N'-cyclohexyl-thiocarbamoyl)piperidine**[40.,75.] **(1); N-(N'-cyclohexyl-thiocarbamoyl)morpholine**[72.,75.] **(2); N-(N'-cyclohexyl-thiocarbamoyl)piperid-4-one (3); N-(N'-cyclohexyl-thiocarbamoyl)-4-(2-hydroxyethyl)piperidine (4); N-(N'-cyclohexyl-thiocarbamoyl)piperidine-4-carboxylic acid hydrate (5) and N,N'-di(cyclohexyl-thiocarbamoyl)piperazine hydrate (6)** followed the general method[8.] of refluxing the reactants in toluene. The compounds are new except for (1) and (2). N-(N'-Cyclohexyl-thiocarbamoyl)piperidine has been made by Tisler[75.] who performed the reaction in Et_2O with an 82-90% yield, and by Dupin and Pesson[40.] who performed the reaction in petroleum ether with a 99% yield. N-(N'-Cyclohexyl-thiocarbamoyl)morpholine has been made by Tisler[75.] as above, and by Takahashi et al[72.] who heated the reactants neat with a 70-80% yield. The general reaction and particular conditions followed for the compounds is shown below.



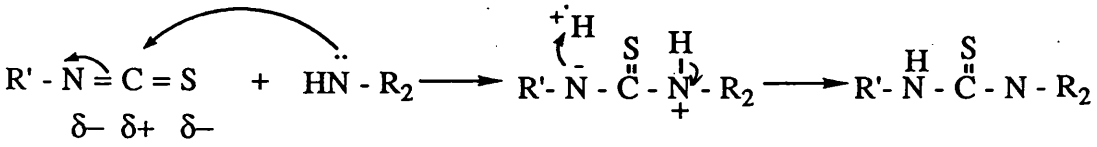
X	'X	Product	Conditions	Yield(overall crude)
CH_2	CH_2	**(1)**	30 mins reflux in toluene	84%, 73% after recrystallization
O	O	**(2)**	30 mins reflux in toluene	91%
$C(OH)_2$	C=O	**(3)**	1 hour reflux in methanol	44%
$CH(CH_2)_2OH$	$CH(CH_2)_2OH$	**(4)**	30 mins reflux in toluene	88%
CHCOOH	CHCOOH	**(5)**	5 hours reflux in $EtOH/H_2O$	30%
N	N-CS-NH-C_6H_{11}	**(6)**	30 mins reflux in toluene, 1/2 mol equivalent piperazine	100%, 37% after recrystallization

Synthesis of compound

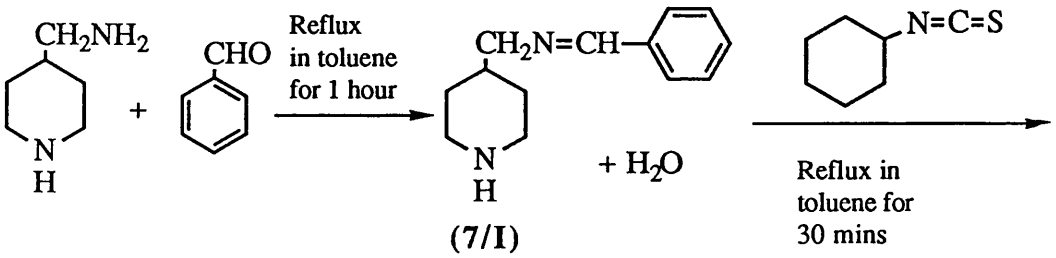
(3) involved converting the HCl salt to base using $Na^{+-}OMe$, then refluxing in MeOH. The starting material 4-piperidone was available in the monohydrated form (gem-diol), but the product (3) was stable in the ketone form. The unusual stability of the gem diol form is probably due to the sp_3 hybridized carbon being unstrained in the ring with bond angles of 109.5°. The energy gained on dehydration probably more than compensates for the sp_2 carbon causing strain in the ring with bond angles of 120° . Due to solubility

problems (5) was synthesized in a heterogenous reaction mixture of aqueous ethanol. Compound (6) required a twofold amount of cyclohexylisothiocyanate, since piperazine has two amine functions.

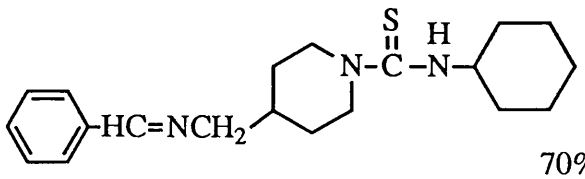
The general mechanism is nucleophilic attack on the isothiocarbonyl carbon by the amine as shown below.



Synthesis of **N(4'-cyclohexylthiocarbamoyl)-4-(benzaldiminomethyl)-piperidine** (7) involved refluxing 4-piperidylmethylamine with benzaldehyde in toluene using a Dean-Stark trap for 1 hour to give the intermediate Schiff's base or imine (7/I), followed by the coupling with cyclohexylisothiocyanate by refluxing in toluene for 30 minutes using the general method[8] as before. The reaction equation is shown below.



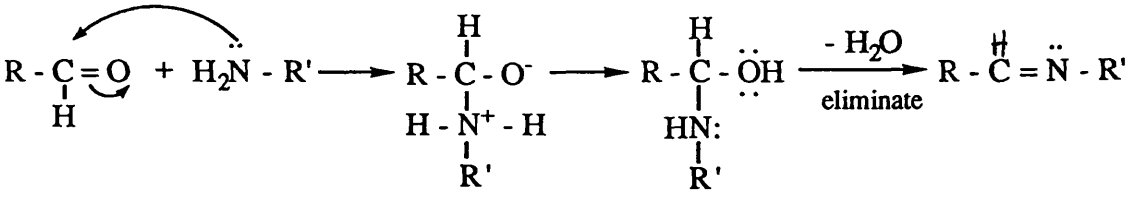
(7/I)



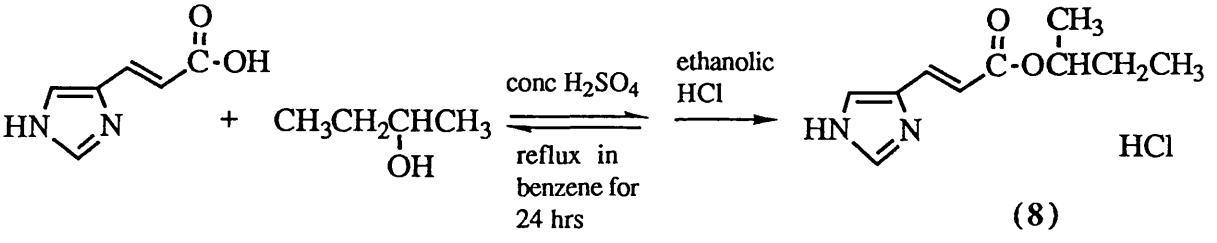
70% overall crude yield

(7)

Mechanism involved nucleophilic attack at the carbonyl carbon by the amine, as shown below.

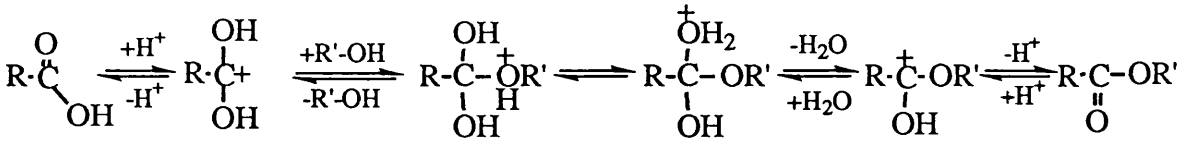


Synthesis of **(2-butyl)-3-(imidazol-4-yl)-2-trans-propenoate hydrochloride (8)** followed the method of Burger et al[29], who prepared the base by refluxing a mixture of trans-urocanic acid, 2-BuOH, C_6H_6 and a catalytic amount of concentrated H_2SO_4 using a Dean-Stark trap for 24 hours. Problems were encountered when attempting to distil the base according to the literature[29], i.e. charring and foaming, and the HCl salt proved to be excessively hygroscopic. The reaction involved acid catalyzed esterification, using an excess of alcohol and removing H_2O formed in the Dean-Stark trap to keep the equilibrium in favour of ester formation. The reaction equation is shown below.

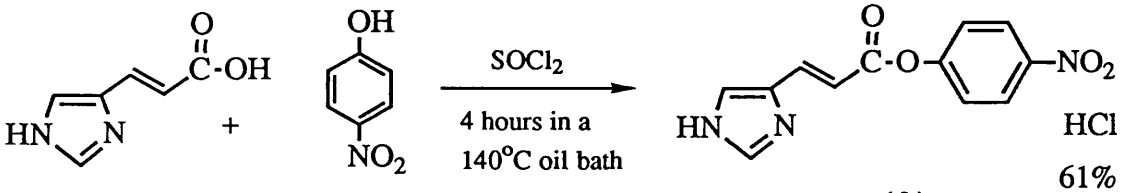


(8)

The mechanism probably involves a tetrahedral intermediate[59] involving acyl-oxygen cleavage, as shown below.

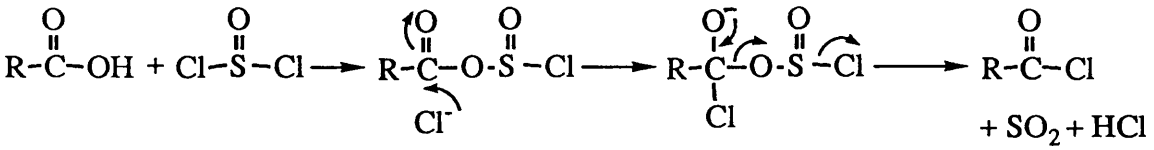


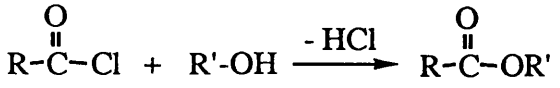
Synthesis of **p-nitrophenyl-3-(imidazol-4-yl)-2-trans-propenoate hydrochloride** (**9**) followed the method of Bruice and Sturtevant[28], who prepared an analogous compound, p-nitrophenyl-γ-(imidazol-4-yl)-butyrate hydrochloride, by reacting a mixture of p-nitrophenol, γ-(imidazol-4-yl)-butyric acid hydrochloride and thionyl chloride in an oil bath at 40°C for 24 hours. In the case of (**9**) it was found necessary to raise the oil bath temperature to 140°C to facilitate reaction. The reaction equation is shown below.



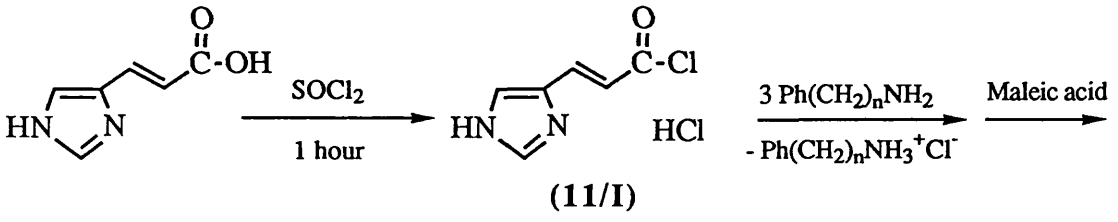
(**9**)

The reaction involved acyl chloride formation from the acid using thionyl chloride and subsequent ester formation by nucleophilic attack at the acyl carbon, the mechanism is shown below.

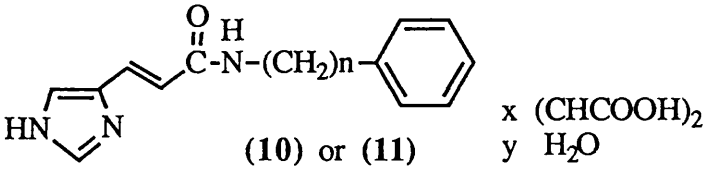




Synthesis of **N-benzyl-3-(imidazol-4-yl)-2-trans-propenamide maleate hydrate (10)** and **N-(2-phenylethyl)-3-(imidazol-4-yl)-2-trans-propenamide maleate hydrate (11)** followed the equation and conditions shown below.



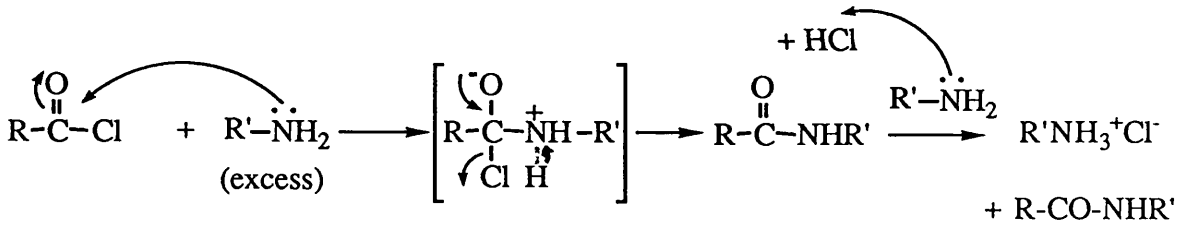
(11/I)



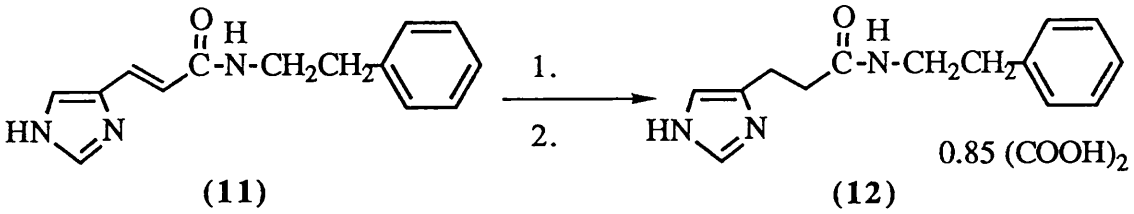
(10) or **(11)**

Compound	n	x	y	Conditions	Yield(crude overall)
(10)	1	1	0.25	3 hours stir at room temperature in CH_2Cl_2	79% base, 47% oxalate
(11)	2	1	0.4	2.5 hours stir at room temperature in $CHCl_3$	92% base, 23% oxalate

An attempt to make the amide from the p-nitrophenyl ester hydrochloride (**9**) and the amine according to Bruice and Sturtevant[28]. who made the butyramide using chloroform saturated with ammonia, proved consistently unsuccessful (room temperature reaction in $CHCl_3$ gave only the hydrochloride salt of phenethylamine; heating under reflux in $CHCl_3$ gave urocanic acid as the product; a further attempt to couple the acid directly with the amine using dicyclohexylcarbodiimide in dimethylfuran also resulted in urocanic acid). Instead, the acyl chloride was obtained from the acid using thionyl chloride as for (**9**). Nucleophilic substitution at the acyl carbon using benzylamine and phenethylamine respectively, produced the amides, which were purified as maleic acid salts. The excess amine served to convert the acyl chloride HCl salt into base during the reaction. Mechanism of the reaction is shown below.



Synthesis of **N-(2-phenylethyl)-3-(imidazol-4-yl)-propanamide oxalate (12)** followed a general procedure by Deulofen et al[37]., but it was eventually established that the reaction required a large excess of Na/Hg amalgam (20-30 molar equivalents of Na). The reaction equation is shown below.

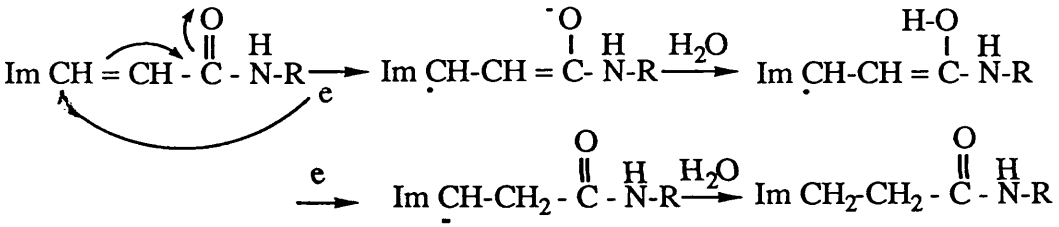


(11) **(12)**

96% crude base
95% crude oxalate

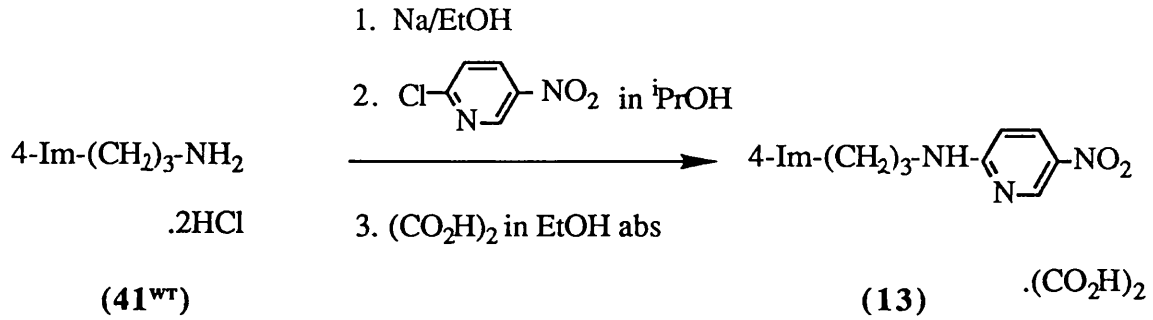
1. Na/Hg, 2.3% amalgam, stir at room temperature for 1 hour, in H_2O
2. Oxalic acid

Mechanism is by direct electron transfer :



where R = CH_2CH_2Ph

Synthesis of **2-(3-(imidazol-4-yl)propyl)-amino-5-nitro-pyridine oxalate (13)** involved liberation of the base using sodium ethoxide, followed by refluxing the 3-(imidazol-4-yl)propylamine (**41**^{WT}) (sample WT76/4396A) provided kindly by Mr. W. Tertiuk) with 2-chloro-5-nitropyridine in isopropanol for 21 hours. Yield after column chromatography was 62%. Purification was continued by formation of the oxalate salt. The reaction equation is shown below.



(41^{WT}) **(13)**

nucleophilic aromatic substitution

A. A mechanism is involved with nucleophilic attack by the amine at the 2-pyridine position.

A novel synthesis (scheme 1) of **3-(imidazol-4-yl)propylamine (41)** was developed, since the supply of **(41^{WT})** ran out, and material seemed to be required for following up some of the most active compounds synthesized, i.e. **(21)** and **(19)**.

Different methods of synthesis have been reported, and these are discussed below.[2., 3., 22., 23., 42., 58.]

Novel synthetic scheme for **3-(imidazol-4-yl)propylamine (41)**

Previous syntheses have been reported, the first being that of Akabori and Kaneko[3.] in 1932, followed by Lure'e et al[58.] in 1939, then Black et al[22., 23.] in 1973, Schunack and Elz[42.] in 1986, and most recently, Adger and Surtees[2.] in 1986. A brief outline of each of the different syntheses follows.

Akabori and Kaneko[3.] converted arginine dihydrochloride to the ethyl ester by heating with HCl (3.5%) in absolute ethanol and benzene. This was treated with Na/Hg amalgam (2.3%) in water with HCl present. The intermediate aldehyde was treated with ammonium rhodanide, then H_2S to give 2-mercapto-4-(ω–guanidinopropyl)imidazole. This was

desulphurized by heating with aqueous iron chloride to give γ-imidazolyl-propylguanidine, which was hydrolyzed by heating with aqueous barium hydroxide to give the product amine, isolated as a dipicrate (mp 243-244°C from H_2O). Alternatively, 2-mercapto-4-(ω–guanidinopropyl)imidazole was converted to 2-mercapto-imidazolylpropylamine by heating with aqueous barium hydroxide and then treating with aqueous iron chloride. The γ-imidazolylpropylamine was isolated as the dipicrate (mp 244-244.5°C from water).

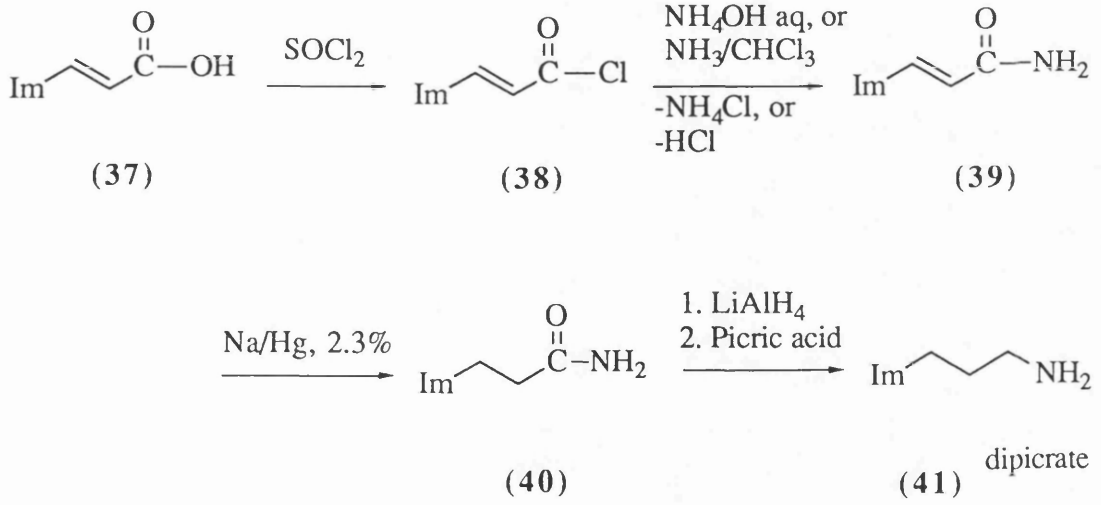
Lure'e et al[58.] heated dibromopropane with phthalimide to obtain γ-bromopropyl phthalimide. This was refluxed with the silver salt of imidazole in dry xylene to give N-γ-imidazolylpropylphthalimide. The phthalimide group was cleaved by refluxing with hydrazine hydrate in ethanol. Refluxing with HCl (10%) gave the N-γ-imidazolyl propylamine dihydrochloride as an extremely hygroscopic solid (mp 117-119°C from EtOH/Et_2O).

Black et al[22., 23.] heated 2-(chloroethyl)-4(5)imidazole hydrochloride with sodium cyanide in DMF or water to give the cyanoethyl derivative. This was dissolved in absolute alcohol and saturated with gaseous ammonia at -20°C, and then hydrogenated using Raney nickel in an autoclave to give the desired amine, which was converted to the phthalimide and then the dihydrochloride (mp 156-158°C from EtOH/Et_2O).

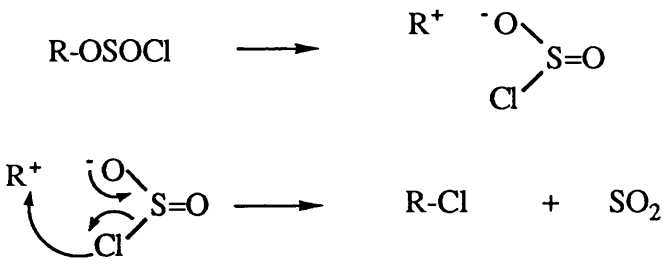
Schunack and Elz[42.] prepared 3-(imidazol-4-yl)propylamine or 'homohistamine' for the synthesis of analogues of impromidine. The six step synthesis started with the protection of the amine function of 4-amino-butyric acid, using phthalic acid anhydride (88%). The phthalimidobutyric acid was converted to the acid chloride using thionyl chloride (92%). This was reacted with N-methyl-N-nitrosourea to give the diazoketone, which was converted to the bromomethyl ketone with aqueous HBr in acetic acid (80%). This was treated with acetamidine in NH_3 in an autoclave, and the phthalimide group cleaved with hot NaOH/EtOH/HCl. The product amine was converted to the dipicrate (60%, mp 237-239°C from H_2O), then the dihydrochloride salt (144-146°C from EtOH/^{i}PrOH).

Adger and Surtees[2] started with fructose, which was converted to 2-(hydroxyethyl)-imidazol-4-yl using the Weidenhagen synthesis.[76] The secondary amine was protected as the N-trityl derivative using trityl chloride with Et_3N/DMF. Oxidation to the aldehyde was carried out with manganese dioxide in dioxane. The aldehyde was converted to the 2-cyanoethenyl derivative by means of a modified Wittig reaction using cyanomethyl diethyl phosphonate with sodium amide as the base. This was converted directly to the 3-(imidazol-4-yl)propylamine by hydrogenation over platinum oxide. The amine was isolated as the dihydrochloride.

Scheme 3.1. Novel synthesis of 3-(imidazol-4-yl) propylamine (**41**).



The starting point was urocanic acid (**37**) (imidazol-4-yl-acrylic acid, imidazol-4-yl-propenoic acid). This was converted to the imidazol-4-yl-propenoic acid chloride (**38**), following a S_Ni (substitution nucleophilic internal) mechanism[59] involving an intimate ion pair and then attack from part of the leaving group as shown below.



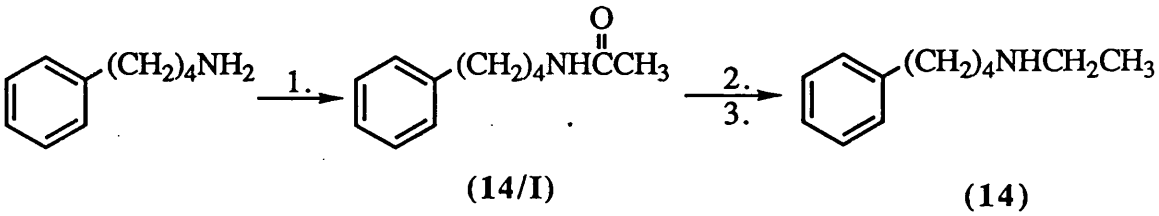
The crude product was converted directly to imidazol-4-yl-propenamide **(39)**, using either NH_4OH (specific gravity, 0.88) or $CHCl_3$ saturated with NH_3 gas (S_N2) with cooling. The next step was reduction of the double bond by direct electron transfer using Na/Hg amalgam (2.3%) to give imidazol-4-yl-propanamide **(40)**.

Efforts to simultaneously reduce the double bond and carbonyl group by reacting imidazol-4-yl-propenamide with $LiAlH_4$ (7mol equivalents in freshly distilled THF, refluxing 9 hours, and 15mol equivalents in freshly distilled THF, refluxing 3 hours) resulted in mixtures of starting material, 3-(imidazol-4-yl)propenamine and 3-(imidazol-4-yl)propylamine.

Reduction of amide to amine was carried out using $LiAlH_4$ (hydride transfer) in THF to give the desired 3-(imidazol-4-yl)propylamine **(41)**, isolated as the picric acid salt (mp 221-223°C).

N-ethyl-4-phenylbutylamine hydrochloride (14) was synthesized by following the methods of Shkylaev et al[68]. who performed the identical reaction, but since the full reference was not available, a similar synthesis by DeVries et al[39]. was used. 4-Phenylbutylamine was treated with acetyl chloride in Et_3N and CH_2Cl_2 under N_2 while cooling to 0°C to give the intermediate amide **(14/I)** with a 62% yield after distillation, by the usual S_N2 mechanism, with Et_3N neutralizing the HCl formed. This was reduced with three molar equivalents of $LiAlH_4$ in THF refluxing overnight, to give the

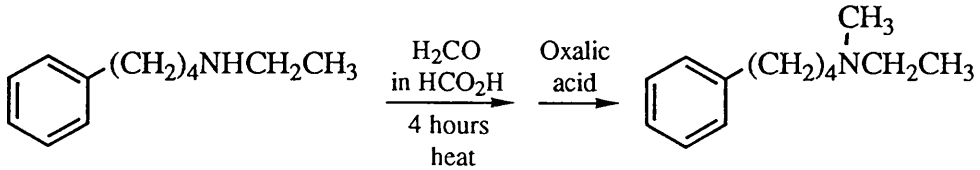
secondary amine as a base with a 92% crude yield and 53% after distillation. This was converted to the HCl salt by treatment with ethanolic HCl to give **(14)** (56% yield after recrystallization). The reaction equation is shown below.



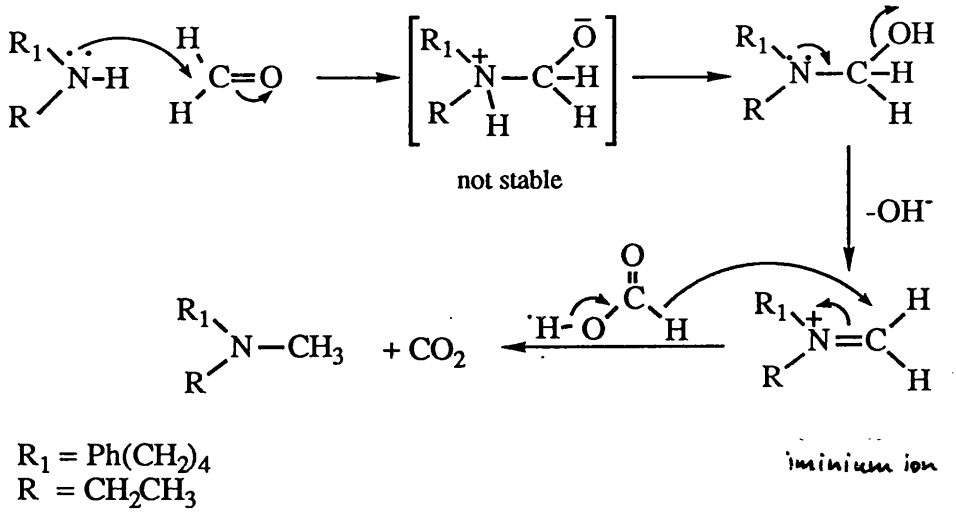
(14/I) **(14)**

1. CH_3COCl in Et_3N and CH_2Cl_2, cool to 0°C, 62% after distillation
2. $LiAlH_4$ in THF, reflux overnight, 92% yield, 53% after distillation
3. Ethanolic HCl, 56% after recrystallization

Synthesis of **N-ethyl-N-methyl-4-phenylbutylamine oxalate (15)** used the Clarke-Eschweiler reaction of Cope and Burrows.[34] N-Ethyl-4-phenylbutylamine **(14)** in formic acid was treated with aqueous formaldehyde (37%) and heated on a steam bath for 4 hours, made strongly alkaline with NaOH and extracted with ether. The crude tertiary amine was distilled (54% overall yield) and converted to the HCl salt. This did not crystallize so the base was reliberated with NaOH and the oxalate formed to give the final product **(15)** (78% after recrystallization). Nucleophilic attack by the amine at the formaldehyde carbon, gives an unstable complex which equilibrates. Elimination of OH^- gives an iminium ion which is protonated by the formic acid to give the product and CO_2. The reaction equation and mechanism are shown below.



(14) **(15)**



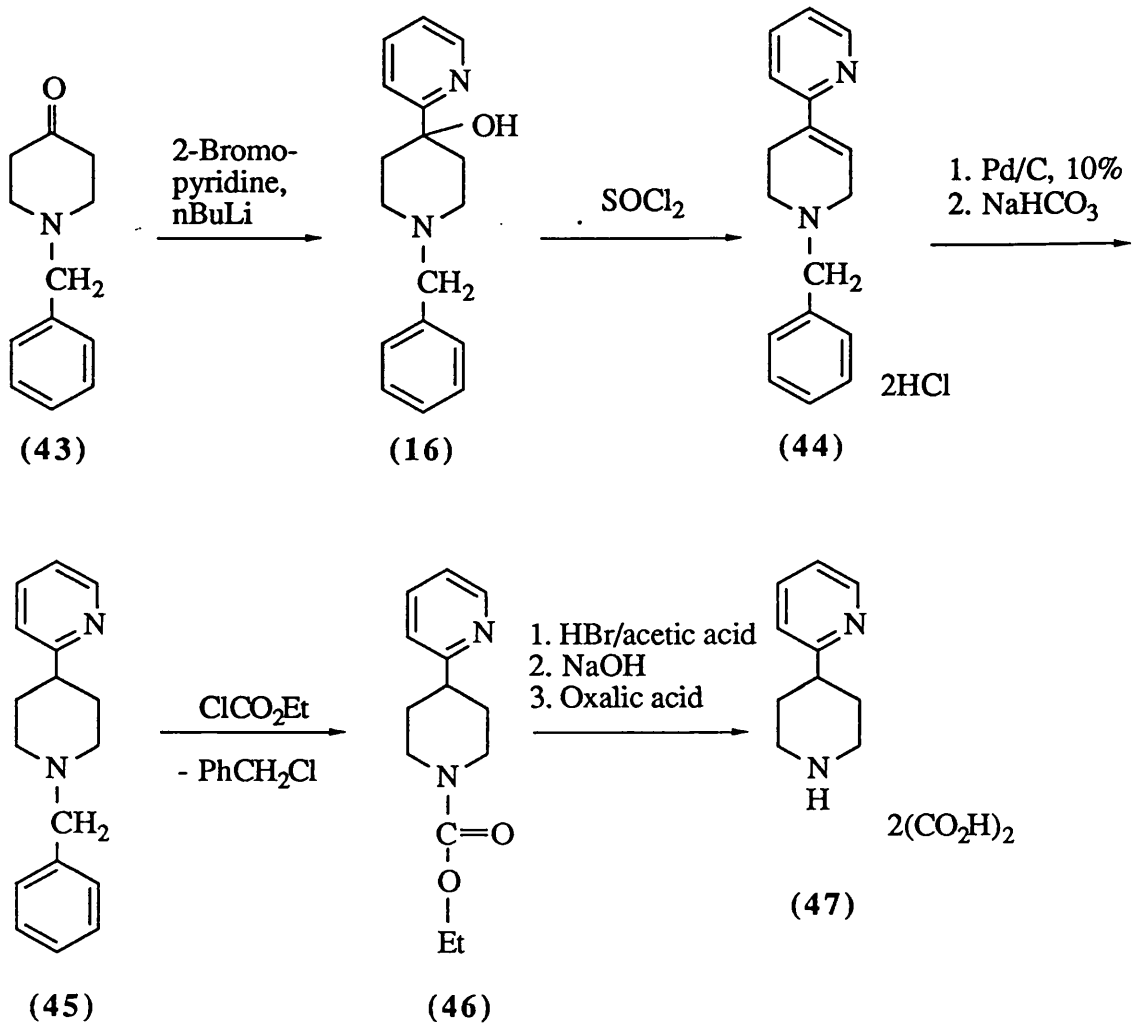
A novel synthesis was developed for **4-(2-pyridinyl)piperidine (47)**, with the ultimate aim of coupling the fragment to cyclohexylisothiocyanate to obtain N-cyclohexyl-4-(2-pyridinyl)-1-piperidine carbothioamide **(17)**. An intermediate compound in the synthesis, **1-benzyl-4-(2-pyridinyl)-piperidin-4-ol hydrochloride (16)** was also submitted for testing.

Novel synthesis of **4-(2-pyridinyl)-piperidine (47)**

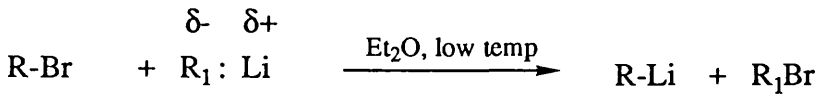
An alternative synthesis in the prior art was included in the patents by Bowden in 1968[26] and 1969[27] who prepared 3-(2-pyridinyl)-pentane-1,5-diol by reacting α-picoline with sodamide and ethylene oxide with a yield of 46%. This was heated under pressure of H_2 in NH_3 with Raney nickel in an autoclave to give 4-(2-pyridinyl)-piperidine with a yield of 100%.

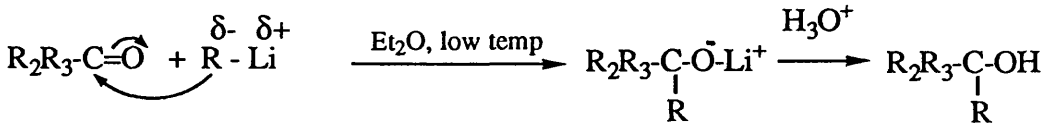
Another patent by McCall, 1981[60] uses 4-(2-pyridinyl)-piperidine but gives no indication of its synthesis.

Scheme 3.2. Novel synthesis of 4-(2-pyridinyl) piperidine **(47)**, a starting material for compound **(17)**.



The starting point was 1-benzyl-4-piperidone **(43)**, which was reacted with 2-pyridinyl lithium (from n-BuLi and 2-bromopyridine) in Et_2O at low temperatures, following a general method in the literature[48]. (preparation of 2-pyridinyl lithium to react with benzaldehyde to give pyridinyl-2-phenyl carbinol, 69%) to give 1-benzyl-4-(2-pyridinyl)-piperidin-4-ol dihydrochloride **(16)**. This was prepared in a similar manner by Raabe et al[61]. as discussed below. The mechanism involved is shown below.





Raabe et al[61]. prepared 1-butyllithium in situ from lithium and 1-bromobutane in ether, added 2-bromopyridine and the 1-benzyl piperidone while cooling to give 1-benzyl-4-(2-pyridinyl)-piperidin-4-ol (87% after distillation, 180-200°C/0.1 torr, mp 58-60°C from hexane/ether). Debenzylation was carried out with bromocyan in $CHCl_3$, stirring at room temperature for 3 hours to give 4-(2-pyridinyl)-piperidin-4-ol (85%). This method of debenzylation could also have been used below.

The next step involved dehydration of the tertiary alcohol, and this was achieved by careful addition of substrate to cooled (ice/water bath) $SOCl_2$. Reaction mechanism involves initial nucleophilic attack by the alcohol on the thionyl chloride to form an alkyl chlorosulphite. This is followed by an S_Ni mechanism, shown previously, to give the alkyl chloride, followed by elimination of HCl to give the alkene, 1-benzyl-4-(2-pyridinyl)-1,2,5,6-tetrahydropyridine dihydrochloride **(44)**.

Efforts to dehydrate the tertiary alcohol group which did not work included: $SOCl_2$ (addition at room temperature and subsequent refluxing for approximately 1 hour; also addition of $SOCl_2$ to cooled starting material) all resulted in dark brown unextractable mess; TosCl (in dry pyridine in fridge over 12 days) gave starting material; $KHSO_4$ anhydrous (fused at 0.03torr for 15 minutes) resulted in starting material; HBr/glacial acetic acid (45%, refluxed 15 minutes; also 49% aqueous refluxed 40 minutes, both using glacial acetic acid as solvent) both resulted in the formation of the HBr salt of the starting material.

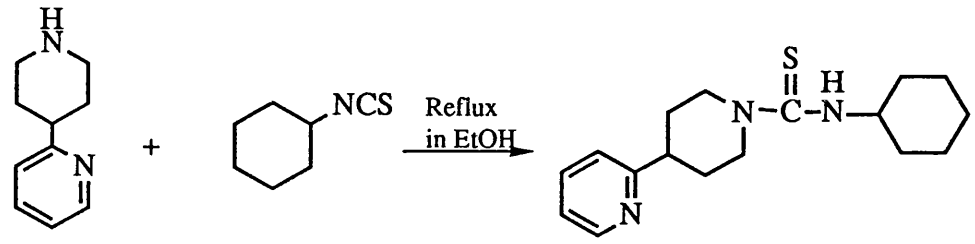
The next step involved reduction of the double bond by catalytic hydrogenation, using 10% Pd/C in EtOH absolute/acetic acid, to give 1-benzyl-4-piperidinyl-2-pyridine **(45)**.

Attempts to simultaneously reduce the double bond and cleave the benzyl group of 1-benzyl-4-(2-pyridinyl)-1,2,5,6-tetrahydropyridine dihydrochloride **(44)** caused problems. It was expected that debenzylation by catalytic hydration would occur, as indicated in the literature[38]. (debenzylation of 1-benzyl-isonipecotic acid). However, Raney nickel initially reduced the double bond, and when pushed further to cleave the benzyl group, the pyridine ring was also reduced. Conditions used were 10-40bar H_2/room temperature to 40°C/15 hours/2 catalyst recharges/MeOH; 7-30bar/room temperature/6 days/1 catalyst recharge/MeOH; 7bar, 40 hours, room temperature/30-45bar/18 days/room temperature-60°C/2 catalyst recharges/MeOH. The same problem was encountered with 10% Pd/C (4bar H_2/EtOH abs, then MeOH with approximately 10% acetic acid/4 catalyst recharges). With Na/NH_3 liquid, 9 equivalents of Na reduced the double bond, but a further 11 equivalents reduced the pyridine ring rather than cleaving the benzyl group.

Reaction of 1-benzyl-4-piperidyl-2-pyridine **(45)** with Na/NH_3 liquid (4 equivalents followed by 2 further equivalents after intermediate workup) showed no reaction apart from liberating the base.

The next step, following a general method[70]. (debenzylation of N-methyl-N-benzylamino-propylides via the amino ester, 63-75%), involved the synthesis of 1-ethoxycarbonyl-2-pyridinyl-4-piperidine **(46)** by reaction with chloroethyl formate (S_N2, with elimination of chloride, followed by elimination of the benzyl group from the quaternary N), and subsequent cleavage of the ethyl formate group with HBr/acetic acid from the crude material, to give 4-(2-pyridinyl)piperidine **(47)**, isolated as an oxalic acid salt.

Synthesis of **N-cyclohexyl-4-(2-pyridinyl)-1-piperidine carbothioamide trifluoroacetate (17)** and the isomeric **N-cyclohexyl-2-(3-pyridinyl)-1-piperidine carbothioamide trifluoroacetate (18)** was by coupling cyclohexylisothiocyanate with 4-(2-pyridinyl)piperidine (**47**) obtained previously and 2-(3-pyridinyl)piperidine obtained commercially, respectively, by the usual nucleophilic attack on the isothiocarbonyl carbon by the amine.

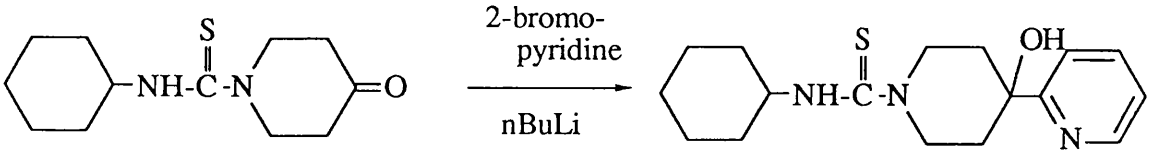


(17)

The mixtures were heated under reflux in absolute ethanol for 51 hours and 10 days respectively, and the products obtained as the trifluoroacetate salts after semi-preparative HPLC, with a 46% and an 89% overall crude yield respectively.

A previous attempt to obtain **(18)** on a smaller scale, heating under reflux in toluene for 8 hours, yielded an approximately 1/1 mixture of starting material and product.

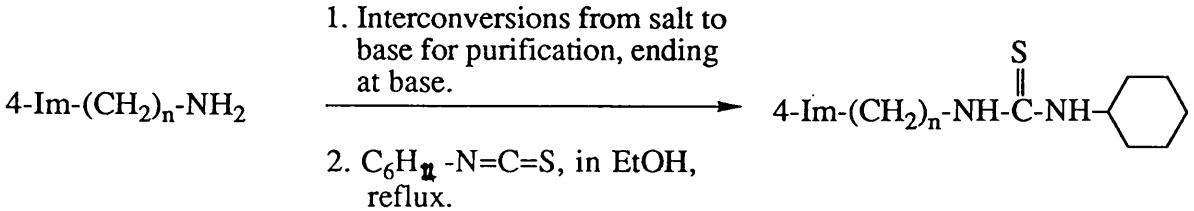
As an alternative approach to N-cyclohexyl-4-(2-pyridinyl)-1-piperidine carbothioamide **(17)** it was attempted unsuccessfully 4 times to synthesize N-(N'-cyclohexyl-thiocarbamoyl-(4-piperidin-4-ol)-2-pyridine **(54)** according to the scheme below.



(3) **(54)**

The starting material was N-(N'-cyclohexyl-thiocarbamoyl)piperidin-4-one, compound **(3)**. The reaction, based on the synthesis of **(16)** was carried out on a 5.2mmol and 2.7mmol scale in Et_2O and THF respectively, and at -80 to -70°C and -20 to -30°C respectively and 1mol equivalent of n-BuLi/2-bromopyridine resulting in both cases in starting material. Since a 2mol equivalent of n-BuLi/2-bromopyridine would be required (allowing for acidic proton abstraction from the starting material), the reaction was repeated in these proportions in THF at -30 to -20°C on 4.16mmol and 20mmol scales. Poor yields were obtained, with an approximate 1/1 mixture of starting material and product, and disintegration into fragments, in the respective cases. This route was abandoned in favour of the original method.

N-(N'-Cyclohexyl-thiocarbamoyl)-3-(imidazol-4-yl)propylamine (19) and N-(N'-cyclohexyl-thiocarbamoyl)-4-(imidazol-4-yl)butylamine oxalate (20) were synthesized as shown below. The starting materials 3-(imidazol-4-yl)propylamine **(41^{WT})** and 4-(imidazol-4-yl)butylamine **(49)** (sample WT76/457A) were kindly provided by Mr. W. Tertiuk. The respective amines were heated under reflux with cyclohexylisothiocyanate for 45 minutes and 3 hours to give the products with 96% and 50% crude overall yields respectively.



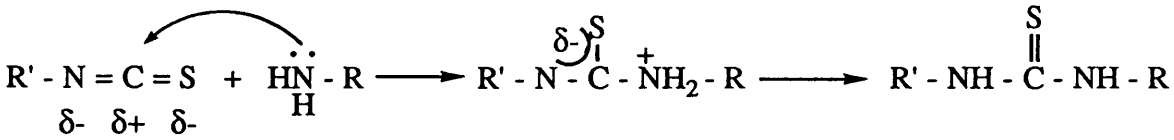
n = 3, **(41^{WT})**, HCl salt

n = 3, **(19)**, base

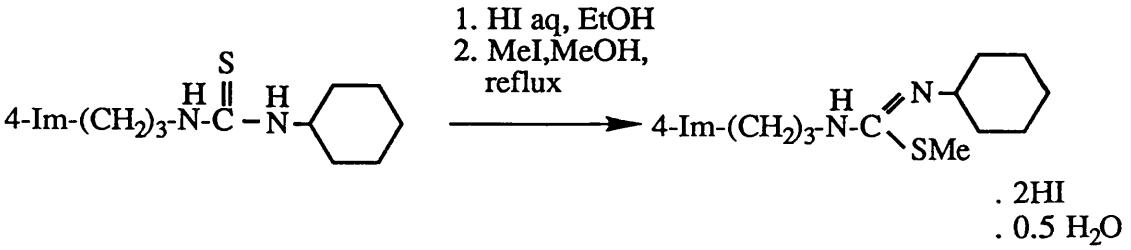
n = 4, **(49)**, crude base

n = 4, **(20)**, converted to oxalate

The reaction involved nucleophilic attack on the isothiocarbonyl carbon by the primary amine group, as shown below.



S-Methyl-N-cyclohexyl-N'-(3-(imidazol-4-yl)propyl)isothiourea dihydroiodide hemihydrate (21), was prepared by following the general procedure[22] of protecting the imidazole basic ring N atoms from methylation by forming the hydroiodide salt, and then allowing nucleophilic (S_N2) attack by the thione S on MeI to result in S-methylation. The synthesis is shown below.



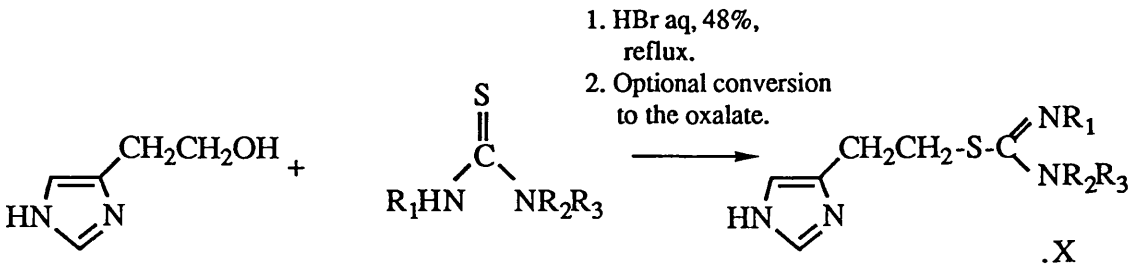
(19) **(21)**

The mixture was refluxed in MeOH for 5 hours to give the product with a 96% crude yield.

The compound was extremely hygroscopic, and thus very difficult to handle.

The imidazolyl alkyl isothioureas :

N,N'-dimethyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dihydrobromide (22); N,N'-diethyl-S-(2-(imidazol-4-yl)ethyl)isothiourea trioxalate (23); N,N'-di-n-propyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dioxalate (24); N,N'-di-n-butyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dioxalate (25); N-methyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dihydrobromide (26); 2-(2-(imidazol-4-yl)ethyl)-thio-imidazoline dihydrobromide (27); N,N,N'-trimethyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dihydrobromide (28); N-cyclohexyl-S-(2-(imidazol-4-yl)ethyl)isothiourea ditrifluoroacetate (29) were synthesized following the literature method[21] of refluxing 2-(hydroxyethyl)-imidazol-4-yl **(50)** (sample WT76/3156) kindly provided by Mr. W. Tertiuk with the appropriate thioureas in 48% aqueous HBr as shown below.



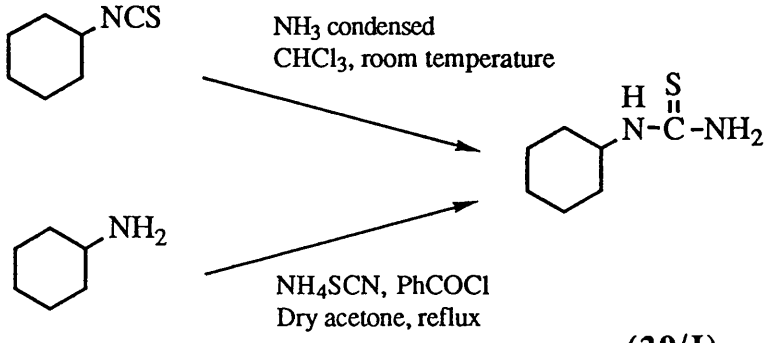
(50) **(22) to (29)**

Compound	R_1	R_2, R_3	X	Reflux hours	Crude yield
(22)	Me	H, Me	2HBr	26	66%, HBr
(23)	Et	H, Et	$2(CO_2H)_2$	89	66%, oxalate
(24)	n-Pr	H, n-Pr	$2.25(CO_2H)_2$	88	72%, base
(25)	n-Bu	H, n-Bu	$2(CO_2H)_2$	89	93%, oxalate
(26)	Me	H, H	2HBr	26	95%, HBr
(27)	CH_2	H, CH_2	2.4HBr	17	95%, HBr
(28)	Me	Me, Me	2HBr	83	100%, HBr
(29)	C_6H_{11}	H, H	$2.5CF_3CO_2H$	70	78%, oxalate

Mechanism of the reaction involves S_N2 to form the 2-(imidazol-4-yl)ethylbromide hydrogen bromide salt, followed by S-alkylation (S_N2) similar to that for the preparation of **(21)**.

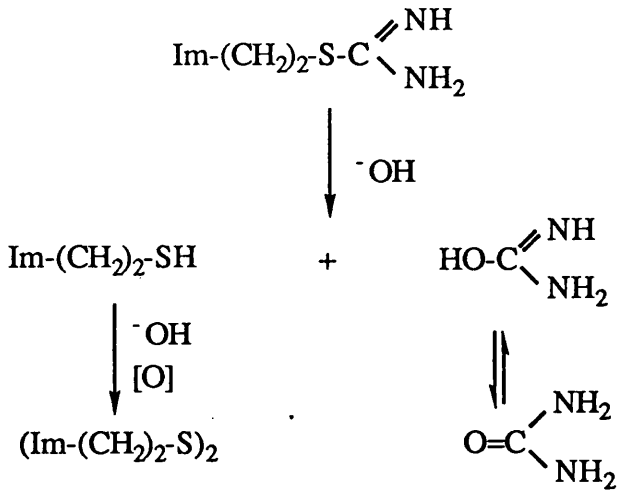
Compounds **(22)**, **(23)**, **(26)** and **(27)** have been prepared by Black et al.[21]. Purification sometimes involved converting the HBr salt (which tended to be very hygroscopic) to the oxalate.

In the case of **(29)** it was necessary to purify by prep-HPLC, and thus the trifluoroacetate was obtained. It was also necessary to prepare the starting material for this compound, monocyclohexylthiourea. This was obtained by condensing NH_3 onto cyclohexylisothiocyanate in chloroform to give **(29/I)** with a yield of 65%. Alternatively, a literature method[64]. was followed, reacting ammonium thiocyanate and benzoyl chloride with cyclohexylamine in dry acetone (45% yield). The thiourea is obtained by the reaction of benzoyl thiocyanate, Ph(CO)SCN, with the amine and subsequent hydrolysis of the reaction products. Both reaction paths are shown below.



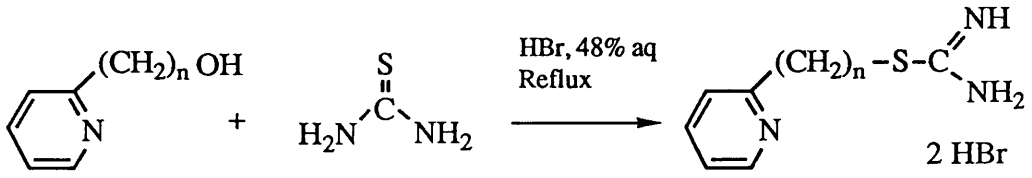
(29/I)

Note that the isothioureas are unstable to aqueous base, decomposing to thiol/disulphide and urea, as shown below.



S-(2-(2-Pyridinyl)ethyl)isothiourea dihydrobromide (30) and **S-(3-(2-pyridinyl)propyl)isothiourea dihydrobromide (31)** were prepared by following the general procedure for the previous isothioureas.[21.] **(30)** was found in existing literature[19., 21., 69.] but **(31)** is new.

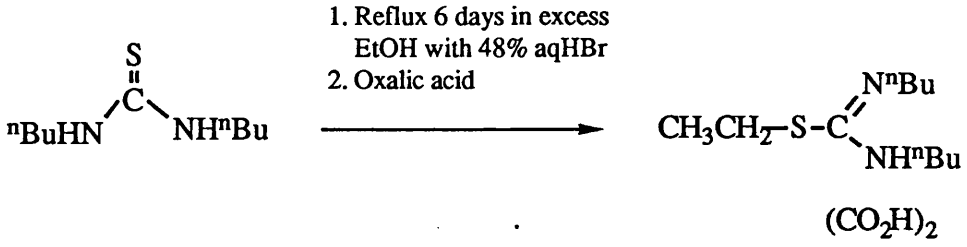
(30) was refluxed for 95 hours and obtained with a 50% overall yield after recrystallization. **(31)** was refluxed for 88 hours and obtained with a 71% overall yield after recrystallization. The reaction equation (S_N2 mechanism as before) is shown below.



n = 2	95 hours	n = 2, **(30)**
n = 3	88 hours	n = 3, **(31)**

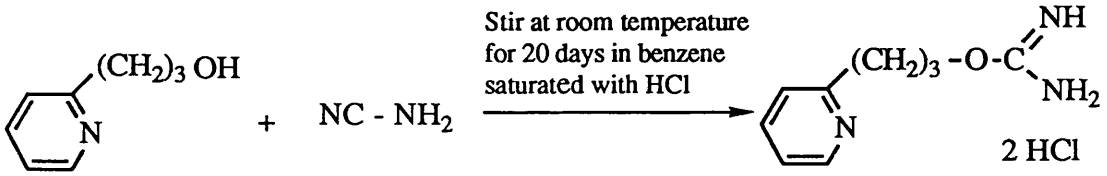
An isothiourea without a heterocycle, **N,N'-di-n-butyl-S-ethyl isothiourea oxalate (32)**, was synthesized following the general method[21.] used previously by

refluxing di-N,N'-butyl thiourea with 48% aqueous HBr in excess absolute ethanol for 6 days. **(32)** was obtained with a 92% crude overall yield. The equation is shown below.



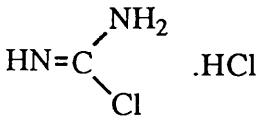
(32)

Synthesis of the isourea **O-(3-pyridin-2-yl-propyl)isourea dihydrochloride (33)** followed the general method[45.,50.] which involves the stirring of 2-(3-hydroxy propyl)-pyridine with cyanamide in dry benzene saturated with dry HCl at room temperature for 20 days, to obtain the product with a 46% yield of the first crop after recrystallization. The reaction equation is shown below.



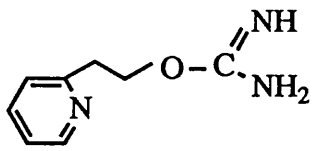
(33)

The reaction occurs via the chloro-amidinium salt (shown below), followed by nucleophilic

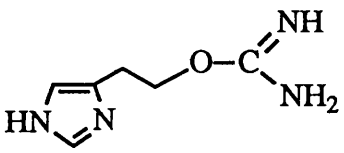


attack (S_N2) of the OH on the amidinium salt carbon atom.

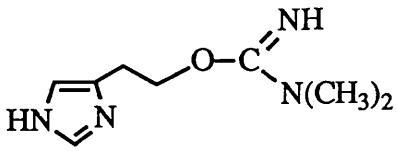
Synthesis of **O-(2-pyridin-2-yl-ethyl)isourea (51), O-(2-imidazol-4-yl-ethyl)isourea (52), N,N-di-methyl-O-(2-imidazol-4-yl-ethyl)isourea (53)**



(51)



(52)



(53)

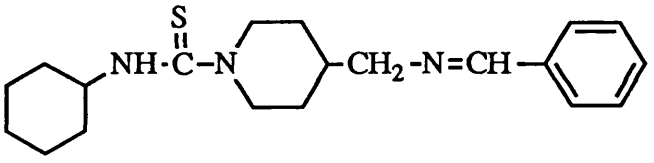
followed the same method using 2-(2-hydroxyethyl)pyridine and cyanamide, stirring for 20 days; (2-hydroxyethyl)imidazol-4-yl **(50)** (sample WT76/3156) and cyanamide, stirring for 18 days; and (2-hydroxyethyl)imidazol-4-yl **(50)** (sample WT76/3156) and di-methyl cyanamide, stirring for 7 days at room temperature and refluxing in CH_2Cl_2 for 15 hours. Problems were encountered with the purification of these three isoureas, probably due to their instability to base and to prolonged standing at room temperature, causing break-up into starting material alcohol and urea.

SELECTED SPECTRA

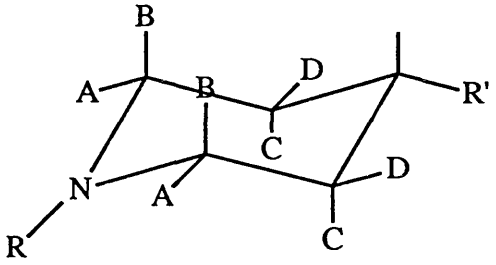
The spectroscopy of the compounds made usually included UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. The spectra served to characterize the compounds, and in most cases this was quite straight-forward. The assignments are given for each compound in Chapter Five.

However, there were some spectra which I found to be more than just characterizations, but interesting in their own right. It is with these that I would like to deal briefly in the following section.

^{1}H NMR (200MHz , $CDCl_3$) of compound (7).



(7)



R is (C_6H_{11}-NH-CS-), R' is (CH_2-N =CH-Ph) and A, B, C and D are hydrogen atoms.

The singlet at δ8.25 is due to N=CHPh, and the two multiplets at δ7.72 and 7.44 are due to the 2-, 6- and 3-, 4-, 5- phenyl hydrogens respectively. The doublet at δ5.29 is due to NH, with J = 8.0Hz, typical for a freely rotating vicinal proton coupling constant. The piperidine ring assumes the stable chair conformation, as shown above, which also

allows the long and bulky substituents R and R' to be substituted in equatorial positions on the ring. The broad doublet at δ4.61 is due to the two equatorial hydrogens A, coupled to their geminal neighbours B with $J_{ae,geminal}$ = 13.06Hz, where a is axial and e is equatorial. The interaction of A with C and D ($J_{ea,vicinal}$, $J_{ee,vicinal}$) is not resolved. The multiplet at δ4.28 is due to C<u>H</u>NH, and the doublet at δ3.52 is due to C<u>H</u>$_2$N, coupled to the piperidine CH, J = 6.63Hz.The broad apparent triplet with doublet splitting at δ2.94 is due to the two axial hydrogens B. Coupling between B and A, and B and C, $J_{ae,geminal}$ and $J_{aa,vicinal}$, respectively is of a magnitude of 12.79Hz each giving rise to a doublet of doublet pattern. Coupling between B and D, $J_{ae,vicinal}$ is of a magnitude of 2.36Hz, resulting in the final 'doublet of doublet of doublet' pattern, which looks like a triplet with each peak split into a doublet. The difference in chemical shift of A and B is probably due to a fixed lone pair of electrons on the nitrogen.The multiplet at δ1.00-2.10 is due to the remaining piperidine and cyclohexyl hydrogen atoms.

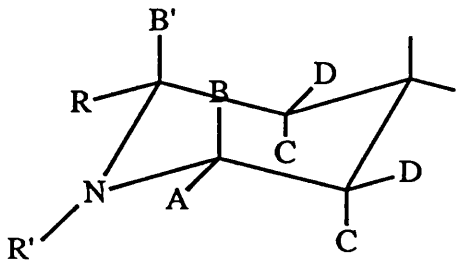
A similar effect is shown by the other compounds incorporating a 1,4-substituted piperidine **(4)**, **(5)**, and **(17)** as shown in the Table 3.1. below.

<u>Table 3.1.</u> Comparison of the axial and equatorial C<u>H</u>NC<u>H</u> ^{1}H NMR signals for compounds **(4)**, **(5)**, **(7)**, and **(17)**. The spectra were recorded at 200MHz (except for **(17)** which was recorded at 400MHz) and using $CDCl_3$ as solvent. The multiplicities (M) given are those observed from the spectra. At higher resolution, the multiplicities of the axial and equatorial hydrogen atoms would be 'doublet of doublet of doublet' and 'doublet of doublet' respectively.

Compound	δ_{axial}	J_{axial}(Hz)	M_{axial}	$\delta_{equatorial}$	$J_{equatorial}$(Hz)	$M_{equatorial}$
(4)	3.02	16.0,4.0	t of d	4.60	14.0	d(br)
(5)	3.05-3.35	12.09,2.99	t of d	4.15-4.50	13.33	d(br)
(7)	2.94	12.79,2.36	t of d	4.61	13.09	d(br)
(17)	3.18	11.93	t	4.82	13.54	d(br)

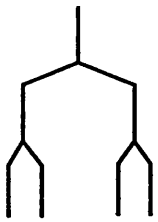
The effect is presumably not shown with compounds **(2)** and **(3)** due to a certain degree of flexibility of the piperidine ring, and with compound **(6)** it is the symmetry of the molecule. Compound **(16)** is di-substituted at the 4-piperidine position, giving rise to boat/chair interconversions of the piperidine to minimize interactions of the substituents, and therefore also does not show differentiated axial and equatorial ring hydrogens.

Compound **(18)** is different, because it has the piperidine ring substituted at the 2-position, as shown below.

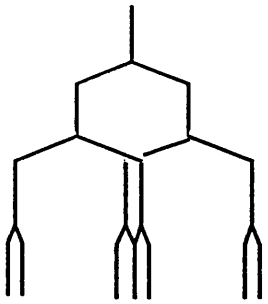


R is 3-pyridine, R' is CS-NH-C_6H_{11}, and A, B, B', C, and D are hydrogen atoms.

The ^1H NMR at 400MHz (in CD_3OD) showed good resolution of most of the individual couplings. The equatorial hydrogen A, couples with B, C and D to give $J_{ae,geminal}$ (14.67Hz), $J_{ea,vicinal}$, $J_{ee,vicinal}$ (both 3.29Hz) respectively, resulting in the 'doublet of doublet' represented below. The axial hydrogen B, couples with A, C, and D to give $J_{ae,geminal}$ (10.86 or 10.47Hz), $J_{aa,vicinal}$ (10.86 or 10.47Hz), $J_{ae,vicinal}$ (3.79Hz) respectively, resulting in the 'doublet of doublet of doublet' represented below. B' couples with C to give $J_{aa,vicinal}$ (14.69Hz), appearing as a broad doublet, since the interaction with D ($J_{ae,vicinal}$) is not resolved. The axial hydrogen B' is at δ4.17 and B is at δ2.90, while the equatorial hydrogen A is at δ2.48, the different order being due to the electron-withdrawing pyridine.



'doublet of doublet'



'doublet of doublet of doublet'

CHAPTER FOUR.

STRUCTURE ACTIVITY.

The thirty-three compounds may be grouped together in various ways in order to compare the effect of structural alterations on activity.

See Table 4.1.

The group constituted of compounds **(1)**, **(2)**, **(3)**, **(4)**, **(5)**, **(6)** and **(7)** shows that removal of the imidazole ring from the thioperamide structure causes loss of activity ($K_i >> 10^{-5}M$). **(1)** is the 'baseline' compound, while the others show that substitution with groups of differing polarities in the 4-piperidine position (an ether linkage, a ketone group, an ethyl alcohol chain, a carboxylic acid group, a cyclohexyl thiourea group and a benzaldiminomethyl group), does not have any effect on activity. It appears that this type of structure does not find an alternative binding site at the receptor, and that either the imidazole ring itself or another heterocycle is required for activity.

The imidazole group is restored in the two esters of urocanic acid **(8)** and **(9)**, and so is antagonistic activity to a moderate extent. The p-nitrophenyl ester is notably more active ($K_i = 8.2 \pm 2.0 \times 10^{-7}M$) than the sec-butyl ester ($K_i = 1.0 \pm 0.2 \times 10^{-5}M$). This was the first indication in this work of the positive influence on activity of the p-nitro group, which was followed up in greater detail by my colleague, Mr. S.K. Hosseini. See discussion of compound **(13)**.

The two amides of urocanic acid **(10)** and **(11)** made, showed moderate activity ($K_i = 6.1 \pm 4.4 \times 10^{-5}M$ and $>> 2 \times 10^{-5}M$). These results were presented as a poster in May 1990.[54]

Table 4.1. Shows the activities of the compounds **(1)** to **(12)**.

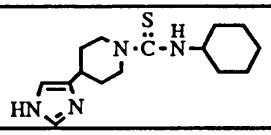
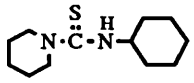
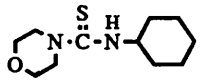
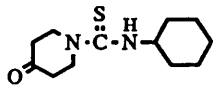
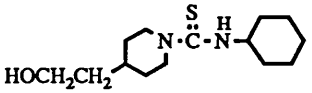
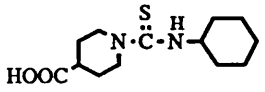
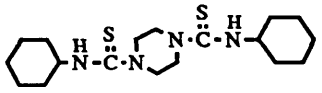
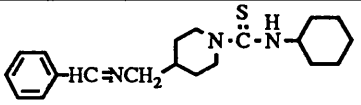
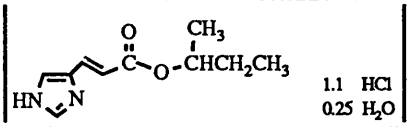
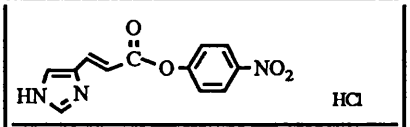
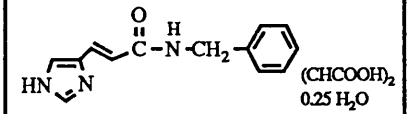
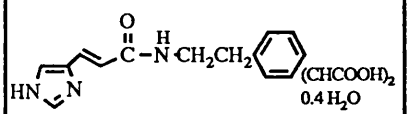
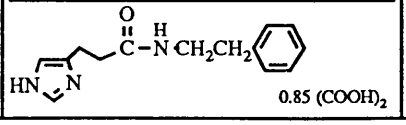
LEAD : Thioperamide (34)					$K_i = 4.3 \times 10^{-9}M$
COMPOUND			**New**	**STRUCTURE**	**POTENCY**
(1)	UCL1010	YK41C			$K_i >> 10^{-5}M$
(2)	UCL1011	YK45A			$K_i >> 10^{-5}M$
(3)	UCL1013	YK69B	*		$K_i >> 10^{-5}M$
(4)	UCL1022	YK57D	*		$K_i >> 10^{-5}M$
(5)	UCL1023	YK89B	*		$K_i >> 10^{-5}M$
(6)	UCL1012	YK49C	*		$K_i >> 10^{-5}M$
(7)	UCL1014	YK73C	*		$K_i >> 10^{-5}M$
(8)	UCL1008	YK34D			$K_i = 1.0 \pm 0.2 \times 10^{-5}M$
(9)	UCL1009	YK37E	*		$K_i = 8.2 \pm 2.0 \times 10^{-7}M$
(10)	UCL1028	YK115C	*		$K_i = 6.1 \pm 4.4 \times 10^{-5}M$
(11)	UCL1027	YK99I	*		$K_i >> 2 \times 10^{-5}M$
(12)	UCL1063	YK218B	*		$K_i = 1.2 \pm 0.1 \times 10^{-5}M$

Table 4.2. Shows the activities of the compounds **(13)** to **(21)**.

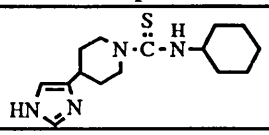
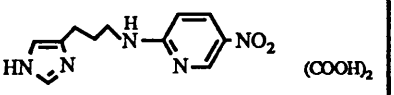
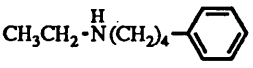
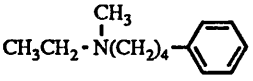
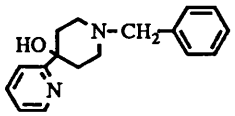
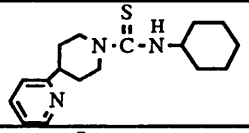
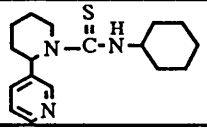
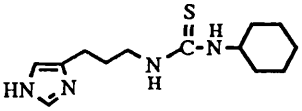
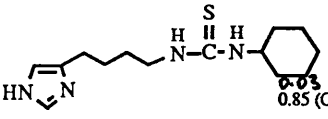
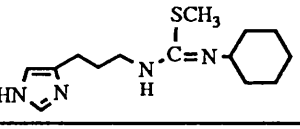
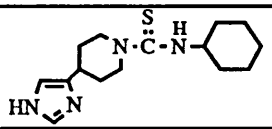
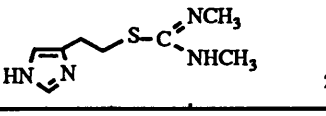
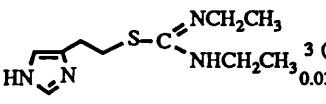
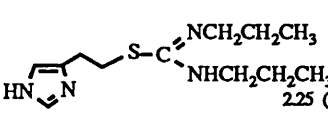
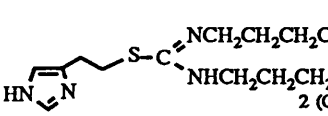
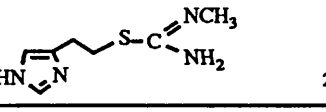
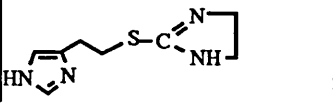
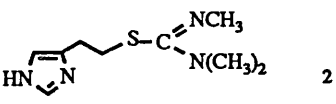
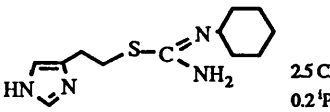
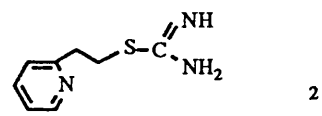
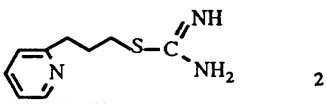
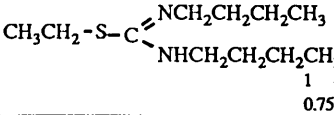
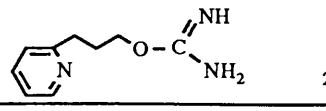
LEAD : Thioperamide (34)					$K_i = 4.3 \times 10^{-9}M$
COMPOUND			**New**	**STRUCTURE**	**POTENCY**
(13)	UCL1073-J	YK215C	*		$K_i = 1.7 \pm 0.4 \times 10^{-7}M$
(14)	UCL1221-A	FS19C			$K_i = 1.3 \pm 0.1 \times 10^{-6}M$
(15)	UCL1229-J	FS37C	*		$K_i = 1.1 \pm 0.1 \times 10^{-6}M$
(16)	UCL1033	YK111F	*		$K_i >> 10^{-4}M$
(17)	UCL1196	YK411B	*		$K_i = 1.3 \pm 0.1 \times 10^{-5}M$
(18)	UCL1220-J	YK427B	*		$K_i = >1 \times 10^{-5}M$
(19)	UCL1053	YK192B	*		$K_i = 1.3 \pm 0.3 \times 10^{-8}M$
(20)	UCL1088	YK251F	*		$K_i = 2.0 \pm 0.7 \times 10^{-8}M$
(21)	UCL1134-C_2	YK336C	*		$K_i = 3.6 \pm 0.7 \times 10^{-9}M$

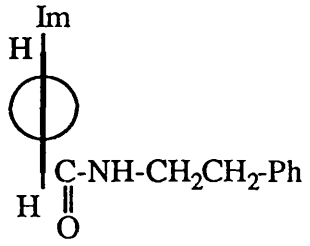
Table 4.3. Shows the activities of compounds (22) to (33).

LEAD : Thioperamide (34)					$K_i = 4.3 \times 10^{-9}M$
COMPOUND			**New**	**STRUCTURE**	**POTENCY**
(22)	UCL1124	YK302C		 2 HBr	$K_i = 5.1 \pm 2.2 \times 10^{-8}M$
(23)	UCL1211-J_2	YK365E		 3 $(CO_2H)_2$ 0.03 ^iPrOH	$K_i = 2.7 \pm 1.7 \times 10^{-8}M$
(24)	UCL1192-J_2	YK403D	*	 2.25 $(CO_2H)_2$	$K_i = 1.7 \pm 0.6 \times 10^{-8}M$
(25)	UCL1176-J_2	YK369C	*	 2 $(CO_2H)_2$	$K_i = 5.4 \times 10^{-9}M$
(26)	UCL1123	YK298D		 2 HBr	Agonist $EC_{50} = 1.5 \pm 0.5 \times 10^{-8}M$ 410% potency of HA
(27)	UCL1133	YK314F		 2.4 HBr	$K_i = 2.6 \pm 0.5 \times 10^{-7}M$
(28)	UCL1140-B_2	YK332F	*	 2 HBr	$K_i = 8.6 \pm 4.1 \times 10^{-7}M$
(29)	UCL1209-J_2	YK392A	*	 2.5 CF_3CO_2H 0.2 ^iPrOH	$K_i = 1.8 \pm 0.8 \times 10^{-8}M$
(30)	UCL1151	YK373C		 2 HBr	Partial Agonist $EC_{50} = 4.5 \pm 3.8 \times 10^{-5}M$ 73% potency of HA
(31)	UCL1152	YK377C	*	 2 HBr	$K_i = 2.3 \pm 0.7 \times 10^{-6}M$
(32)	UCL1213-J	YO16A	*	 1 $(CO_2H)_2$ 0.75 H_2O	$K_i = 3 \times 10^{-5}M$
(33)	UCL1208-A	YK423B	*	 2 HCl	$K_i = 1.0 \pm 0.4 \times 10^{-7}M$

Compound **(12)** was synthesized to determine whether it is the reverse amide nature or/and the ability of the compound to adopt the gauche form, that accounts for the greater activity of compound **(35)** (prepared by Schunack et al [56].) compared to compound **(11)**.

Im-CH=CH-CO-NH-CH_2CH_2-Ph	**(11)**	K_i >>10^{-5}M
Im-CH_2CH_2-CO-NH-CH_2CH_2-Ph	**(12)**	$K_i = 1.2 \pm 0.1 \times 10^{-5}$M
Im CH_2 CH_2 NH CO CH_2CH_2Ph	**(35)**	$K_i = 6.3 \times 10^{-7}$M

Although the compounds are isosteres, they are obviously different in the sense that the more active compound **(35)** is a reverse amide, and is not constrained by a trans double bond, as is **(11)**. The implication of the trans double bond is that **(11)** is limited to an anti conformation only (shown below), while Schunack's more active compound is free to adopt the conformations shown in Fig. 4.1.[59].

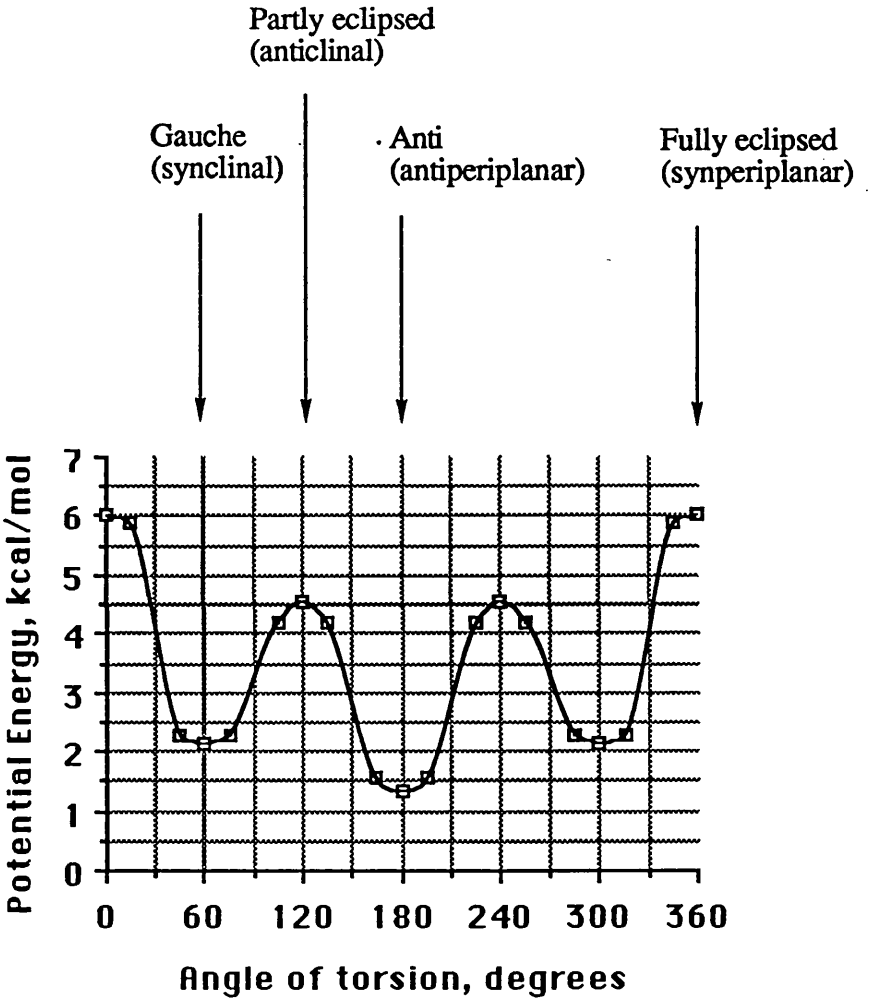


(11) anti conformation

The activity of **(12)** seems to indicate that it is the reverse amide function which affects activity, and that there may be a dipole interaction with the receptor which is direction dependent.

See Table 4.2.

Fig. 4.1. Shows the conformations which can be adopted by (35). The diagram is general for the conformational energy for molecules of the type YCH_2-CH_2Y or YCH_2-CH_2X. The potential energy figures are for n-butane.



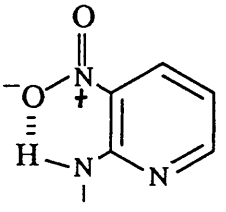
Compound **(13)** (K_i = 1.7±0.4 x 10^{-7}M) was synthesized to follow up compound (55) (K_i = 2.9±1.1 x 10^{-8}M) shown in overleaf . Extending the side chain by one methylene group reduces activity in this case, which is contrary to expectations, based on examples in the literature in the development of cimetidine[46]. and also in an H_3 antagonist series shown later, (Table 4.6.).

See Table 4.4.

Table 4.4. 4-Im -A- NH-2(5-nitro)pyridine : activity decreases with increasing methylene side chain length. Compounds **(55)** and **(56)** were made by Mr. S.K.Hosseini.

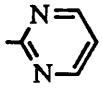
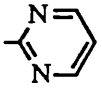
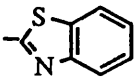
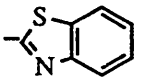
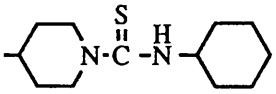
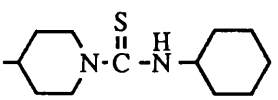
A	Compound	Activity (K_i(M))
$(CH_2)_2$	**(55)**	$2.9\pm1.1 \times 10^{-8}$
$(CH_2)_3$	**(13)**	$1.7\pm0.1 \times 10^{-7}$
$CH_2S(CH_2)_2$	**(56)**	$1.3\pm0.2 \times 10^{-6}$

Note the apparent importance of the para orientation of the nitro group, and the drop in activity observed by Mr. S.K. Hosseini with ~~o~~-NO_2, using 4-Im -$(CH_2)_2$- NH-2(3-nitro)pyridine, **(57)**, $K_i = 2.4\pm1.2 \times 10^{-7}$M for comparison with **(55)** above. Reasons for this effect could be the change in dipole direction, or intramolecular hydrogen bonding (see below), which may diminish receptor attachment.



Compounds **(14)** ($K_i = 1.3\pm0.1 \times 10^{-6}$M) and **(15)** ($K_i = 1.1\pm0.1 \times 10^{-6}$M) are derived from compound **(42)** $ImCH_2CH_2NH(CH_2)_4Ph$ and their activities clearly demonstrate that the imidazole group or an alternative heterocycle is not absolutely necessary for antagonistic activity at the H_3 receptor. This is discussed further later in this chapter.

Table 4.5. Shows H_3 affinity ratios for pairs of 4-Imidazole and 2-Pyridine compounds. Thanks are given to Mr. S.K. Hosseini for supplying the second and third pairs of results. The compounds are shown as free bases.

COMPOUND		Histamine release K_i (μM)	Ratio $\frac{K_i\ (2\text{-Py})}{K_i\ (4\text{-Im})}$
4-Im - $(CH_2)_2$ - $NHCH_3$	N^{α}-Methyl histamine	0.01 *	690
2-Py - $(CH_2)_2$ - $NHCH_3$	Betahistine	6.9	
4-Im - $(CH_2)_2$ - NH 	UCL1017	2.1	5
2-Py- $(CH_2)_2$ - NH 	UCL1030	10	
4-Im - $(CH_2)_2$ - NH 	UCL1029	0.33	9
2-Py - $(CH_2)_2$ - NH 	UCL1031	3.1	
4-Im 	Thioperamide (34)	0.0043	3020
2-Py 	UCL1196 (17)	13	

* N^{α}- Methyl histamine is an H_3-agonist. Its K value for inhibition of histamine release is taken as equivalent to K_i (histamine release), if there are assumed to be no spare receptors.

On the other hand, replacing the imidazole ring in thioperamide with 2-pyridine in compound **(17)** resulted in significant loss of activity. See Table 4.5.

The first pair of compounds is perhaps not so representative anymore, since there do seem to be spare receptors[74]. involved. However, the second and third pairs clearly show that no great loss in activity is necessarily incurred by replacing the imidazole group with pyridine. This is however not true for the fourth pair, when the imidazole group in thioperamide **(34)** is replaced in this way to give compound **(17)**, activity being reduced by 3000 times. This is discussed further later in this chapter.

Compound **(16)** was obtained as an intermediate to **(17)** and is not active ($K_i >> 10^{-4}M$). Compound **(18)** is the 3-pyridine-2-piperidine isomer of **(17)** and is also not active.

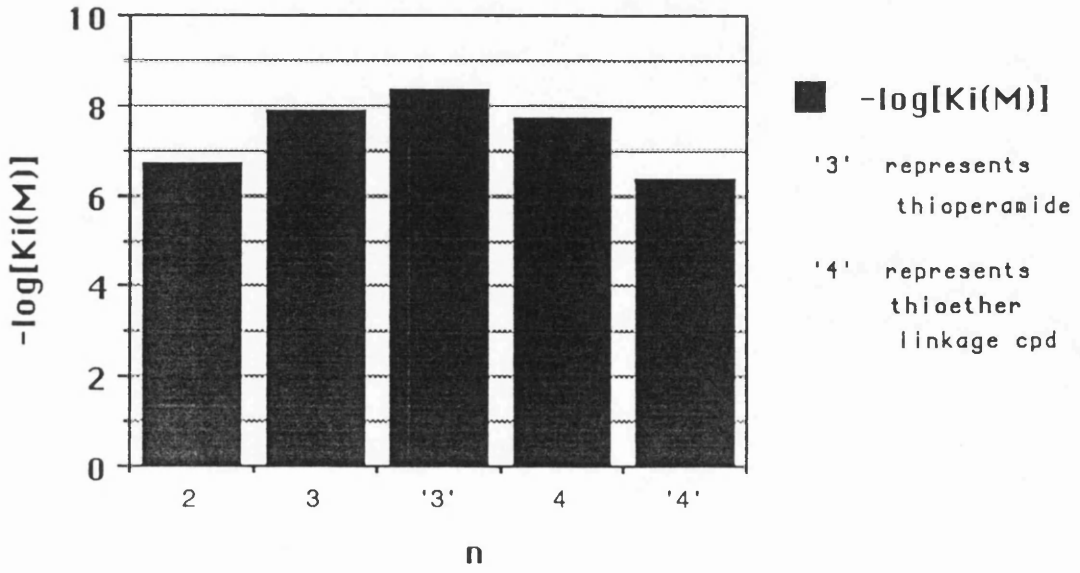
Opening up the piperidine ring of thioperamide, resulting in the more flexible molecules **(19)** and **(20)**, showed that activity is only reduced by a factor of 3 (for **19**). Compound **(20)** is identical to burimamide except that the one thiourea nitrogen atom is substituted with a cyclohexyl group and not a methyl group as in burimamide. The activities are also of the same order ($10^{-8}M$). The compounds may be compared with two prepared by Mr. S.K. Hosseini shown in Table 4.6 and Graph 4.1. below.

The chain of three methylene groups in **(19)** represents the same number of C-C bonds as the piperidine ring in thioperamide **(34)** (although possibly a longer distance in real space), while the tertiary amine group has been replaced by a secondary one, and the semi-rigidity of the piperidine ring (capable of boat/chair interconversions) is now flexible. Increasing the methylene groups from three to four does not seem to have any significant effect on activity, and so it seems that the optimum chain length is between 3 and 4 methylene groups. Compound **(19)** was tested further in vivo, see later in this chapter.

Table 4.6 H_3 antagonist activity trends (K_i(M)) with methylene side chain length. Note that the thioether linkage (-S-) is not interchangeable with the methylene group (-CH_2-) here, despite their similar sizes and bioisosteric nature in some cases.[46]

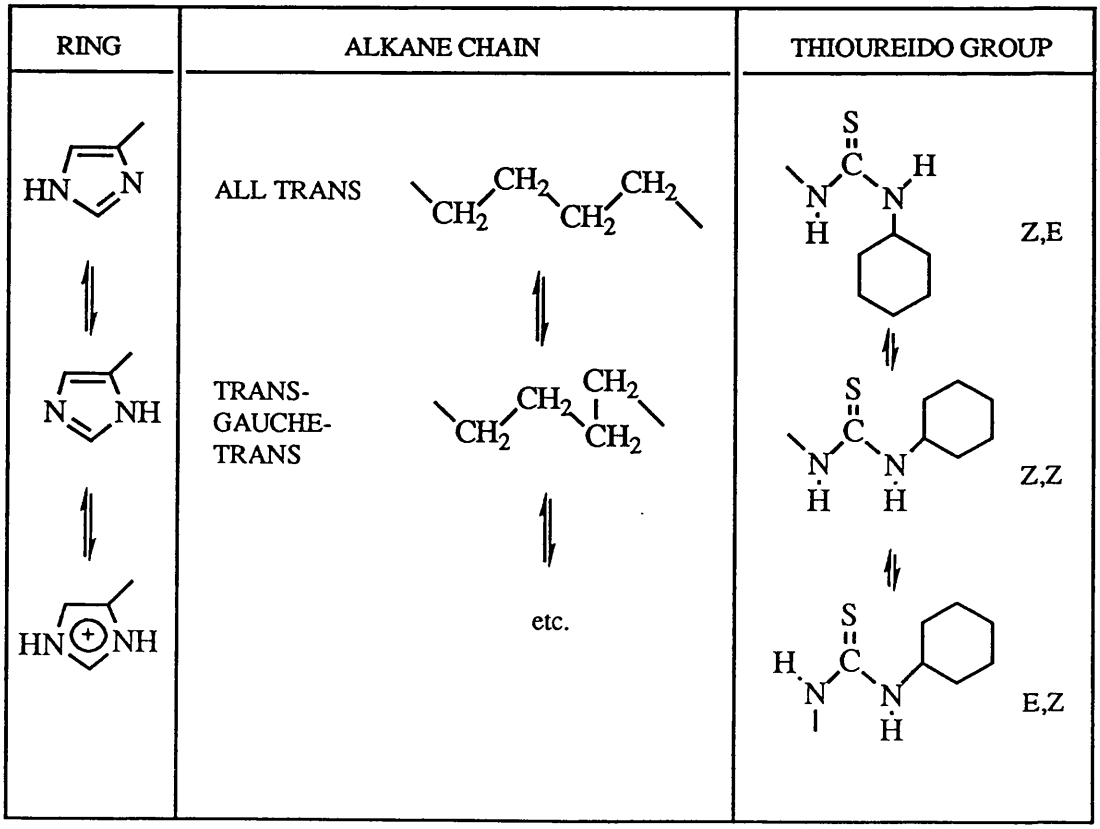
Compound	No.	Activity
4(5)-Im-$(CH_2)_2$-NH-(C=S)-NH-C_6H_{11}	**(58)**	$K_i = 2.0\pm1.0 \times 10^{-7}$M
4(5)-Im-$(CH_2)_3$-NH-(C=S)-NH-C_6H_{11}	**(19)**	$K_i = 1.3\pm0.3 \times 10^{-8}$M
4(5)-Im-$(CH_2)_4$-NH-(C=S)-NH-C_6H_{11}	**(20)**	$K_i = 2.0\pm0.7 \times 10^{-8}$M
4(5)-Im-$CH_2S(CH_2)_2$-NH-(C=S)-NH-C_6H_{11}	**(59)**	$K_i = 4.7\pm2.3 \times 10^{-7}$M

Graph 4.1. Shows the variation of activity with chain length for $Im(CH_2)_n$-NH-CS-NH-C_6H_{11} where n = 2, 3, 4, '4' for compounds **(58)**, **(19)**, **(20)** and **(59)** respectively.



The thioureas in aqueous solution[44.,46.] are a mixture of many chemical species in equilibrium, see Fig. 4.2. At physiological pH there are three main forms of the imidazole ring, three major planar configurations of the thioureido group, and various trans and gauche rotamer combinations of the side-chain CH_2-CH_2 bonds. There are substantial energy barriers to interconversion between the species, so that it is quite likely that a drug molecule, presenting itself in a form unfavourable for drug-receptor interaction, might diffuse away again before having time to rearrange into a more favourable form. The existence of a number of different species leads one to question which may be biologically active and whether altering drug structure to favour a particular species would alter drug potency.

Fig. 4.2. Compounds **(19)** and **(20)** in aqueous solution, an equilibrium mixture of various interconverting species (where E is 'entgegen' and Z is 'zusammen').



Thioureas are also weakly amphoteric (exist almost exclusively in the uncharged form), and tautomeric. In water, they are mainly in the thione form in equilibrium with a minute amount of thioenol, as shown below.

thione thioenol

The thione : thioenol ratio for N-methylthiourea : S-methylisothiourea is suggested[44] to be of the order 10^{11} : 1.

The S-methylated derivative **(21)** (K_i = 3.6±0.7 x 10^{-9}M) of **(19)** was as active as the lead **(34)**, and was tested further in vivo, see later in this chapter. This was the first indication of the effectiveness of the isothiourea function at the H_3 receptor.

See Table 4.3.

Remarkable results were obtained with the isothiourea compounds **(22)** through to **(33)** which were a direct follow-up on **(48)**, the 'super-agonist' (EC_{50} = 1 x 10^{-9}M, see Chapter Two) and compound **(21)**.

Blocking off the NH sites of the isothiourea group in an effort to determine which one/s is/are necessary for binding to the receptor, has shown some intriguing results, see Table 4.7. below.

With no methyl substituents, agonist activity is maximal, decreasing with one methyl, and changing to a definite antagonist activity with two methyl groups. However, blocking off all the NH sites, either with three methyl groups or the ethylene bridge, results in weaker antagonistic activity, the obvious conclusion being that three free NH sites are most favourable for agonism, while one free NH site is most favourable for

antagonism. The decrease in sites available for H-bonding in the dialkyl substituted isothiourea is a positive feature for improved brain penetration. Moreover the dialkyl substituted thioureas required for synthesis of further analogues (see later in this section) were more accessible than mono or trialkyl substituted thioureas.

Table 4.7. H_3 activities of some (imidazol-4-yl)ethyl isothioureas.

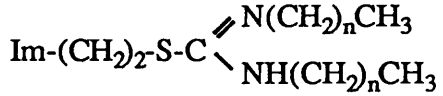
4-Im-$(CH_2)_2$-S-C(=NH)-NH_2	**(48)**	Agonist, $EC_{50} = 1 \times 10^{-9}M$
4-Im-$(CH_2)_2$-S-C(=NMe)-NH_2	**(26)**	Agonist, $EC_{50} = 1.5 \pm 0.5 \times 10^{-8}M$
4-Im-$(CH_2)_2$-S-C(=NMe)-NHMe	**(22)**	Antag, $K_i = 5.1 \pm 2.2 \times 10^{-8}M$
4-Im-$(CH_2)_2$-S-C(=NMe)-NMe_2	**(28)**	Antag, $K_i = 8.6 \pm 4.1 \times 10^{-7}M$
4-Im-$(CH_2)_2$-S-C(=N)-NH (ring)	**(27)**	Antag, $K_i = 2.6 \pm 0.5 \times 10^{-7}M$

However, comparing **(29)** with **(26)**, the only difference is that the single isothiourea nitrogen substituent is a bulky cyclohexyl group instead of a methyl group, and yet **(29)** is a potent antagonist ($K_i = 1.8 \pm 0.8 \times 10^{-8}M$) while **(26)** is a potent agonist ($EC_{50} = 1.5 \pm 0.5 \times 10^{-8}M$).

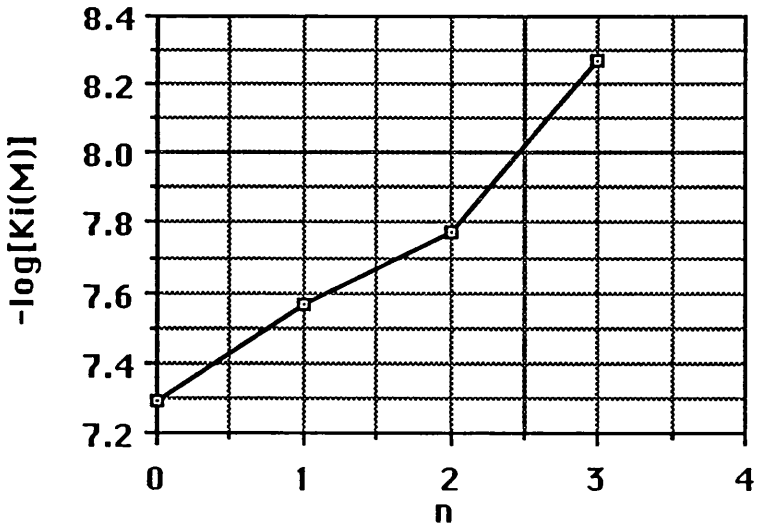
It appears that the longer the alkyl substituents, the more active the compound, with the maximum reached in this series with the di-N,N'-n-butyl derivative **(25)**, which was tested further in vivo, see the appropriate section later in this chapter.

Due to the constraints of time and the combination of carrying out most of the isothiourea and isourea syntheses in parallel (due to time-consuming purification procedures) and the time gap of receiving testing results from Paris, a restricted series of (imidazol-4-yl)ethyl isothioureas was made. The results indicated that derivatives with alkyl chain substituents longer than n-butyl would be necessary for a determination of the optimum chain length substituent, and this is identified in the 'Conclusions and further suggestions' section at the end of this chapter.

Graph 4.2. Shows variation of activity with length of alkyl substituents on the isothiourea nitrogen atoms for



where n = 0, 1, 2 and 3 for compounds **(22)**, **(23)**, **(24)** and **(25)** respectively.



Removal of the imidazole group of structure **(25)** to give structure **(32)** resulted in loss of activity. This is discussed further later in this chapter.

Compound (30) is another example of replacing 4-imidazole with 2-pyridine, with the result that the 'superagonist' (48) becomes a partial agonist (EC_{50}= 4.5±3.8 x 10^{-5}M).

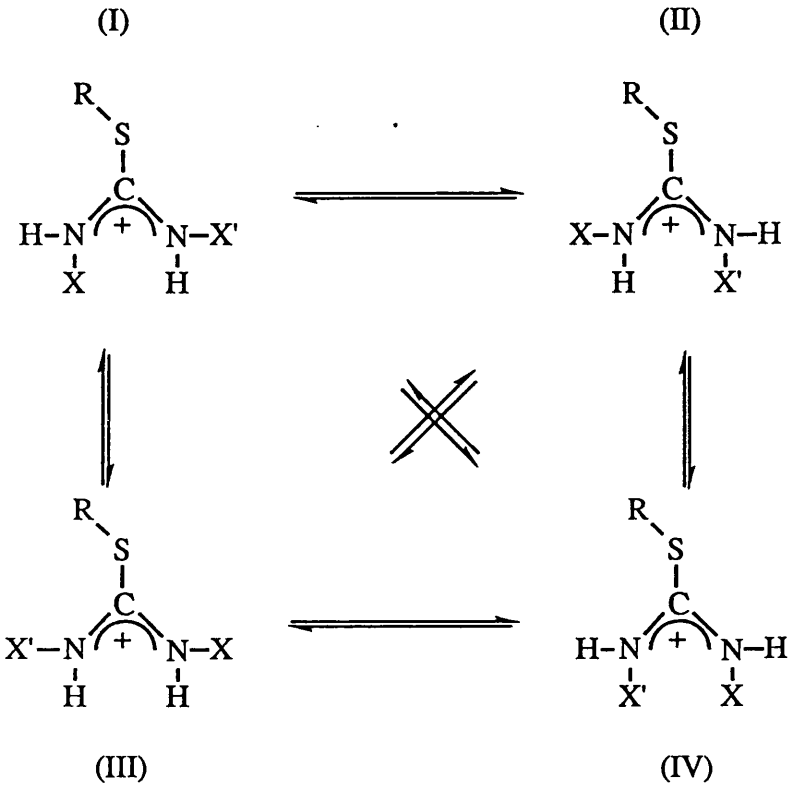
Compound (31) shows the effect of extending the length of the methylene chain in (30), which is to go from partial agonist to antagonist (K_i = 2.3±0.7 x 10^{-6}M).

The isourea analogue (33) of (31) is a 20 times more potent antagonist than (31), with K_i = 1.0±0.4 x 10^{-7}M.

The amidine moiety in the unsubstituted isothiourea (or isourea) group has two types of nitrogen atoms: the single-bonded -NH_2, and the double-bonded =NH, which has a lone pair of electrons. The amidine moiety can undergo a 1,3-prototropic tautomerism, i.e. it can transfer a proton from -NH_2 to =NH.
However, since isothioureas are basic (pK_a >9)[41], they are mainly protonated at physiological pH (7.4), and it is probable that they act in the cationic form. See Fig. 4.3.

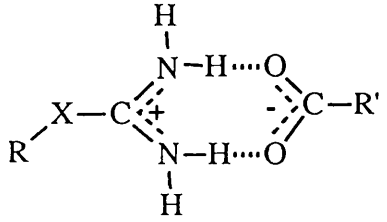
As shown by Graph 4.2., antagonistic activity increases with length of alkyl substituents in the N,N'-disubstituted derivatives, in which case stereochemical factors would probably favour structure (I) . Table 4.7. indicates that the agonists are not at all or only singly substituted on the isothiourea nitrogens, while the change to antagonist occurs from the more stereochemically hindered N,N'-disubstituted compounds onwards. The unsubstituted agonist has all the structures (I), (II), (III) and (IV) available. The monosubstituted agonist (26), with X'=Me and X=H, also has all the structures available, except (III), which would probably be less favoured. However, compound (29) with its single bulky and lipophilic substituent, the cyclohexyl group (X'=C_6H_{11} and X=H), is probably limited to structure (I), typical of the disubstituted antagonists. Hence, it could be inferred that structures (II) and (IV) are necessary for agonism.

Fig. 4.3. Conformations of the protonated isothiourea group, when disubstituted (X'=X=alkyl group), monosubstituted (X'=alkyl group and X=H), and unsubstituted (X'=X=H). R = 4-Im-$(CH_2)_2$. Not all the structures are shown for the case of disubstitution with different alkyl groups (i.e. X' is not the same alkyl group as X).
The C-S rotated conformers (with R on the other side of C-S) are found within the structures (I) to (IV).



It seems clear that activity at the H_3 receptor of the isothiourea compounds is a combination of conformational preference and hydrophobicity, both of which vary with the size of alkyl group substituted on the isothiourea nitrogens.

The isothioureas and isoureas as their oxyacid salts might act at the receptor through the type of H-bonding suggested by Walker[52.,81.] for the 'doublet ion pairing' between isothioureas/isoureas and oxyacids, as shown.



Walker referred to the special stability of isothiouronium salts of carboxylic and sulphonic acids, stating that 'anion and cation hold each other electostatically and rigidly in a preferred orientation, and this behaviour may well underlie the activities of amidines, guanidines, isothioureas and isoureas'. The antagonists **(23)**, **(24)** and **(25)** could act in this way.

The observation that there may be a progression from agonist through partial agonist to antagonist, was first made by Prof. C.R. Ganellin on a retrospective analysis of H_2 antagonists and H_1 antagonists (using work of van der Brink[78].), presented as a lecture at the Society of Drug Research meeting in December 1989.

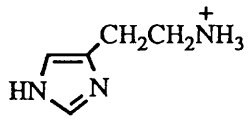
See Fig.4.4. below.

When the agonist, histamine, is compared to the partial agonist 3-(imidazol-4-yl)propyl guanidine (monocation) SKF 91486, there is a decrease in efficacy, but an increase in affinity. The additional binding must be provided by the guanidine function. SKF 91486 can be altered in structure until binding and efficacy improve to give a potent partial agonist, impromidine. Removing the positive charge by replacing the guanidine group by thiourea and cyanoguanidine, results in antagonists, i.e. efficacy has been removed. Finally, removal of that portion of the structure common to all H_2 agonists, i.e. Im-CH_2CH_2, gives the equally potent H_2 antagonists, metiamide and cimetidine.
Thus the Im$(CH_2)_3$ group used to locate the receptor in the agonist does not contribute additional affinity in the antagonist.

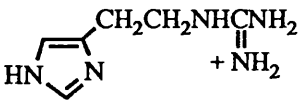
Similarly for H_1 antagonists, see Fig.4.5. below.

The agonist, betahistine, becomes a partial agonist N-ethyl-2-(2-pyridinyl)ethylamine on replacing the N-methyl group with N-ethyl. Substituting the side-chain nitrogen with an N-(4-chloro-phenyl)-N-benzyl-aminoethyl group results in a potent antagonist structure. Removal of the heterocycle 2-Pyr-CH_2 gives an equally potent antagonist.

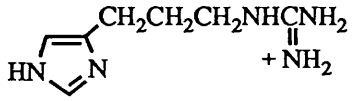
Fig. 4.4. Impromidine and non-basic analogues (H_2, guinea pig atrium)[33.,25.]



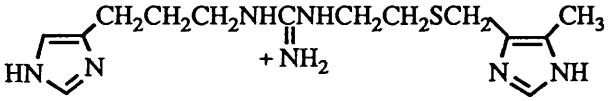
Agonist — Histamine



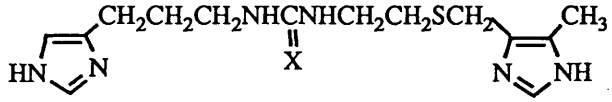
Weak Partial Agonist — N^{α}-guanyl histamine; 0.007 x histamine; 60% maximum; $K_B = 1.3 \times 10^{-4}$M



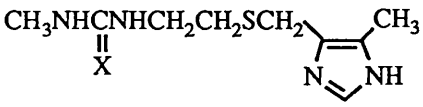
Partial Agonist — SKF 91486; 0.04 x histamine; 70% maximum; $K_B = 2.2 \times 10^{-5}$M



Potent Partial Agonist — Impromidine; 48 x histamine; $K_B = 4.8 \times 10^{-7}$M



Antagonist — X = S, $K_B = 1.2 \times 10^{-6}$M. X = NCN, $K_B = 5.7 \times 10^{-7}$M.

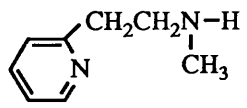


Antagonist — X = S, metiamide, $K_B = 9.1 \times 10^{-7}$M. X = NCN, cimetidine, $K_B = 7.9 \times 10^{-7}$M.

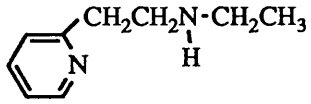
A similar progression was searched for at the H_3 receptor.

In the first approach, the structure of the endogenous agonist histamine is modified by extending the ethylamine chain, including the nitrogen atom in a piperidine ring, and adding a cyclohexyl thioamide group, resulting in the potent antagonist thioperamide (**34**). See Chapter One for the development[8.] of the analogues, activities of which have

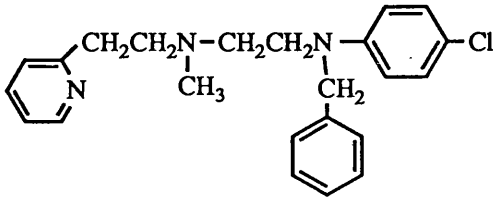
Fig. 4.5. 2-pyridinyl ethylamine derivatives (H_1, guinea pig ileum)[78].



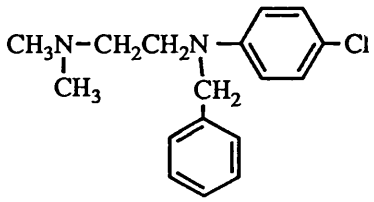
Agonist — Betahistine, 0.05 x histamine



Partial Agonist — 0.005 x histamine, 91% maximum



Antagonist — $K_B = 4.7 \times 10^{-9}$M.



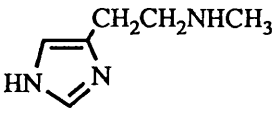
Antagonist — $K_B = 5.7 \times 10^{-9}$M.

not as yet been published. Removing the imidazole ring from thioperamide to give compound **(1)**, resulted in total loss of activity. See Table 4.1.

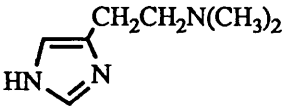
In the second approach, the very potent isothiourea agonist **(48)** is modified structurally to give the di-N,N'-n-butyl derivative, antagonist **(25)**, see Table 4.3. Removing the imidazole ring from **(25)** to give compound **(32)** results in a 5500-fold reduction of activity.

However in the third approach, the result is different, see Fig. 4.6. below.
4-(2-(1-pyrrolidinyl)ethyl)imidazole is a partial agonist.[7] Affinity and efficacy is much improved when the histamine side-chain terminal nitrogen is methylated as in the two

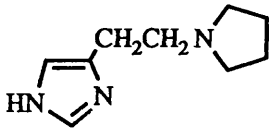
Fig. 4.6. A series of H_3 active compounds (rat cerabral cortex).



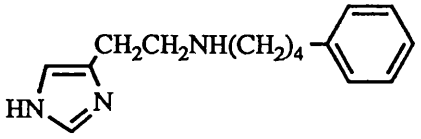
Agonist — 2.7 x histamine



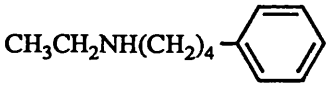
Agonist — 1.7 x histamine



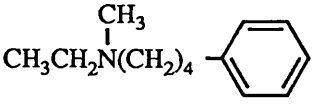
Partial agonist — 0.07 x histamine; 56% maximum; $K_i = 2.2 \times 10^{-6}M$



Antagonist — **(42)**; $K_i = 6.3 \times 10^{-7}M$



Antagonist — **(14)**; $K_i = 1.3 \times 10^{-6}M$



Antagonist — **(15)**; $K_i = 1.1 \times 10^{-6}M$

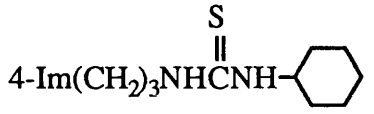
H_3 agonists N^{α}-methyl and N^{α}, N^{α}-dimethyl histamine. Replacing methyl with n-butyl phenyl results in an antagonist, compound **(42)** made by Schunack et al.[56]. Removal of the Im- moiety leaves an antagonist , compound **(14)**, though slightly less potent. The tertiary amine **(15)** has similar potency to **(14)**.

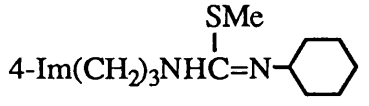
This seems to indicate that H_3 antagonists do not have to possess the imidazole group, once an additional binding site with the receptor which is sufficiently strong has been located.

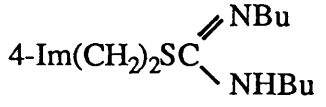
IN VIVO RESULTS

The following results were obtained by courtesy of Prof. J.-C. Schwartz and Dr. M. Garbarg, (who carried out the experiments) at Inserm.

Due to their high in vitro activity, compounds **(19)**, **(21)** and **(25)** were tested in vivo[47], by measuring their effect on the level of τ-methylhistamine (τ-MeHA) in the mouse brain. Thioperamide **(34)** was tested at the same time for comparison.

(19)  $K_i = 13$ nM

(21)  $K_i = 3.6$ nM

(25)  $K_i = 5.4$ nM

The results are represented in Table 4.8. shown below.

Table 4.8. Percentage changes of τ-MeHA level after treatment in relation to dose.

Dose (mg/kg)	**(34)**	**(19)**	**(21)**	**(25)**
3	+ 49 ± 7 (6) *			+ 13 ± 9 (6) NS
10	+ 76 ± 18 (12) *	+ 43 ± 12 (12) *	+ 17 ± 20 (6) NS	+ 21 ± 10 (12) NS
20	+ 71 ± 6 (12) *		+ 37 ± 9 (6) *	
30				+ 23 ± 6 (12) *

( ) number of mice, * $p < 0.05$, NS non significant.

The increase in the catabolite of histamine level (τ-MeHA) is an index of the increase in neuronal histamine release elicited by the H_3 receptor antagonists.

Animals were sacrificed 90 minutes after oral administration of the drug.

A similar increase in neuronal histamine release is elicited by thioperamide (**34**) at 3mg/kg, by (**19**) at 10mg/kg, and by (**21**) at 20 mg/kg. Even at 30mg/kg (**25**) shows only half that effect. Maximal effect is shown by thioperamide at 10mg/kg.

The effect of (**19**) and (**25**) on the τ-MeHA level in the mouse brain over a period of time was also determined. The effect was measured for (**34**) again for comparatative purposes. The results are shown in Table 4.9. below.

Table 4.9. Percentage changes of τ-MeHA level after treatment in relation to time.

Time (hours)	(34)	(19)	(25)
(Dose (mg/kg)	3	10	10)
1.5	+ 49 ± 7 (6) *	+ 43 ± 12 (12) *	+ 21 ± 10 (12) NS
3	+ 43 ± 13 (6) *	- 12 ± 13 (6) NS	- 3 ± 11 (6) NS
6	+ 7 ± 9 (6) NS	- 30 ± 11 (6) *	- 20 ± 9 (6) NS

( ) number of mice, * $p < 0.05$, NS non significant.

Animals were sacrificed at the times shown above in Table 4.9. after oral administration of 3 mg/kg of thioperamide and 10 mg/kg of the two other drugs.

Results shown using 6 mice were obtained from single experiments, while those using 12 mice were obtained from the mean of two experiments (one in the dose response, the other in the kinetic study).

The thioperamide result (+49 ± 7%) is common to both studies, which were performed together during one day.

The kinetic study shows that maximal histamine release is reached by 1.5 hours with thioperamide (**34**) at 3mg/kg and (**19**) and (**25**) at 10 mg/kg.

The result of **(19)** at 6 hours (Table 4.9.) is negative and significant. Such negative results are said to have been observed at Inserm on previous occasions for H_3 antagonists. The effect has not been studied further by them, but two possible reasons are suggested : either, after the receptor is blocked by an antagonist for some time it becomes hypersensitive to endogenous histamine, resulting in the reduction of histamine release observed; or, the effect is non-specific.

None of the three compounds showing nanomolar activity in vitro, is more effective than thioperamide in vivo, but **(19)** is the most potent in vivo of the three.

CONCLUSIONS AND FURTHER SUGGESTIONS

Some of the effects of altering the structure of leads such as thioperamide on the activity at the H_3 receptor have been shown. The alterations have been made with the aims of increasing brain penetration, avoiding toxicity and achieving high activity, but not to the exclusion of a more general investigation of behaviour at the receptor.

1. Thioperamide probably uses the imidazole group for binding to the receptor, since activity is lost by its removal (and subsequent substitution at the 4-piperidine position with groups of different polarities), or its replacement with an alternative heterocycle, 2-pyridine. (Compounds **(1)** to **(7)**).

2. The ester and amide derivatives of urocanic acid are weak antagonists, except for the p-nitrophenyl ester **(9)**, the p-nitrophenyl substituent being followed up by a colleague. (Compounds **(8)** to **(11)**).

3. The possibility of a dipole interaction with the receptor which is direction-dependent is indicated by the comparison of compound **(12)** to the reverse amide **(35)**.

4. Increasing the alkyl side chain length in imidazol-4yl-alkylamino-5-nitro-2-pyridines causes a decrease in activity **(13)**.

5. The theory that the heterocycle-sidechain moiety (e.g. Im-$(CH_2)_3$ or Pyr-$(CH_2)_2$) typical of agonists can be used to locate the receptor, and once an additional binding site which is sufficiently strong has been found on the receptor, it may no longer contribute additional affinity in the antagonist, seems to hold not only for the H_1 and H_2 receptors, but also for the H_3 receptor. This is exemplified by compounds **(14)** and **(15)**, which are active antagonists without the usually necessary imidazole group (c.f. (1.) and (8.)).

6. Opening up the piperidine ring in thioperamide, and changing the now flexible alkyl side chain length shows that three or four methylene groups give the optimum, yet

slightly lower activity than thioperamide ((**19**) and (**20**)). Subsequently methylating the sulphur atom (conversion from thiourea to isothiourea) increases activity (**21**).

7. The ethyl-(imidazol-4-yl)-isothioureas show that substitution of alkyl groups on the isothiourea nitrogens has a profound influence on activity at the H_3 receptor. Generally, three free NH sites (unsubstituted) are most favourable for agonism, while one free NH site is most favourable for antagonism. Moreover, the longer the n-alkyl substituents, the greater the antagonistic effect, suggesting a hydrophobic binding interaction with the receptor. With two free NH sites, the bulk of the monosubstituent may determine that the molecule is an antagonist rather than an agonist. The number of substituents on the isothiourea nitrogens and activity as agonist or antagonist has been linked to conformational preference of the molecule. (Compounds (**22**) to (**28**)).

8. Removing the imidazole group from (**25**) to give (**32**) resulted in loss of activity. Perhaps the additional binding site found is not strong enough.

9. Replacing the imidazole group of the superagonist (**48**) with 2-pyridine results in a partial agonist (**30**) while subsequently extending the side chain by one methylene group, gives and antagonist (**31**). The isourea analogue of this (**33**) is a more potent antagonist. This shows that replacing imidazole with 2-pyridine can work (c.f. (1.)), and that the H_3 receptor is very sensitive to minor structural changes in this type of molecule, a small change in side chain length determining whether a molecule is a partial agonist or an antagonist. Also, making isourea analogues of isothioureas is promising, despite the synthetic difficulties.

10. The three compounds ((**19**), (**21**) and (**25**)) which had nanomolar activity in vitro, did not surpass thioperamide in vivo, with (**19**) being the most potent.

Some suggestions for further work

1. Synthesizing longer n-alkyl chain derivatives of (imidazol-4-yl)ethyl isothiourea, following up the di-n-butyl substituted compound **(25)**, to determine the optimum substituent chain length.
2. Preparing compounds with a longer side chain than S-(3-(2-pyridinyl)propyl) isothiourea **(31)**, to follow up the transition from partial agonist, S-(2-(2-pyridinyl)ethyl)isothiourea **(30)**, to antagonist **(31)**.
3. Making more derivatives with bulky substituents (e.g. Ph, t-Bu)) to follow up the transition from agonist, N-methyl-S-(2-(imidazol-4-yl)ethyl)isothiourea **(26)** to antagonist, N-cyclohexyl-S-(2-(imidazol-4-yl)ethyl)isothiourea **(29)**.
4. Preparing the isourea analogue of N,N'-di-n-butyl-S-(2-(imidazol-4-yl)ethyl) isothiourea **(25)**, N,N'-di-n-butyl-O-(2-(imidazol-4-yl)ethyl)isourea, despite the synthetic difficulties.
5. Synthesizing at least one trial guanidine analogue of the potent isothioureas, for example, N,N'-di-n-butyl-S-(2-(imidazol-4-yl)ethyl)guanidine.

CHAPTER FIVE.

EXPERIMENTAL.

GENERAL PROCEDURES

Starting materials were obtained commercially, except for **(50)** (sample WT76/3156), **(49)** (sample WT76/457A) and **(41^{WT})** (sample WT76/4396A) which were provided by courtesy of Mr. W. Tertiuk. Compounds with the identification YO or FS were made by Miss Y. Ohtake and Miss F. Siddiqi respectively, as part of their 3rd year Medicinal Chemistry projects (supervised by me) with Prof. C. R. Ganellin. Melting points were determined using an Electrothermal (open capillary) melting point apparatus and are uncorrected. TLC was carried out on Merck Kieselgel 60 F254 plates, visualized at 254nm and then with either gaseous iodine or potassium iodoplatinate spray reagent. HPLC (analytical and preparative) was carried out by Mr. S. Corker or myself on a Gilson Binary Gradient System combined with Gilson 714 Software or a Hewlett Packard Integrator System for data analysis. UV spectra were recorded on a Perkin-Elmer 554 UV-VIS Spectrophotometer, using cells of 1cm path length and methanol as solvent. IR spectra were recorded on a Perkin-Elmer 983 Infrared Spectrophotometer, using Nujol mulls between sodium chloride plates, or KBr discs. ^{1}H NMR and ^{13}C NMR spectra 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 being run by the UCL NMR spectroscopy service. NMR spectra are referenced to TMS unless stated otherwise. Mass spectra (FAB and EI) were recorded by Dr. M. Mruzek on a VG 7070H Double Focussing Mass Spectrometer with a Finnigan Incos data system. Microanalyses were carried out by Mr. A. Stones and Mrs. J. Maxwell of the departments' microanalysis service. All chemicals, unless otherwise stated, were obtained from the commercial sources Aldrich and Lancaster.

N-(N'-Cyclohexyl-thiocarbamoyl)piperidine[40., 75.]

(1) (sample YK41C/UCL1010)

Cyclohexylisothiocyanate (1.98g, 0.014mol) was added to piperidine (1.07g, 0.0126mol). A yellow solid was formed with the evolution of heat. The mixture was allowed to attain ambient temperature and toluene (150ml) was added. The solid dissolved, anti-bumping granules were added, and the mixture was heated under reflux for 30 minutes according to the general method[8.]. The colourless solution was decanted from the granules. On reducing the volume to approximately 50ml under vacuum, crystals appeared. The flask was kept in the fridge. The two crops obtained YK41A and YK41B (1.33g and 1.08g respectively, 84% overall crude yield) were recrystallized from toluene, washed with Et_2O, to obtain YK41C, 2.07g, 73% overall yield after recrystallization.

YK41C was obtained as a white crystalline solid : mp 130-132°C (from toluene, washed with Et_2O; reported 132°C[40.] from petroleum ether, 99% and 133°C[75.] from EtOH/H_2O, 3/1, 82-90%).

Solubility, sol (MeOH, $CHCl_3$, DMSO), sp sol (toluene), insol (H_2O).

TLC R_f 0.9 (silica; NH_4OH-MeOH-EtOAc, 1:1:5; also in MeOH; and EtOAc-MeOH, 1:1; and $CHCl_3$/MeOH, 9:1) and 0.82 (silica; $CHCl_3$).

HPLC 99.75% at 7.67 minutes (Lichrosorb RP Select B 250x4mm, 1.0ml/min, UV 254nm 0.05aufs, A/B (30/70) where A is water with 0.5% ortho-phosphoric acid and B is methanol).

UV (MeOH) λ_{max} 246nm (ε 12,900; logε 4.11).

IR (Nujol mull) 3287 (m, NHstr), 2852, 2924 (s, CHstr, Nujol), 1531 (w, NH bend), 1448 (m, CH def, Nujol), 1462 (s, thioamide I), 1231 (s, thioamide II), 1119 (s, CS str), 721 (CH_2 rock) cm^{-1}.

^1H NMR (200MHz, $CDCl_3$) δ 5.28 (s(br), 1H, N<u>H</u>), 4.34 (s(br), 1H, C<u>H</u>NH), 3.76 (s, 4H, C<u>H</u>$_2$NC<u>H</u>$_2$), 2.10, 1.64, 1.42, 1.18 (m, m, m, m, 16H, Cyclohexyl, piperidine).

^{13}C NMR δ (50MHz, $CDCl_3$) 179.9 (<u>C</u>S), 54.2 (<u>C</u>HNH), 48.6 (<u>C</u>H_2N), 33.2 (<u>C</u>H_2CHNH), 25.6 (<u>C</u>$H_2$$CH_2$$CH_2$CHNH), 25.3 (<u>C</u>$H_2$$CH_2$CHNH), 25.0 (<u>C</u>$H_2$$CH_2$N), 24.2 (<u>C</u>$H_2$$CH_2$$CH_2$N).

Mass spectrum (EI) m/e 226 (M^+), 141 $(C_6H_{11}NCS)^+$, 128 (Piperidine-CS)$^+$, 98 $(C_6H_{11}$-NH)$^+$, 84 (Piperidine)$^+$.

Anal. Calcd for $C_{12}H_{22}N_2S$: C, 63.67; H, 9.80; N, 12.37; S, 14.16.

Found : C, 63.68; H, 9.76; N, 12.47; S, 14.36.

<u>N-(N'-Cyclohexyl-thiocarbamoyl)morpholine</u>[72., 75.]

(2) (sample YK45A/UCL1011)

Cyclohexylisothiocyanate (1.98g, 0.014mol) was added to morpholine (1.10g, 0.013mol). Reaction was exothermic, and after initial fuming, a yellow/white solid formed. Toluene (100ml) and antibumping granules were added. The solid dissolved, and the mixture was refluxed for 30 minutes according to the general method.[8.] The pale yellow solution was decanted from the granules, reduced in volume to about 50ml, and allowed to stand in the fridge for 6 days. Big glassy crystals were filtered off. The two

crops obtained, YK45A and YK45B (1.93g and 0.68g respectively) gave a crude overall yield of 2.61g, 91%.

YK45A was obtained as a colourless translucent crystalline solid : mp 137-138°C (from the reaction solvent toluene, not recrystallized because of the quality of the crystals; reported 133-135°C[72] from dilute MeOH, 70-80% and 136°C[75] from EtOH/H_2O, 2/1, 82-90%).

Solubility, sol (MeOH, $CHCl_3$), sp sol (toluene), insol (H_2O, Et_2O).

TLC R_f 0.22 (silica; $CHCl_3$).

HPLC 99.65% at 6.89 minutes (Lichrosorb RP Select B 250x4mm, 1.0ml/minutes, UV 254nm 0.05aufs, A/B (30/70) where A is water with 0.5% ortho-phosphoric acid and B is methanol).

UV (MeOH) λ_{max} 224, 247nm (ε 11,900, 14,500; logε 4.07, 4.16).

IR (Nujol mull) 3273, 2358 (m,w, NHstr), 2853, 2928 (s, CHstr, Nujol), 1528 (s, NH bend), 1454 (s, CH def, Nujol, thioamide II), 1210 (s, thioamide I), 1122 (m, CO str), 1106 (m, CS str), 722 (CH_2 rock) cm^{-1}.

^1H NMR (200MHz, $CDCl_3$) δ 5.60 (d, J = 8.02Hz, 1H, N<u>H</u>), 4.30 (m, 1H, C<u>H</u>NH), 3.74 (m, 8H, morpholine ring), 2.06-1.84 (m, 10H, piperidine ring).

^{13}C NMR (50MHz, $CDCl_3$) δ 181.42 (<u>C</u>S), 66.12 (<u>C</u>H_2 O), 54.38 (<u>C</u>HNH), 47.32 (<u>C</u>H_2N), 33.04 (<u>C</u>H_2CHNH), 25.55 (<u>C</u>$H_2CH_2CH_2$CHNH), 24.94 (<u>C</u>H_2CH_2CHNH).

Mass spectrum (EI) m/e 228 (M^+), 195 (Cyclohexyl-NC-morpholine)$^+$, 141 ($C_6H_{11}NCS)^+$, 98 (C_6H_{11}-NH)$^+$, 86 (Morpholine)$^+$.

Anal. Calcd for $C_{11}H_{20}N_2OS$: C, 57.85; H, 8.83; N, 12.27; S, 14.04.

Found: C, 57.59; H, 8.86; N, 12.10; S, 14.07.

N-(N'-Cyclohexyl-thiocarbamoyl)piperid-4-one

(3) (sample YK69B/UCL1013)

Sodium (0.45g, 19.5mmol) was dissolved in MeOH (anhydrous, 20ml). This was added to a warm solution of piperidone monohydrate hydrochloride (3.0g, 19.5mmol) in MeOH (40ml). The NaCl was alowed to precipitate in a fridge overnight, and then filtered off under gravity. Cyclohexylisothiocyanate (3.03g, 21.45mmol) was added to the filtrate to give a clear lemon yellow solution. Antibumping granules were added, and the mixture refluxed for 1 hour. After filtering under gravity, solvent was removed under vacuum to give an orange solid. This was recrystallized from toluene. Two crops were obtained, YK69B and YK69C (1.4g and 0.65g respectively) giving an overall yield after recrystallization of 44%. Mp YK69C was 145-146°C.

YK69B was obtained as a pale yellow crystallline solid : mp 146-148°C (from toluene).

Solubility, sol (MeOH, $CHCl_3$), sp sol (toluene, H_2O, Et_2O).

TLC R_f 0.82 major, ketone form and 0.70 minor monohydrate form (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 90.40% at 5.15 minutes (ketone form) and 7.46% at 13.65 minutes (monohydrate form) (Lichrosorb RP Select B 250x4mm, 1.0ml/min, UV 254nm 0.05aufs, A/B (50/50) where A is water with 0.1% triethylamine and B is acetonitrile with 0.1% triethylamine).

UV (MeOH) λ_{max} 218, 246nm (ε 12,000, 13,500; logε 4.08, 4.13).

IR (Nujol mull) 3307 (s, NHstr), 2853, 2929 (s, CHstr, Nujol),1711 (s, CO str), 1534 (s, thioamide II), 1501 (w, NH bend), 1460(m, CH def, Nujol), 1217 (m, thioamide I), 1142 (m, CS str), 722 (CH_2 rock) cm^{-1}.

^{1}H NMR (200MHz, $CDCl_3$) δ 5.38 (d, J = 8.0Hz, 1H, NH), 4.34 (m, 1H, CHNH), 4.12 (t, J = 6.17, 6.46Hz, 4H, CH_2NCH_2), 2.62 (t, J = 6.35, 6.28Hz, 4H, CH_2COCH_2), 1.00-2.20 (m, 10H, C_6H_{10}).

^{13}C NMR (50MHz, $CDCl_3$) δ 207.0 (CO), 180.7 (CS), 54.7 (CHNH), 45.0 (CH_2N), 39.8 (CH_2CO), 33.1 (CH_2CHNH), 25.5 ($CH_2CH_2CH_2CHNH$), 24.9 ((CH_2CH_2CHNH).

Mass spectrum (EI) m/e 240 (M^+), 141 $(C_6H_{11}NCS)^+$, 98 $(C_6H_{11}\text{-}NH)^+$, (piperidone)$^+$, 83 $(C_6H_{11})^+$.

Anal. Calcd for $C_{12}H_{20}N_2OS$: C, 59.96; H, 8.39; N, 11.65; S, 13.34.

Found: C, 60.05; H, 8.50; N, 11.56; S, 13.17.

N-(N'-Cyclohexyl-thiocarbamoyl)-4-(2-hydroxyethyl)piperidine

(4) (sample YK57D/UCL1022)

Cyclohexylisothiocyanate (1.69g, 0.012mol) was added to 4-piperidine ethanol (1.42g, 0.011mol). Toluene (100ml) was added, and refluxed for 30 minutes with antibumping granules. After 6 days in the fridge, voluminous small shiny white crystals were filtered off. The two cops obtained (2.3g and 0.33g, both mp 98-9°C, 88% crude yield) were combined and recrystallized from toluene. The solid initially oiled out, then crystallized out overnight in the fridge, 2.5g (mp 98-9°C). This was recrystallized from absolute ethanol with an equivalent volume of H_2O added to induce crystallization. Three crops were obtained, YK57D and YK57E (0.47g and 0.11g respectively, both mp 100-101°C), and YK57F (0.45g, which was attempted to purify by silica gel column chromatography, using $CHCl_3$/MeOH, 9/1 as eluant), giving and overall yield after the

second crystallization of 35%. The starting material, 4-piperidine-ethanol was obtained at 95% purity, and its distillation (65°C, 0.04torr) did not improve purity (by tlc) significantly.

YK57D was obtained as a white crystallline solid : mp 100-102°C (from ethanol/H_2O, 1:1).

Solubility, sol (abs EtOH, $CHCl_3$, Et_2O), sp sol (toluene), insol (H_2O).

TLC R_f 0.69(major) and 0.72(minor), 0.46(major) and 0.59(minor), 0.84 (silica; NH_4OH-MeOH-EtOAc, 1:1:5; EtOAc; Acetone).

HPLC 91.7% at 3.9 minutes and 8.3% at 5.3 minutes (Sphensorb OD52 10x4.5cm, 1.0ml/min, UV 254nm, A/B (40/60) where A is water with 0.1% triethylamine and B is methanol with 0.1% triethylamine).

UV (MeOH) λ_{max} 222, 244nm (ε 12,400, 15,100; logε 4.09, 4.18).

IR (Nujol mull) 3347 (m(br), OH str), 3210 (m, NHstr), 2929, 2851 (s, CHstr, Nujol), 1532 (s, NH bend), 1464, 1449 (s, thioamide II, Nujol, CH def), 1357 (s, OH bend), 1217 (m, thioamide I), 1184 (m, CS str), 1112 (m, CO str), 722 (CH_2 rock) cm^{-1}.

^1H NMR (200MHz, $CDCl_3$) δ 5.34 (d(br), J = 10.0Hz, 1H, N<u>H</u>), 4.60 (d(br), J = 14.0Hz, 2H, C<u>H</u>NC<u>H</u>, equatorial), 4.38 (m, C<u>H</u>NH), 3.76 (t, J = 5.0Hz, 2H, C<u>H</u>$_2$OH), 3.02 (t of d, J = 4.0, 16.0Hz, 2H, C<u>H</u>NC<u>H</u>, axial), 1.00-2.30 (m, 18H, (C<u>H</u>$_2$)$_2$C<u>H</u>C<u>H</u>$_2$, O<u>H</u>, C$_6$<u>H</u>$_{10}$).

^{13}C NMR (50MHz, $CDCl_3$) δ 179.7 (<u>C</u>S), 59.8 (<u>C</u>H_2OH), 54.2 (<u>C</u>HNH), 47.8 (<u>C</u>H_2N), 38.7 (<u>C</u>H_2CH_2OH), 33.1 (<u>C</u>H_2CH_2N), 32.4 (<u>C</u>HCH_2CH_2OH), 31.6 (<u>C</u>H_2CHNH), 25.5 (<u>C</u>$H_2CH_2CH_2$CHNH), 24.9 ((<u>C</u>H_2CH_2CHNH).

Mass spectrum (EI) m/e 270 (M^+), 141 ($C_6H_{11}NCS)^+$, 128 (Piperidine-$CH_2CH_2OH)^+$, 98 (C_6H_{11}-NH)$^+$, 83 (C_6H_{11})$^+$, (Piperidinyl)$^+$.

Anal. Calcd for $C_{14}H_{26}N_2OS$: C, 62.18; H, 9.69; N, 10.36; S, 11.85.

Found: C, 62.36; H, 9.84; N, 10.56; S, 12.10.

N-(N'-Cyclohexyl-thiocarbamoyl)piperidine-4-carboxylic acid hydrate

(5) (sample YK89B/UCL1023)

Isonipecotic acid or piperidine-4-carboxylic acid (1.42g, 0.011mol) was ground up, and cyclohexylisothiocyanate (1.69g, 0.012mol) was added. A mixture of EtOH/H_2O (100ml, 1:1) was added and the solution refluxed for 5 hours, stirring magnetically. The volume was reduced to about 50ml under vacuum, then stored in the fridge. A white solid was filtered off (mp 110-5°C, 0.88g, 30% overall yield), and recrystallized from $CHCl_3$ to obtain YK89B (0.25g).

YK89B was obtained as a white crystallline solid : mp approx 275°C(dec) (from ethanol/H_2O, 1:1).

Solubility, sol (MeOH, DMSO), sp sol (H_2O, $CHCl_3$), insol Et_2O).

TLC R_f 0.05, 0.71 (silica; NH_4OH-MeOH-EtOAc, 1:1:5; MeOH).

UV (MeOH) λ_{max} 218, 246nm (ε 13,500, 14,600; logε 4.13, 4.16).

IR (Nujol mull) 3341 (w, NH str), 3200, 2500 (w(br), OH str), 2951, 2851 (s, CHstr, Nujol), 1695 (s, CO str), 1532 (s, NH bend), 1460 (s, thioamide II, Nujol, CH def), 1334 (m, OH bend), 1200 (m, thioamide I), 1184 (w, CS str), 1096 (w, CO str), 722 (CH_2 rock) cm^{-1}.

^1H NMR (200MHz, $CDCl_3$) δ 5.10–5.40 (d(br), J = 6.35Hz, 1H, N<u>H</u>), 4.15-4.50 (d(br), J = 13.33Hz, 3H, C<u>H</u>NC<u>H</u>, equatorial, C<u>H</u>N), 3.05-3.35 (t of d, J = 12.09,

2.99Hz, 2H, (CHNCH, axial), 2.50-3.75 (m/quint, J_{av} = 2.5Hz, 1H, CHCOOH), 1.00-2.20 (m, 14H, $(CH_2)_2$CHCOOH, cyclohexyl).

^{13}C NMR (50MHz, DMSO/$CDCl_3$) δ 180.0 (CS), 176.1 (COOH), 54.7 (CHNH), 47.0 (CH_2N), approx 40 (CHCOOH, obscured by DMSO), 32.6 (CH_2CHNH), 25.3 ($CH_2CH_2CH_2CHNH$), 25.2 ((CH_2CH_2CHNH).

Mass spectrum (EI) m/e 270 (M^+), 141 $(C_6H_{11}NCS)^+$, 128 (Piperidine-COOH)$^+$, 98 $(C_6H_{11}$-NH)$^+$, 83 $(C_6H_{11})^+$, (Piperidinyl)$^+$.

Anal. Calcd for $C_{13}H_{22}N_2O_2S.0.25H_2O$: C, 56.80; H, 8.25; N, 10.19; S, 11.66.
Found: C, 56.74; H, 8.39; N, 10.16; S, 11.22.

N,N'-Di(cyclohexyl-thiocarbamoyl)piperazine hydrate

(6) (sample YK49C/UCL1012)

Cyclohexylisothiocyanate (3.25g, 0.023mol) was added to piperazine hydrate (1.86g, 0.0096mol). Toluene (140ml) and antibumping granules were added, and the mixture refluxed for 30 minutes. After storing in the fridge overnight, a profuse white solid was filtered off (mp 260-1°C, 3.54g, 100% overall crude yield). This was recrystallized from $CHCl_3$ to obtain YK49C (0.47g). Trituration with Et_2O yielded further crops, YK49D (0.55g, 262-3°C), YK49E (0.11g, 263-5°C), YK49F (0.18g, approx 270°C), giving an overall yield after recrystallization of 37%.

YK49C was obtained as a white crystallline solid : mp 265-266°C (from $CHCl_3$).

Solubility, sol (DMSO), sp sol ($CHCl_3$), insol (Et_2O, MeOH, H_2O).

TLC R_f 0.91 (silica; $CHCl_3$/MeOH, 9:1).

UV (MeOH) λ_{max} 222, 246nm (ε 24,600, 30,700; logε 4.39, 4.49).

IR (Nujol mull) 3193, 2358 (m, NHstr), 2949 (w, CHstr, Nujol), 1532 (s, NH bend), 1462 (s, thioamide II, Nujol, CH def), 1209 (s, thioamide I), 1144 (w, CS str), 720 (CH_2 rock) cm^{-1}.

^1H NMR (200MHz, DMSO) δ 7.18 (d, J = 8.0Hz, 2H, NH), 4.16 (m, 2H, CHNH), 3.82 (s, 8H, piperazine),3.34 (s, H_2O), 1.90-2.96 (m, 20H, cyclohexyl).

^{13}C NMR (50MHz, DMSO) δ 194.68 (CS), 54.32 (CHNH), 45.76 (CH_2N), 31.96 (CH_2CHNH), 25.09 ($CH_2CH_2CH_2CHNH$), 25.0 ((CH_2CH_2CHNH).

Mass spectrum (EI) m/e 368 (M^+), 227 (Piperazine-CSNH-cyclohexyl)$^+$, 141 ($C_6H_{11}NCS$)$^+$, 98 (C_6H_{11}-NH)$^+$, 83 (C_6H_{11})$^+$.

Anal. Calcd for $C_{18}H_{32}N_4S_2.0.25H_2O$: C, 57.95; H, 8.78; N, 15.02; S, 17.18.
Found: C, 57.83; H, 8.71; N, 15.02; S, 17.34.

N-(N'-Cyclohexylthiocarbamoyl-4-(benzaldiminomethyl)-piperidine

(7) (sample YK73C/UCL1014)

Benzaldehyde (2.12g, 20mmol) was added to 4-aminomethylpiperidine (2.28g, 20mmol) in toluene (70ml). The mixture was magnetically stirred and refluxed for 1 hour using a Dean-Stark apparatus to collect H_2O (approx 0.4ml). The colourless solution was reduced in volume to obtain a very pale yellow oil, YK73RM, 4.5g crude identified as the product by NMR (^1H, 60MHz, $CDCl_3$).

Cyclohexylisothiocyanate (3.13g, 22mmol) was added to YK73RM from above. Significant exothermicity was observed as the reaction mixture became more viscous and

yellow. Toluene (50ml) was added, and the solution refluxed for 30 minutes. The clear yellow solution was reduced to dryness under vacuum, toluene (20ml) added and the pale yellow solid, YK73A (mp 135-6°C, 4.83g, 70% overall crude yield) was filtered off. Of this, 1.0g was recrystallized from ^{i}PrOH to obtain white shiny crystals (mp 135-6°C, 0.78g) which were recrystallized again from ^{i}PrOH to obtain translucent shiny crystals of YK73C (0.35g).

YK73C was obtained as a translucent crystallline solid : mp 135-6°C (from ^{i}PrOH).

Solubility, sol (abs EtOH, MeOH, $CHCl_3$), sp sol (Et_2O, H_2O).

TLC R_f 0.88 and 0.39, 0.73 and 0.07 (silica; NH_4OH-MeOH-EtOAc, 1:1:5; $CHCl_3$; two spots possibly due to cis/trans isomers of -N=CHPh).

HPLC 92.65% at 5.48 minutes and 3.40% at 3.35 minutes and 2.13% at 8.52 minutes (Lichrosorb RP Select B, 250x4mm, 1.0ml/min, UV 254nm, 0.05aufs, A/B (30/70) where A is water with 0.1% triethylamine and B is acetonitrile with 0.1% triethylamine).

UV (MeOH) λ_{max} 246nm (ε 34,500; logε 4.54).

IR (Nujol mull) 3353 (m, NHstr), 2925, 2851 (s, CHstr, Nujol), 1642 (m, CN str), 1601, 1580, 1494 (w, CC Ar str), 1536 (s, NH bend), 1494, 1450 (s, thioamide II, Nujol, CH def), 1298 (s, thioamide I), 1122 (s, CS str), 752, 695 (s, CH oopb, monosubst Ar), 722 (CH_2 rock) cm^{-1}.

^{1}H NMR (200MHz, $CDCl_3$) δ 8.25 (s, 1H, N=CHPh), 7.72 (m, 2H, Ph-2,6), 7.44 (m, 3H, Ph-3,4,5), 5.29 (d, J = 8.0Hz, 1H, N<u>H</u>), 4.61 (d, J = 13.09Hz, 2H, C<u>H</u>NC<u>H</u>, equatorial), 4.28 (m, 1H, C<u>H</u>NH), 3.52 (d, J = 6.63Hz, 2H, C<u>H</u>$_2$N), 2.94 (t of d, J = 12.79, 2.36Hz, 2H, C<u>H</u>NC<u>H</u>, axial), 1.00-2.10 (m, 15H, (C<u>H</u>$_2$)$_2$C<u>H</u>C<u>H</u>$_2$, C$_6$<u>H</u>$_{10}$).

^{13}C NMR (50MHz, $CDCl_3$) δ 180.1 (<u>C</u>S), 161.6 (N=<u>C</u>H), 136.0 (Ph-1), 130.7 (Ph-4), 128.6/128.1 (Ph2/3), 67.0 (C<u>H</u>$_2$N), 54.3 (<u>C</u>HNH), 47.7 (<u>C</u>H$_2$N), 37.2 (<u>C</u>HCH$_2$),

33.2 ($\underline{C}H_2CHNH$), 29.9 ($NCH_2\underline{C}H_2CH$), 25.6 ($\underline{C}H_2CH_2CH_2CHNH$), 25.0 ($\underline{C}H_2CH_2CHNH$).

Mass spectrum (EI) m/e 345 $(M+2)^+$ possibly due to self saturation, 204 (Piperidine-$CH_2NHCH_2Ph)^+$, 141 $(C_6H_{11}NCS)^+$, 98 $(C_6H_{11}\text{-}NH)^+$, 91 $(CH_2Ph)^+$, 83 $(C_6H_{11})^+$, (Piperidinyl)$^+$.

Anal. Calcd for $C_{20}H_{29}N_3S$: C, 69.93; H, 8.51; N, 12.23; S, 9.33.

Found: C, 70.04; H, 8.46; N, 12.18; S, 9.76.

(2-Butyl)-3-(imidazol-4-yl)-2-trans-propenoate[29]. hydrochloride

(8) (sample YK34D/UCL1008)

A mixture of trans-urocanic acid (21.0g, 0.15mol), butan-2-ol (700ml, 7.6mol), benzene (300ml), and H_2SO_4 (10ml, 98%), was refluxed using a Dean-Stark trap for 24 hours, according to the method of Burger, Bernabe and Collins[29]. who prepared the base only. The cooled solution was poured into Et_2O (1l). The ether layer was washed with NaOH (100ml, 10% aqueous), dried ($MgSO_4$) and solvent removed under vacuum. A distillation was attempted, but resulted in the material becoming foamy and charring. Extracted with Et_2O (30ml) over 7 days. Formed the HCl salt by treatment with ethanolic HCl. Recrystallized the several crops obtained from absolute ethanol. The material was found to be very hygroscopic. The several crops obtained again were recrystallized from absolute ethanol, triturated with Et_2O to obtain YK34D (0.98g, 3%). YK34D (0.90g) was recrystallized again in the same manner, to give YK34E (0.14g). Both YK34D and YK34E were used for characterization.

YK34D and YK34E were obtained as white crystallline solids : mp 176-177°C and 179-180°C respectively (from EtOH/Et_2O).

Solubility (YK34D), sol (MeOH, H_2O), sp sol (abs EtOH), insol (Et_2O).

TLC (YK34D) R_f 0.75, lower shadow present (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC (YK34E) 95.13% at 8.51 minutes and 4.42% at 3.43 minutes (Lichrosorb RP Select B, 250x4mm, 1.0ml/min, UV 254nm, 0.02aufs, A/B (70/30) where A is water with 0.5% ortho-phosphoric acid and B is methanol).

UV (YK34E) (MeOH) λ_{max} 267nm (ε 18,700; logε 4.3).

IR (YK34D) (Nujol mull) 3400 (vw, NHstr), 3031 (m, >C=CH), 2924, 2850 (s, CHstr, Nujol), 1780 (s, CO str), 1582, 1653 (m,s, CN str, CC str), 1525 (s, NH bend), 1466 (m, CH def, Nujol), 1378 (s, CH_3 sym def), 1099, 1238 (s, CO str), 928 (s, CH str, CH=CH, trans), 841 (m, CH oopd >C=CH), 733 (w, CH_2 rock).

^1H NMR (YK34E) (400MHz, DMSO) δ 9.08 (s, 1H, Im-2), 8.02 (s, 1H, Im-5), 7.55 (d, J = 16.01Hz, 1H, CH=C<u>H</u>), 6.75 (d, J = 16.26Hz, 1H, C<u>H</u>=CH), 4.86 (t of q, J = 6.25Hz, 1H, OC<u>H</u>), 3.1-3.9 (s(vbr), NH, HOD), 1.60 (d of q, J = 1.4, 6.12, 7.30, 7.30Hz, 2H, C<u>H</u>$_2$CH$_3$), 1.22 (d, J = 6.40Hz, 3H, C<u>H</u>$_3$CH), 0.88 (t, J = 7.30, 7.52Hz, 3H, CH$_2$C<u>H</u>$_3$) .

^{13}C NMR (YK34E) (100MHz, DMSO) δ 165.09 (CO), 136.46 (Im-2), 129.76 (Im-5), 129.10 (CH=<u>C</u>H), 121.64 (Im-4), 119.91 (<u>C</u>H=CH), 72.01 (O<u>C</u>H), 28.14 (<u>C</u>H$_3$CH), 19.20 (<u>C</u>H$_2$CH$_3$), 9.43 (CH$_2$<u>C</u>H$_3$).

Mass spectrum (YK34D) (EI) m/e 194 (M^+) 138 (Im-CH=CH-COOH)$^+$, 121 (Im-CH=CH-CO)$^+$, 66 (Im)$^+$.

Anal. (YK34D) Calcd for $C_{10}H_{14}N_2O_2$.1HCl.0.25H_2O :

C, 50.29; H, 6.58; N, 11.73; Cl, 16.33.

Found : C, 50.31; H, 6.40; N, 11.94; Cl, 15.94.

p-Nitrophenyl-3-(imidazol-4-yl)-2-trans-propenoate hydrochloride

(9) (sample YK37E/UCL1009)

Freshly distilled thionyl chloride (2ml, 27.5mmol) was added to a finely ground mixture of p-nitrophenol (1.51g, 10.8mmol) and trans-urocanic acid (1.5g, 10.8mmol) in a flask fitted with a $CaCl_2$ drying tube. The method is similar to that used by Bruice and Sturtevant[28]. who prepared the analogous butyrate compound. The mixture was heated gradually to an oil bath temperature of 140°C. Heating was maintained for 4 hours, and the mixture cooled to room temperature slowly. The brown powder was extracted with $CHCl_3$/ MeOH (150ml, 2:1) with heating. Undissolved material was filtered off, and the filtrate treated with charcoal. On reducing the volume under vacuum, Et_2O was added to induce precipitation of solid (1.96g, 61%, crude overall yield). This was recrystallized from MeOH, and the product washed with Et_2O to remove any p-nitrophenol traces, giving YK37E and YK37F (0.67g and 1.01g respectively, overall yield after recrystallization, 60%).

YK37E was obtained as an off-white crystallline solid : mp 220-222°C (from MeOH).

Solubility, sol (DMSO), sp sol (MeOH, H_2O), insol ($CHCl_3$, Et_2O).

TLC R_f 0.73 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 99.99% at 8.89 minutes (Lichrosorb RP Select B, 250x4mm, 1.0ml/min, UV 254nm, 0.02aufs, A/B (70/30) where A is water with 0.5% ortho-phosphoric acid and B is methanol).

UV (MeOH) λ_{max} 286nm (ε 27,000; logε 4.43).

IR (Nujol mull) 3500 (vw, NHstr), 3040 (m, Ar-H str), 2885, 2880 (s, CHstr, Nujol), 1720 (vs, CO str), 1660 (w, CN str), 1590, 1650 (s, CC str), 1500, 1540, 1590 (m,

Ar· str), 1500 (m, NH bend), 1340, 1540 (s, NO str), 1210, 1260 (s, CO str), 990, 710 (m, CH oopd), 860 (m, CH oopv, p-substit Ar).

^{1}H NMR (200MHz, DMSO) δ 9.34 (s, 1H, Im-2), 8.34 (d, J = 8.97 Hz, 2H, Ph-2,6), 8.18 (s, 1H, Im-5), 7.86 (d, J = 16.19Hz, 1H, CH=C<u>H</u>), 7.56 (d, J = 7.55 Hz, 2H, Ph-3,5), 7.11 (d, J = 16.19Hz, 1H, C<u>H</u>=CH).

^{13}C NMR (50MHz, DMSO) δ 163.5 (CO), 155.0 (Ph-1), 145.0 (Ph-4), 136.6 (Im-2), 132.0 (Im-5), 128.3 (CH=CH) , 125.2 (Ph-2), 123.1 (Ph-3), 122.6 (Im-4), 118.5 (<u>C</u>H=CH).

Mass spectrum (EI) m/e 260 $(M+1)^+$ trace, 121 $(Im\text{-}CH{=}CH\text{-}CO)^+$, 139 $(HO\text{-}C_6H_4\text{-}NO_2)^+$, 93 $(Im\text{-}CH{=}CH)^+$.

Anal. Calcd for $C_{12}H_9N_3O_4$.HCl: C, 48.74; H, 3.41; N, 14.21; Cl, 11.99.

Found: C, 48.43; H, 3.48; N, 14.16; Cl, 11.85.

<u>N-Benzyl-3-(imidazol-4-yl)-2-trans-propenamide maleate hydrate</u>

(10) (sample YK115C/UCL1028)

Thionyl chloride (10ml) was placed in a flask fitted with a $CaCl_2$ drying tube. trans-Urocanic acid (3.0g, 0.022mol) was added in small portions. The mixture was stirred magnetically. The exothermic orange solution was heated with an oil bath, bath temperature rising to 140°C in 1 hour. Most of the $SOCl_2$ was distilled off and the remainder removed under vacuum (0.1torr/room temperature/0.5 hour). The acyl chloride was an orange powder, YK97A (4.1g, 98% crude yield) identified by TLC and NMR (^{1}H, 60 MHz, DMSO).

A warm suspension of urocanyl chloride, HCl (YK97A, 1.71g, 8.8mmol) in CH_2Cl_2 (50ml, anhydrous) was added in Pasteur pipette portions to a magnetically stirred solution of benzylamine (2.85g, 26.5mmol) in CH_2Cl_2 (50ml, anhydrous). The orange suspension became yellow/brown with slight exothermicity. The mixture was stirred at room temperature for 3 hours, then left unstirred overnight, fitted with a $CaCl_2$ drying tube. Sticky yellow/brown solids were filtered off, and the filtrate reduced in volume under vacuum to give a brown oil (1.58g, 79% crude yield). This was dissolved in absolute ethanol (5ml) and a solution of maleic acid (1.77g, 15mmol) in absolute ethanol (10ml) was added. Precipitation of a yellow solid was induced by addition of Et_2O (10ml, anhydrous), and subsequent storage in the fridge for 3 hours. A yellow solid was filtered off (1.43g, 47% overall crude maleate yield) which was recrystallized from ^{i}PrOH to give YK115C and YK115D (0.81g and 0.1g respectively, 64% recrystallization yield).

YK115C was obtained as a pale yellow crystallline solid : mp 149-150°C (from ^{i}PrOH).

Solubility, sol (MeOH, H_2O), sp sol (^{i}PrOH), insol ($CHCl_3$, Et_2O).

TLC R_f 0.56, 0.78 (silica; NH_4OH-MeOH-EtOAc, 1:1:5; MeOH).

HPLC 93.02% at 6.85 minutes and 5.80% at 7.86 minutes (Lichrosorb RP Select B, 250x4mm, 1.0ml/min, UV 254nm, 0.02aufs, A/B (70/30) where A is water with 0.5% ortho-phosphoric acid and B is methanol).

UV (MeOH) λ_{max} 214, 284nm (ε 24,400, 24,900; logε 4.39, 4.40).

IR (Nujol mull) 3410, 3364 (w, m, NHstr), 2951, 2921, 2852, 2663 (s, CH str, OH str, NH str, Nujol), 1685 (s, CO str, amide I), 1624 (CC str, α-β-unsat CO), 1624, 1567, 1495 (m, aromatic), 1567 (m, CO_2^- antisym str, NH bend, amide II), 1463 (s,

CH def, Nujol), 1358 (s, CO_2^-sym str), 1000 (w, CH oopd, C=C-C=O), 728 (m, CH_2 rock, CH oopv, monosubstit. Ar), 697 (m, CH oopv, monosubstit. Ar).

^1H NMR (200MHz, D_2O, referenced to maleic acid δ 6.28) δ 9.00 (s, 1H, Im-2), 7.86 (s, 1H, Im-5), 7.30-7.60 (m, 6H, Ph, CH=C<u>H</u>), 6.71 (d, J = 16.8Hz, 1H, C<u>H</u>=CH), 6.28 (s, 2H, HOOC-C<u>H</u>=C<u>H</u>-COOH), 4.27 (s, 2H, C<u>H</u>$_2$).

^{13}C NMR (50MHz, D_2O, referenced to maleic acid δ 170.6, 137.6) δ 170.6 (<u>C</u>OOH), 166.8 (<u>C</u>O), 137.6 (<u>C</u>H-COOH), 135.0 (Im-2), 134.3 (Ph-1), 129.0 (Im-5), 128.8 (Ph-2), 127.5 (CH=<u>C</u>H), 127.3 (Ph-3), 125.1 (Ph-4), 123.3 (Im-4) , 119.7 (<u>C</u>H=CH), 43.3 (NH-<u>C</u>H$_2$).

Mass spectrum (EI) m/e 227 $(M)^+$ 281 (trace), 136 $(Im\text{-}CH{=}CH\text{-}CO\text{-}NH)^+$, 121 $(Im\text{-}CH{=}CH\text{-}CO)^+$, 106 $(Ph\text{-}CH_2\text{-}NH)^+$, 93 $(Im\text{-}CH{=}CH)^+$, 77 $(Ph)^+$, 66 $(Im)^+$.

Anal. Calcd for $C_{13}H_{13}N_3O.C_4H_4O_4.0.25H_2O$: C, 58.70; H, 5.07; N, 12.08.

Found: C, 58.57; H, 4.91; N, 11.82.

N-(2-Phenylethyl)-3-(imidazol-4-yl)-2-trans-propenamide maleate hydrate

(11) (sample YK99I/UCL1027)

Urocanyl chloride hydrochloride (YK97A) was prepared as for YK115C/UCL1028 **(10)**.

A suspension of YK97A (2.0g, 0.0104mol) in $CHCl_3$ (50ml, anhydrous) was added in small portions over 10 minutes to a magnetically stirred solution of phenethylamine (3.77g, 0.031mol) in $CHCl_3$ (50ml, anhydrous). Reaction was exothermic in the first 15 minutes, and the reaction was then stirred at room temperature for 2.5 hours, a $CaCl_2$ drying tube having been fitted. The mixture was filtered under vacuum. The solid obtained was $PhCH_2CH_2NH_3^+Cl^-$, shown by NMR (1H, DMSO). The filtrate was reduced in volume under vacuum, then treated with charcoal in a $CHCl_3$ solution, to give a crude yield of 2.32g, 92%. This was passed through a silica gel column, using MeOH as the eluant. The purer fractions were combined, reduced in volume under vacuum, treated with maleic acid in absolute ethanol/Et_2O. The salt thus obtained (YK99B, mp 141-142°C, 0.25g) was recrystallized from ^iPrOH to obtain two crops, YK99D ans YK99E (0.14g and 0.07g respectively). The remaining fractions were combined and treated in the same way as the purer fractions, to give cude maleate YK99F (mp 138-139°C, 1.5g). Overall yield of maleate was 1.25g, 23%. YK99F was recrystallized from ^iPrOH to give YK99G (mp 145-146°C, 0.93g), which was recrystallized from absolute ethanol to give YK99I and YK99J (0.55g and 0.20g respectively).

YK99I was obtained as a pale yellow crystallline solid : mp 152-153°C (from ^iPrOH).

Solubility, sol (MeOH, ^iPrOH, H_2O), insol ($CHCl_3$, Et_2O).

TLC R_f 0.56, 0.78 (silica; NH_4OH-MeOH-EtOAc, 1:1:5; MeOH).

HPLC 99.12% at 7.81 minutes (Lichrosorb RP Select B, 250x4mm, 1.0ml/min, UV 254nm, 0.02aufs, A/B (70/30) where A is water with 0.5% orthophosphoric acid and B is methanol).

UV (MeOH) λ_{max} 211, 282nm (ε 24,200, 25,700; logε 4.38, 4.41).

IR (Nujol mull) 3419, 3323 (w, NH str), 2951, 2922, 2851, 2664 (s, CH str, OH str, NH str, Nujol), 1679 (s, CO str, amide II), 1642 (C=C str, α–β-unsat CO), 1642, 1565, 1512 (m, aromatic), 1565 (m, CO_2^- antisym str, NH bend, amide II), 1461 (s, CH def, Nujol), 1362 (m, CO_2^- sym str), 996 (w, CH oopd, C=C-C=O), 723 (w, CH_2 rock), 747, 699 (m, CH oopv, monosubstituted Ar).

^{1}H NMR (200MHz, D_2O, referenced to maleic acid δ 6.28) δ 8.96 (s, 1H, Im-2), 7.83 (s, 1H, Im-5), 7.30-7.55 (m, 6H, Ph, CH=C<u>H</u>), 6.55 (d, J = 18.0Hz, 1H, C<u>H</u>=CH), 6.28 (s, 2H, HOOC-C<u>H</u>=C<u>H</u>-COOH), 3.24 (t, J = 7.0Hz, 2H, HN-C<u>H</u>$_2$), 2.44 (t, J = 9.0Hz, 2H, C<u>H</u>$_2$-Ph).

^{13}C NMR (50MHz, D_2O, referenced to maleic acid δ 170.8, 139.1) δ 170.8 (<u>C</u>OOH), 166.9 (<u>C</u>O), 139.1 (<u>C</u>H-COOH), 135.0 (Im-2), 134.4 (Ph-1), 129.1 (Im-5), 128.9 (Ph-2), 128.6/126.5 (CH=<u>C</u>H/Ph-3), 124.9 (Ph-4), 123.3 (Im-4), 119.6 (<u>C</u>H=CH), 40.8 (NH-<u>C</u>H$_2$), 34.5 (NH-CH$_2$<u>C</u>H$_2$).

Mass spectrum (EI) m/e 241 $(M)^+$ 150 $(Im\text{-}CH{=}CH\text{-}CO\text{-}NH\text{-}CH_2)^+$, 137 $(Im\text{-}CH{=}CH\text{-}CO\text{-}NH_2)^+$, 121 $(Im\text{-}CH{=}CH\text{-}CO)^+$, 93 $(Im\text{-}CH{=}CH)^+$, 66 $(Im)^+$.

Anal. Calcd for $C_{14}H_{15}N_3O.C_4H_4O_4.\ 0.4H_2O$: C, 59.30; H, 5.47; N, 11.54.

Found: C, 59.32; H, 5.43; N, 11.39.

N-(2-Phenylethyl)-3-(imidazol-4-yl)propanamide oxalate

(12) (sample YK218B/UCL1063)

1-(Phenylethyl)-3-(imidazol-4-yl)-2-trans-propenamide maleate hydrate **(11)**, sample YK99I/UCL1027 obtained previously (0.252g, 0.69mmol) was suspended in water (15ml), stirring magnetically. Na/Hg amalgam[37] (2.3%, 20.08g, equivalent to 20 mmol Na) in small portions, causing much frothing. After stirring at room temperature for 1hour the grey solution was decanted from the mercury and filtered through celite. A clear solution (pH 14) was obtained, extracted with $CHCl_3$ (4 x 15ml) and dried ($MgSO_4$). The aqueous layer was evaporated to a small volume (5ml) and extracted with $CHCl_3$ (3 x 5ml) and dried ($MgSO_4$). The extracts were combined, filtered and evaporated to yield a colourless oil (YK218A, 0.162g, 96% crude yield). YK218A (0.162g, 0.66mmol) was dissolved in absolute ethanol (3ml) and treated with oxalic acid (0.070g, 0.78mmol) dissoved in absolute ethanol (2ml). The mixture was evaporated to dryness (0.22g), stirred with Et_2O and the supernatant layer decanted to remove excess oxalic acid. Evaporation yielded crude oxalate (0.21g, 95%). This was recrystallized from ^{i}PrOH, yielding a first crop of white crystalline solid, YK218B (0.107g) and a second crop of white sticky solid, YK218C (0.07g).

YK218B was obtained as a white crystalline solid : mp 122-123°C (from ^{i}PrOH).

Solubility, sol (H_2O, DMSO), insol ($CHCl_3$, Et_2O, EtOAc).

TLC R_f 0.49 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 99.34% at 7.40min (and 0.33% at 9.50min due to starting material YK99I) (Lichrosorb RP Select B 250x4mm, 0.75ml/min, UV 211nm 0.05aufs, A/B (3/1) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 5% water).

UV (MeOH) λ_{max} 211nm (ε 11,400; logε 4.06).

IR (Nujol mull) 3313, 3099 (w, NH str), 2851, 2922, 2951 (s, CH str, OH str, Nujol), 1736 (w, C=O str, COOH), 1644 (m, C=O str, CONH, amide I), 1612, 1546,1524 (m, aromatic ring), 1546 (m, , CONH, amide II, NH bend), 1460 (m, CH def), 775, 698 (w, CH oopv, monosubstituted phenyl), 721 (w, CH_2 rock) cm^{-1}.

^1H NMR (400MHz, DMSO) δ 8.40 (s, 1H, Im-2), 7.99 (t, J = 5.48Hz, 1H, C=O-NH) , 7.26-7.31 (m, 2H, Ph-2,6), 7.16-7.22 (m, 3H, Ph-3,4,5), 7.09 (s, 1H, Im-5), 3.90-6.10 (s(vbr), ImNH, obscured by HOD), 3.27 (q, J = 7.65, 5.90, 6.80Hz, 2H, $HNC\underline{H}_2$), 2.80 (t, J = 7.58, 7.16Hz, 2H, $C\underline{H}_2C{=}O$), 2.69 (t, J = 7.72, 7.02Hz, 2H, $C\underline{H}_2Ph$), 2.41 (t, J = 7.86, 7.16Hz, 2H, Im-$C\underline{H}_2$).

^{13}C NMR (100MHz, DMSO, referenced to DMSO δ39.5) δ 170.71 $(\underline{C}O_2H)_2$, 164.68 (C=O), 139.48 (Im-2), 133.83 ($\underline{C}H_2Ph$) 133.62 (Im-5), 128.64 (Ph-2), 128.34 (Ph-3), 126.10 (Ph-4), 115.85 (Im-4), 40.24 ($NH\underline{C}H_2$), 35.16 ($ImCH_2\underline{C}H_2$), 34.14 (Ph-1), 20.87 (Im-CH_2).

Mass spectrum (EI) m/e 243 (M^+), 152 ($Im(CH_2)_2CONHCH_2)^+$, 139 ($Im(CH_2)_2C(OH)NH)^+$, 123 ($Im(CH_2)_2C{=}O)^+$, 104 ($CH_2CHPh)^+$, 95 ($Im(CH_2)_2)^+$, 91 ($CH_2Ph)^+$, 81 ($ImCH_2)^+$, 77 $(Ph)^+$, 68 $(Im)^+$, 45 $(COOH)^+$.

Anal. Calcd for $C_{14}H_{17}N_3O.0.85C_2H_2O_4$: C, 58.96; H, 5.89; N, 13.14.

Found: C, 58.94; H, 5.83; N, 13.25.

2-(3-(Imidazol-4-yl)propyl)-amino-5-nitro-pyridine oxalate

(13) (sample YK215C/UCL1073-J)

3(Imidazol-4-yl)propylamine dihydrochloride (**41**^{WT}) (0.192g, 0.97mmol) was dissolved in absolute ethanol (2ml). A solution of Na (0.05g, 2.17mmol) in absolute

ethanol (21ml) was added. The NaCl precipitated overnight was filtered off and the solvent evaporated to give the base as a pale yellow oil, YK214A (0.158g, >100% probably due to some NaCl carryover). A solution of imidazol-4-yl-3-propylamine, YK214A (0.158g, max. 0.97mmol) in ^{i}PrOH (10ml) was added to a solution of 2-chloro-5-nitropyridine (0.154g, 0.97mmol) in ^{i}PrOH (10ml). Colour changes were observed from pale yellow, through bright yellow, green and orange. The reaction mixture was refluxed for 21 hours. Purification was by silica column chromatography, using $CHCl_3$/MeOH (1/1) as the eluant mixture. The selected middle fractions, on solvent evaporation, gave YK218B (0.112g, 0.45mmol). This was extracted with ^{i}PrOH (10ml) at room temperature to remove any traces of silica. A couple of slightly less pure fractions gave a yellow oil, YK214C (36mg, 0.15mmol). Combined yield : 0.148g, 0.60mmol, 62%). To a solution of YK214B and YK214C (0.148g, 0.60mmol) in absolute ethanol (10ml) was added oxalic acid (0.124g, 1.41mmol) in absolute ethanol (5ml). The mixture was left at room temperature over 2 days and evaporated to dryness to yield a yellow solid (0.138g, 0.41mmol, 68%). This crude solid was recrystallized from ^{i}PrOH (50ml), to give as a first crop a pale yellow crystalline solid, YK215C (50mg), and a second crop YK215D (13mg). Combined yield : 0.065g, 31%.

YK215C was obtained as a pale yellow hygroscopic crystalline solid : mp 181-182°C (from ^{i}PrOH).

Solubility, sol (H_2O, DMSO), insol (Et_2O, $CHCl_3$).

TLC R_f 0.59 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 95.26% at 5.79min (Lichrosorb RP Select B 4+250x4mm, 1ml/min, UV 254nm, A/B (55/45) where A is water and B is acetonitrile with 5% water).

UV (MeOH) λ_{max} 218, 356 nm (ε 13,000, 15,700; logε 4.11, 4.19).

IR (Nujol mull) 3237 (w, NH str, >NH), 2951, 2921, 2851 (s, CH str, Nujol), 2327 (w, NH str, $>NH_2^+$), 1610 (s, C=C str, aromatic, C=O antisymmetric str, CO_2^-), 1542

(w, NO asymmetric str, conjugated C-NO_2), 1507 (C=C str, aromatic, NH bend, >NH), 1460 (m, CH def, >CH_2, Nujol), 1329 (s, C=O symmetric str, CO_2^-, NO symmetric str, conjugated C-NO_2) cm^{-1}.

^1H NMR (400MHz, DMSO) δ 8.90 (d, J = 2.77Hz, 1H, Pyr-6), 8.46 (s, 1H, Im-2), 8.18 (br s, 1H, NH), 8.10 (br d, J = 8.12Hz, 1H, Pyr-4), 7.21 (s, 1H, Im-5), 6.56 (d, J = 9.40Hz, 1H, Pyr-3), 3.40 (br s, 2H, CH_2N), 3.0-5.5 (v br s, ImNH, PyrNH, OH), 2.67 (t, J = 7.52, 7.62Hz, 2H, ImCH_2), 1.88 (quintet, J = 7.23Hz, 2H, Im$CH_2C\underline{H}_2$).

^{13}C NMR (100MHZ, DMSO, referenced to DMSO δ39.05) δ 163.62 $(CO_2H)_2$, 161.37 (Pyr-5), 146.91 (Pyr-6), 134.22 (Pyr-2), 133.99 (Im-2), 133.67 (Pyr-4), 131.69 (Im-5), 115.95 (Pyr-3), 108.60 (Im-4), 27.79 (CH_2NH), 25.52 (ImCH_2), 22.16 (Im$CH_2\underline{C}H_2$).

Mass spectrum (EI) m/e 247 (M^+), 230 $(Im(CH_2)_3N(Pyr\text{-}N{=}O))^+$, 200 $(Im(CH_2)_3N\text{-}Pyr)^+$, 152 $(CH_2NH\text{-}Pyr\text{-}NO_2)^+$, 95 $(Im(CH_2)_2)^+$, 82 $(ImCH_2\text{-}H)^+$, 78 $(Pyr\text{-}H)^+$, 68 $(Im\text{-}H)^+$, 45 $(CO_2H)^+$.

Anal. Calcd for $C_{11}H_{13}N_5O_2.C_2H_2O_4$: C, 46.29; H, 4.48; N, 20.77
Found : C, 46.11; H, 4.17; N, 20.21.

N-Ethyl-4-phenylbutylamine hydrochloride[68].

(14) (sample FS19C/UCL1221-A)

Since the paper of Shklyaev and Cheryslikin[68] which reports **(14)** was unavailable except for a chemical abstract, the method of DeVries, Bloom, Dutia, Katocs and Largis[39] for an analogous reaction was followed.

***Synthesis of 4-phenyl-n-butylmethylamide*: (14/I)**: 4-Phenylbutylamine (1.498g, 10.0mmol), Et_3N (2.024g, 20.0mmol) and dry CH_2Cl_2 (10ml, molecular sieves) in a flask fitted with a thermometer, condenser and septum, was flushed with N_2 via a bubbler, and cooled to 0°C with an ice/salt bath. Acetyl chloride (1.02g, 0.93ml, 13.0mmol) was added dropwise by injection causing a total temperature rise of 25°C during the 20 minutes of reaction. The pale yellow reaction mixture was stirred for 3 hours. Water was added, and the CH_2Cl_2 layer seperated. The organic layer was washed with HCl (4M) and saturated NaCl solution, dried ($MgSO_4$), to give crude amide FS1A (1.71g, 89% crude yield, reported 98%[68.]). This was distilled (160-164°C/0.1torr) to give the amide FS1B as a pale yellow oil (1.177g, 62% after distillation), which was characterized by ^1H NMR, IR (C=O stretch at 1646 cm^{-1}) and mass spectrometry (M^+ = 191).

Note: This reaction was repeated on a 50mmol scale to yield more amide FS9B (8.34g, 87% after distillation, 142-144°C/0.2torr).

***Synthesis of N-Ethyl-4-phenylbutylamine* (14)** : Dry THF (25ml, distilled over Na) was placed in a flask fitted with a thermometer, condenser, septum, and stirred magnetically. This was flushed with N_2 and cooled with an ice/water bath. $LiAlH_4$ (1.14g, 30mmol) was added slowly, followed by dropwise addition of 4-phenyl-n-butylmethylamide, FS9B (1.91g, 10mmol). The reaction mixture was refluxed overnight. $Na_2SO_4.10H_2O$ was added to the reaction mixture in small portions while cooling. The mixture was creamy white after standing 2 hours at room temperature. It was filtered through a layer of dry $MgSO_4$ washing with Et_2O. Removal of solvent gave the crude amine as a liquid FS11A (1.63g, 92% overall yield, reported 46%[68.]). This was distilled to give FS11B as a colourless liquid (0.942g, 53%, 80-82°C/0.25torr).

Note: This reaction was repeated on a 37mmol scale to give more amine FS17B (56% yield after distillation, 90-94°C/0.3torr).

The amine (1.08g, 6.1mmol) was converted to the HCl salt by treatment with ethanolic HCl (6.1mmol in 5ml). Removal of solvent under vacuum, followed by azeotroping and washing with Et_2O gave crude HCl salt (1.352g). This was recrystallized from Et_2O with the minimum amount of MeOH to give FS19C (0.726g, 56% yield).

FS19C was obtained as a white crystalline solid : mp 152-154°C (from EtOH/MeOH, 9/1).

Solubility, sol (H_2O, MeOH), sp sol (EtOAc, $CHCl_3$), insol (Et_2O).

TLC R_f 0.43 (silica; MeOH-$CHCl_3$-Et_3N, 80:20:1drop).

HPLC 99.94% at 4.89 minutes (Lichrosorb RP Select B 250x10mm, 5ml/min, UV 215nm, A/B (50/50) where A is water with 0.1% trifluoroacetic acid and B is methanol with 0.1% trifluoroacetic acid).

UV (MeOH) λ_{max} 209, 257 nm (ε 5,800, 500; logε 3.76, 2.68).

IR (Nujol mull) 2948 (NH str), 748, 700 (CH oop) cm^{-1}.

^1H NMR (200MHz, DMSO) δ 9.2-9.0 (m, 2H, $-NH_2^+$), 7.4-7.1 (m, 5H, Ph), 2.96-2.76 (m, 4H, $C\underline{H}_2NHC\underline{H}_2$), 2.68-2.54 (m, 2H, $PhC\underline{H}_2$), 1.80-1.55 (m, 4H, $PhCH_2C\underline{H}_2C\underline{H}_2$), 1.30-1.14 (t, 3H, $C\underline{H}_3$, J = 8.0Hz).

Mass spectrum (EI) m/e 177 (M^+), 92 $(PhCH_2-H)^+$, 60 $(CH_3NHCH_2CH_3-H)^+$.

Anal. Calcd for $C_{12}H_{19}N.HCl$: C, 67.49; H, 9.43; N, 6.55; Cl, 16.59.
Found : C, 67.64; H, 9.49; N, 6.50; Cl, 16.87.

N-ethyl-N-methyl-4-phenylbutylamine oxalate

(15) (sample FS37C/UCL1229-J)

The Clarke-Eschweiler reaction of Cope and Burrows[34] was followed. To N-ethyl-4-phenylbutylamine, **(14)** sample FS17B (2.09g, 0.012mol) in formic acid (2.75g, 0.06mol) was added aqueous formaldehyde (37%, 1.10g, 0.013mol). After bubbling had ceased, the mixture was heated on a steam bath for four hours. The mixture was poured into ice/water and made strongly basic by the addition of NaOH pellets. The product was extracted with Et_2O. Removing solvent under vacuum gave crude product as a dark yellow liquid (1.59g, 70% crude base yield). This was distilled (90-94°C/0.8torr) to give FS31B as a colourless liquid (1.23g, 54% overall yield of base). Most of this (1.14g) was converted to the HCl salt by treatment with ethanolic HCl and cooling with an ice/water bath. Precipitation with Et_2O gave a yellow oil, FS35A. FS35A (1.00g, 4.4mmol) was converted to base by treatment with aqueous NaOH (4M) followed by extraction with Et_2O, and drying ($MgSO_4$). This was converted to oxalate by addition of oxalic acid (1.1 molar equivalent) in EtOH, removal of solvent under vacuum and azeotroping with Et_2O. The crude oxalate was recrystallized from Et_2O with the minimum of MeOH required, to give FS37C (0.964g, 78%).

FS37C was obtained as a white crystalline solid : mp 110-112°C (from EtOH/MeOH, 9/1).

Solubility, sol (H_2O, MeOH), sp sol (EtOAc, $CHCl_3$), insol (Et_2O).

TLC R_f 0.28 (silica; MeOH-$CHCl_3$-Et_3N, 80:20:1drop).

HPLC 99.95% at 5.09 minutes (Lichrosorb RP Select B 250x10mm, 5ml/min, UV 215nm, A/B (50/50) where A is water with 0.1% trifluoroacetic acid and B is methanol with 0.1% trifluoroacetic acid).

UV (MeOH) λ_{max} 209, 260 nm (ε 6,800, 1,000; logε 3.83, 3.00).

IR (Nujol mull) 2921 (NH str) 748, 700 (CH oop) cm^{-1}.

^1H NMR (200MHz, DMSO) δ 7.42-7.11 (m, 5H, Ph), 3.15-2.90 (m, 4H, $C\underline{H}_2NHC\underline{H}_2$), 2.80-2.54 (m, $PhC\underline{H}_2$, $NC\underline{H}_3$, obscured by DMSO), 1.80-1.40 (m, 4H, $PhCH_2C\underline{H}_2C\underline{H}_2$), 1.35-1.00 (t, 3H, $C\underline{H}_3$, J = 8.0Hz).

Mass spectrum (EI) m/e 191 (M^+), 91 $(PhCH_2)^+$, 72 $(CH_2NMeEt)^+$, 58 $(NMeEt)^+$, 44 $(COO^-)^+$.

Anal. Calcd for $C_{13}H_{21}N.(COOH)_2$: C, 64.04; H,8.24; N, 4.98.
Found : C, 63.66; H, 8.16; N, 4.95.

1-Benzyl-4-(2-pyridinyl)-piperidin-4-ol hydrochloride[61].

(16) (sample YK111F/UCL1033)

The general method of Gilman and Spatz[48]. was used, resulting in a method similar to the patent of Raabe et al[61].,which prepares **(16)**, but was obtained after this preparation was completed. A round bottom 3 neck flask was fitted with a septum, pressure equalizing dropping funnel, air condenser/bubbler, and flushed with N_2. Et_2O (200ml, anhydrous) was added, and nBuLi (0.045mol, 18ml of 2.5M in hexanes) was injected. The apparatus was continuously flushed with N_2, and the vessel cooled using acetone/Drikold, and stirred magnetically. A solution of 2-bromopyridine (6.32g, 0.04mol) in Et_2O (50ml, anhydrous) was added over 5 minutes to the nBuLi via the dropping funnel. The solution turned orange, then blood red, while stirring for 7 minutes 1-benzyl piperidone (9.46g, 0.05mol) in Et_2O (50ml, anhydrous) was added over 5 minutes to the solution of 2-pyridyl lithium. Colour changes over the next 30 minutes were from red to brown to a yellow solution with brown precipitate. Bath

temperature was -40 to -18°C. The reaction mixture was hydrolyzed with ice cold NH_4Cl (0.045mol, 2.4g in 80ml H_2O), turning bright yellow. The clear yellow ether layer was sepeated from the aqueueous layer. It was extracted with aqueous HCl (10%, 40ml, 0.12mol) to turn univeral indicator paper red. The acid extract was neutralized with $NaHCO_3$ (7.72g, 0.09mol). After much frothing, obtained pH7 (universal paper), and a dark red oil appeared. Extracting this mixture ('RM') with Et_2O (220ml), drying ($MgSO_4$), and removing solvent under vacuum, gave and orange oil (5.62g) which was shown to be 1-benzyl-4-piperidone on vacuum distillation (2.17g, 88°C/0.02torr) by NMR (^1H, 60MHz, $CDCl_3$). The mixture ('RM') after standing for several days, was extracted with $CHCl_3$ (75ml). dried ($MgSO_4$) and solvent removed to give a red oil (11.0g, 100% crude overall yield). The red oil was passed through a silica gel column ($CHCl_3$/MeOH, 3:1 as eluant), without significant seperation (combined yield after column, 7.85g, 75%, reported 87%[61]. after distillation 180-200°C/0.1 torr). The middle fractions (most pure) obtained, yielded a red viscous oil (3.0g, 0.011mol), which was dissolved in $CHCl_3$ (5ml) and triturated with Et_2O. The orange solid obtained (3.45g) was recrystallized from ^iPrOH and Et_2O to induce crystallization, to yield YK111C (0.64g) identified as product by NMR (^1H, 200MHz, D_2O). The other fractions obtained from the column were combined to give an orange oil (4.85g, 0.018mol) in absolute ethanol (25ml). Addition of Et_2O (50ml, anhydrous) caused oiling/precipitating out of YK111E (2.2g). YK111E and solid obtained from the filtrate of YK111C, were combined and recrystallized from absolute ethanol, washing with ^iPrOH, to give YK111F (0.45g) and YK111G (0.35g, on Et_2O trituration).

Note: The reaction was repeated several times since the product is an intermediate in the synthesis of 4-(2-pyridinyl) piperidine (see later in this chapter): on a 0.05mol scale to give crude HCl salt YK135C (mp 243-244°C, 73% yield); on a 0.1mol scale to give crude base YK173A (yield >100%, contains some starting ketone); on a 0.24mol scale to give crude HCl salt (15%), and on recrystallization from EtOH the major crop obtained was YK223A (mp 240-242°C, total recrystallization yield 83%).

YK111F was obtained as a white crystallline solid : mp 254-255°C (from EtOH, reported[61]. base mp 58-60°C from hexane/ether).

Solubility, sol (H_2O), sp sol (absolute EtOH), insol (^iPrOH, $CHCl_3$, Et_2O).

TLC R_f 0.81 (silica; $CHCl_3$-MeOH, 3:1).

HPLC 100.00% at 4.33 minutes (Lichrosorb RP Select B, 250x4mm, 1.0ml/min, UV 254nm, A/B (90/10) where A is water with 0.1% triethylamine and 0.5% ortho-phosphoric acid and B is methanol with 0.1% triethylamine).

UV (MeOH) λ_{max} 209, 259nm (ε 18,900, 7,100; logε 4.28, 3.85).

IR (Nujol mull) 3149 (m, OH str), 2951, 2922, 2851, 2664 (s, CH str, Nuj), 2513 (s(br), NH str), 1607, 1521, 1449 (m, C=C str, Ar), 1521 (m, NH bend), 1449 (s, CH def, Nuj), 1376 (m, OH bend), 1147 (m CO str), 719 (m, CH_2 rock), 768, 699 (s, CH oopv, monosubstituted Ar).

^1H NMR (200MHz, D_2O, referenced to Ph δ 7.3) δ 8.6 (d, J = 7.0 Hz, 1H, Pyr-6), 8.4 (t, J = 8.0 Hz, 1H, Pyr-5), 8.1 (d, J = 9.0 Hz, 1H, Pyr-3), 7.8 (t, J = 8.0 Hz, 1H, Pyr-4), 7.3 (s, 5H, Ph), 4.0 (s, 2H, CH_2), 2.7-3.2 (m, 4H, $(CH_2)_2N$), 1.3-2.0 (m, 4H, $(CH_2)_2C$).

^{13}C NMR (50MHz, D_2O, referenced to Ph-1 of the benzyl group δ 141.4) δ 158.0 (Pyr-2), 147.4 (Pyr-6), 141.4 (Ph-5), 131.3 (Ph-1), 130.4 (Ph-2), 129.4 (Ph-3), 128.4 (Ph-4), 126.5 (Ph-3), 123.8 (Pyr-4), 69.1 (CH_2), 60.9 (COH), 47.6 (CH_2N), 33.5 ($\underline{C}H_2CH_2N$).

Mass spectrum (EI) m/e 268 $(M)^+$ 250 $(M - H_2O)^+$, 177 $(M - CH_2\text{-}Ph)^+$, 159 $(M - CH_2\text{-}Ph - H_2O)^+$, 79 $(Pyr + H)^+$.

Anal. Calcd for $C_{17}H_{20}N_2O.2HCl$: C, 59.83; H, 6.50; N, 8.21.
Found : C, 59.85; H, 6.47; N, 8.23.

N-Cyclohexyl-4-(2-pyridinyl)-1-piperidine carbothioamide trifluoroacetate

(17) (sample YK411B/UCL1196)

Crude 4(2-pyridinyl)piperidine (**47**), samples YK399C and YK399G (70mg and 44mg respectively, 0.70mmol) obtained previously, and cyclohexylisothiocyanate (0.100g, 0.70mmol) in absolute ethanol (30ml) was refluxed for 51 hours. Solvent was removed under vacuum to give the product a crude brown oil YK411A (240mg, 113% due to solvent retention). This was purified by semi-preparative HPLC (Lichrosorb RP Select B 250x10mm column, UV 254nm detector, 1ml/min flow, A/B = 95/5 to 60/40 over 20 minutes, where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 5% water).On removal of solvent under vacuum the product was obtained as a brown oil (0.145g, 46% overall yield). This was dissolved in ^{i}PrOH at room temperature and filtered through a sinter (to remove any traces of silica). Solvent was removed under vacuum (room temperature/0.5torr/8 hours) to give YK411B.

YK411B was obtained as an off-white partial solid: mp 110-112°C (from preparative HPLC/^{i}PrOH).

Solubility, sol (H_2O, ^{i}PrOH, $CHCl_3$).

TLC R_f 0.84 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 98.98% at 21.26 minutes (Lichrosorb RP Select B 250x4mm, 1ml/min, UV 254nm 0.1aufs, A/B (95/5 to 60/40 at 20 minutes) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 5% water).

UV (MeOH) λ_{max} 212-221(br), 246 nm (ε 17,800, 21,800; logε 4.25,4.34).

IR (KBr) 3432, 2932 (m, NH str, salt), 3242 (s, OH str, H bonded), 2946, 2932, 2851 (s, CH str), 1675, 1624, 1531 (m, Ar str, C=O str, NH bend), 1480 (m, amide II),

1451, 1446 (w, CH def), 1383 (m, CH_3 sym def), 1369 (m, OH bend), 1250 (m, amide I), 1184, 1116 (s, CF str, CS str, CO str), 717 (w, CH_2 rock) cm^{-1}.

^1H NMR (400MHz, $CDCl_3$) δ 8.84 (d, J = 5.33 Hz, 1H, Pyr-6), 8.30 (t, J = 7.86Hz, 1H, Pyr-5), 7.74 (t, J = 5.89, 6.52Hz, 1H, Pyr-4), 7.68 (d, J = 7.68Hz, 1H, Pyr-3). 5.45 (s(br), 1H, NH), 4.82 (d(br), J = 13.54Hz, 2H, CHNCH, equatorial), 4.34 (s(br), 1H, CHNH), 3.60 (t of t, J = 12.81, 3.54Hz, 1H, CH-Pyr), 3.18 (t, J = 11.93Hz, 2H, CHNCH, axial), 2.13 (m, 4H, Pyr-CH(CH_2)$_2$), 1.87-1.63, 1.47-1.37, 1.22-1.13 (m, 10H, C_6H_{10}), 1.22 (d, J = 6.14Hz, 0.9H, $2CH_3$ of ^{i}PrOH).

^{13}C NMR (100MHz, $CDCl_3$) δ 180.71 (CS), 160.16 (Pyr-2), 144.72 (Pyr-6), 142.61 (Pyr-5), 124.40 (Pyr-4), 123.62 (Pyr-3), 54.52 (Cyclohexyl-1), 47.17 (CH_2N), 40.20 (Pyr-CH), 33.04 (CH_2CH_2N), 30.68 (Cyclohexyl-2), 25.49/24.90 (Cyclohexyl-3/4).

Mass spectrum (EI) m/e 303 $(M)^+$, 205 (Pyr-Pip-CSNH)$^+$, 161 (Pyr-Pip)$^+$, 83 (Pip)$^+$, $(C_6H_{11})^+$, 78 (Pyr)$^+$.

Anal. Calcd for $C_{17}H_{25}N_3S.1.2CF_3CO_2H.0.15^iPrOH$:

C, 53.06; H, 6.15; N, 9.35; S, 7.14.
Found: C, 53.34; H, 6.30; N, 9.59; S, 7.34.

N-Cyclohexyl-2-(3-pyridinyl)-1-piperidine carbothioamide trifluoroacetate

(18) (sample YK427B/UCL1220-J)

2(3-Pyridinyl)piperidine or (±) anabasine (0.200g, 1.23mmol, 89% purity, checked by HPLC) obtained from Sigma and cyclohexylisothiourea (0.174g, 1.23mmol) were refluxed in EtOH (30ml) for 10 days. Solvent was removed under vacuum, and the

residue dried (0.1torr/room temperature/4 hours) to give the crude product YK427A (0.332g, 89% overall crude yield). Part of this (223mg) was purified by semi-preparative HPLC (Lichrosorb RP Select B 250x10mm column, UV 220nm detector, 4ml/min flow, A/B is 95/5 going to 60/40 over 20 minutes and 40/60 at 30 to 37 minutes, where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 0.1% trifluoroacetic acid and 5% water) to give the product as a yellow oil (110mg). This was dried and dissolved in ^{i}PrOH and filtered under gravity and dried (0.3torr/room temperature/24 hours) to give YK427B (85mg, semi-preparative HPLC yield 38%).

YK427Bwas obtained as a brown oil.

Solubility, sol (H_2O, MeOH), insol (Et_2O).

TLC R_f 0.86 with trace at 0.62 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 99.96% at 22.55 minutes (Lichrosorb RP Select B 250x4mm, 1ml/min, UV 254nm, A/B (95/5 at 0 to 60/40 at 20 minutes and 40/60 at 30-37 minutes) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 0.1% trifluoroacetic acid and 5% water).

UV (MeOH) λ_{max} 216, 244 nm (ε 18,700, 15,900; logε 4.27,4.20).

IR (Nujol mull) 3600-3100 (br, OH str, NH str), 2923, 2851 (s, CH str, Nujol), 1683, 1674, 1528 (m, aromatic ring system, C=O str, NH bend), 1460 (m, CH def, Nujol, Amide II), 1375 (m, CH_3 sym def, Nujol, OH bend), 1254 (m, Amide I), 1200, 1133 (s, C-F str, C=S str, C-O str), 798, 686 (m, CH oopd, Pyr), 721 (CH_2 rock) cm^{-1}.

^{1}H NMR (400MHz, CD_3OD) δ 8.71 (d, J = 5.53Hz, 1H, Pyr-6), 8.65 (s, 1H, Pyr-2), 8.39 (d, J = 8.22Hz, 1H, Pyr-4), 8.00-7.96 (m, 1H, Pyr-5), 6.85 (s(br), 1H, NH), 4.40-4.35 (m, 1H, C<u>H</u>NH), 4.17 (d(br), J = 14.69Hz, 1H, C<u>H</u>N), 3.92 (septet, J = 6.15Hz, 0.5H, CH from ^{i}PrOH), 2.90 (d of d of d, J = 10.86, 10.47, 3.79Hz, 1H, C<u>H</u>N, axial), 2.48 (d of d, J = 14.67, 3.29Hz, 1H, C<u>H</u>N, equatorial), 2.08-1.99 (m,

4H, $C\underline{H}_2CH_2NCHC\underline{H}_2$), 1.81-1.17 (m, 12H, $C_6\underline{H}_{10}$, $C\underline{H}_2(CH_2)_2N$), 1.15 (d, J = 6.12Hz, 3H, $2CH_3$ from ^iPrOH).

Mass spectrum (FAB) m/e 304 $(M+H)^+$, 205 $(Pyr\text{-}Pip\text{-}CSNH)^+$, 161 $(Pyr\text{-}Pip)^+$, 84 $(Pip+H)^+$, $(C_6H_{12})^+$, 78 $(Pyr)^+$.

Anal. Calcd for $C_{17}H_{25}N_3S.1.5CF_3CO_2H.0.5C_3H_8O$:
C, 51.18; H, 6.09; N, 8.33; S, 6.36.
Found : C, 51.21; H, 6.21; N, 8.08; S, 6.21.

N-(N'-Cyclohexyl-thiocarbamoyl)-3(4-imidazolyl)propylamine

(19) (sample YK192B/UCL1053)

3(4-Imidazolyl)propylamine dihydrochloride (**41**^{WT}) (1.0g, 5.05mmol) in absolute ethanol (20ml) was converted to the base by treatment with Na (0.23g, 0.01mol) in absolute ethanol (20ml). The NaCl precipitated overnight in the fridge was filtered off under gravity and solvent evaporated to give the base as a pale yellow oil, which yielded off-white 'fishscale' crystals overnight, YK188B (0.45g, 3.6mmol, 75% yield).

3(4-Imidazolyl) propylamine, YK188B (0.45g, 3.6mmol) was dissolved in absolute ethanol (40ml). Cyclohexylisothiocyanate (0.56g 4mmol) was added dropwise to give a pale yellow transparent solution. The mixture was refluxed for 45 minutes, evaporated, azeotroping with Et_2O to obtain a white amorphous solid, (0.92g, 96% crude yield). This was recrystallized from EtOAc, oiled out, and on scratching obtained a white crystalline solid, YK192B (0.69g), and a second crop of white solid YK192C (0.105g). Combined yield : 0.795g, 86%.

YK192B was obtained as a white crystalline solid : mp 114-115°C (from EtOAc).

Solubility, sol (^{i}PrOH), sp sol (H_2O, $CHCl_3$, toluene), insol (Et_2O).

TLC R_f 0.30 with trace on baseline (silica; $CHCl_3$-MeOH, 5/1); 0.51 with trace at 0.30-0.38 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 100% at 7.29min (Lichrosorb RP Select B 5 μm 4+250x4mm, 1ml/min, UV 254nm 0.05 aufs, A/B (1/4) where A is acetonitrile and B is water with 0.3% ortho-phosphoric acid).

UV (MeOH) λ_{max} 215, 240 nm (ε 16,500, 14,900; logε 4.22,4.17).

IR (Nujol mull) 3580, 3262 (w, m, NH str, >NH, S=C-NH), 3072 (m, CH str, C=C-H), 2923, 2852 (s, CH str, CH2, Nujol), 1551 (s, NH bend, >NH), 1462 (s, CH def, amide II, S=C-NH, Nujol), 1258 (m, amide I, S=C-NH), 1167 (w, C=S str), 840 (w, CH oopd, R_2C=CHR), 723 (w, CH_2 rock) cm^{-1}.

^{1}H NMR (200MHz, $CDCl_3$) δ 7.56 (s,1H, Im-2), 6.98 (br s, 2H, NH), 6.79 (s, 1H, Im-5), 3.98 (br s, 1H, C<u>H</u>NH), 3.55 (br s, 1H, C<u>H</u>N), 2.64 (t, J = 6.67, 6.42Hz, 2H, Im-C<u>H</u>$_2$), 2.05-1.17 (m, 12H, Im-CH_2C<u>H</u>$_2$, remaining cyclohexyl CH_2's).

^{13}C NMR (50MHz, $CDCl_3$) δ 180.22 (C=S), 137.01 (Im-2), 134.35 (Im-5), 116.58 (Im-4), 53.16 (cyclohexyl-1), 43.63 (<u>C</u>H_2NH), 32.90 (cyclohexyl-2), 28.92 (Im-<u>C</u>H_2), 25.47 (Im-CH_2<u>C</u>H_2), 24.88 (cyclohexyl-3), 23.63 (cyclohexyl-4).

Mass spectrum (EI) m/e 266 (M^+), 232 $(Im(CH_2)_3NCN\text{-}C_6H_{11})^+$, 141 $(SCN\text{-}C_6H_{11})^+$, 109 $(Im(CH_2)_3)^+$, 95 $(Im(CH_2)_2)^+$, 82 $(ImCH_2)^+$.

Anal. Calcd for $C_{13}H_{22}N_4S$: C, 58.61; H, 8.32; N, 21.03.
Found : C, 58.56; H, 8.43; N, 21.02.

N-(N'-Cyclohexyl-thiocarbamoyl)-4(4-imidazolyl)butylamine oxalate

(20) (sample YK251F/UCL1088)

4-(Imidazol-4-yl)butylamine, a crude brown oil (**49**) WT76/457A (4.67g, 33.6mmol) was dissolved in absolute ethanol and treated with oxalic acid (8.40g, 93.3mmol) in absolute ethanol. A cream solid (8.65g) was filtered off, washing thoroughly with Et_2O to remove excess oxalic acid. The solid was stired in Et_2O (100ml) at room temperature for 1hour, filtered and dried at 0.02torr/90°C/6hr, to obtain an off-white solid, YK189B (8.51g, 26.65mmol, 79% yield), mpt. 164-166°C. 4-(Imidazol-4-yl)butylamine dioxalate was satisfactorally analyzed by ^1H NMR (400MHz), MS and microanalysis.

YK189B (1.12g, 3.5mmol) was suspended in absolute ethanol (75ml), stirring and warming gently. A solution of Na (0.18g, 7.7mmol) in absolute ethanol (10ml) was added at room temperature. The NaCl precipitated in the fridge over 2 days was filtered off and on solvent evaporation the base was obtained as a yellow solid, YK250A (0.391g, 2.8mmol, 81% yield).

YK250A (0.391g, 2.8mmol) was dissolved in absolute ethanol (20ml). Cyclohexyl isothiocyanate (0.44g, 3.1mmol) was added dropwise. More absolute ethanol (10ml) was added and the mixture refluxed for 3 hours. Solvent was evaporated, azeotroping with Et_2O and petroleum spirit (60-80°C) was added to solidify the crude in the fridge overnight. A yellow hygroscopic solid, (0.392g, 1.39mmol, 50% yield) was filtered off, and dried over P_2O_5 in a vacuum desiccator for several days. Attempted recrystallization of the base from EtOAc and from ^{i}PrOH proved unsuccessful, and the fractions obtained were recombined to give crude base, YK250B (0.375g).

YK250B (0.357g, 1.27mmol) in ^{i}PrOH/Et_2O, was treated with oxalic acid (0.172g, 1.9mmol) in ^{i}PrOH. After 2 hours at room temperature a creme solid was filtered off, washing thoroughly with Et_2O, to give YK251A (0.281g), mpt. 102-104°C. A second crop was obtained as a pale yellow sticky solid, YK251B (56mg). Crude oxalate yield :

0.274g, 58%. It was attempted to recrystallize YK251A from absolute ethanol with insufficient yield. The fractions were recombined, together with YK251B (0.211g total) and the cream solid recrystallized from ^{i}PrOH (10ml). An off-white crystalline solid, YK251F (0.110g, 0.31mmol, 24%) was obtained.

YK251F was obtained as an off-white crystalline solid : mp 107-108°C (from ^{i}PrOH).

Solubility, sol (H_2O, DMSO, MeOH), insol (Et_2O, $CHCl_3$).

TLC R_f 0.64 with a trace at 0.57 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 97.06% at 15.23min (Lichrosorb RP Select B 5μm 4+250x4mm, 0.75ml/min, UV 254nm 0.05aufs, A/B (3/1) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 5% water).

UV (MeOH) λ_{max} 240, 213 nm (ε 12,800, 18,400; logε 4.11, 4.26).

IR (Nujol mull) 3253, 2323 (w, NH str, NH, N^+H), 2921, 2851 (s, CH str, CH_2, Nujol), 1628, 1376 (m, antisymmetric str, CO_2^-), 1546 (m, NH bend), 1460 (s, CH def, CH_2, Nujol, amide II, S=C-NH), 1229 (m, amide I, S=C-NH), 1124 (w, C=S str), 848 (w, CH oopd, R_2C=CHR), 722 (CH_2 rock) cm^{-1}.

^{1}H NMR (400MHz, DMSO) δ 8.47 (s, 1H, Im-2), 7.42 (t, J = 4.98Hz, 1H, $CH_2N\underline{H}$-C=S), 6.95 (br s, 1H, CS-N$\underline{H}$), 7.19 (s, 1H, Im-5), 4.10-3.80 (v br s, 1H, C$\underline{H}$NH), 3.37 (br s, 2H, CH_2N), 2.60 (t, J = 7.14, 7.51Hz, 2H, ImCH_2), 1.81 (br d, J = 9.99Hz, 2H, Im$CH_2CH_2C\underline{H}_2$), 1.67-1.46 & 1.27-1.09 (m, 12H, Im$CH_2C\underline{H}_2$, remaining cyclohexyl CH_2's), 3% ^{i}PrOH present.

^{13}C NMR (100MHz, DMSO, referenced to DMSO δ39.50) δ 180.80 (C=S), 163.79 $(CO_2H)_2$, 134.12 (Im-2), 133.76 (Im-5), 116.02 (Im-4), 51.58 (cyclohexyl-1), 42.84 (CH_2NH), 32.21 (cyclohexyl-2), 28.16 (Im-CH_2), 25.68 (Im-$CH_2CH_2\underline{C}H_2$), 25.09 (Im-$CH_2\underline{C}H_2$), 24.44 (cyclohexyl-3), 24.27 (cyclohexyl-4).

Mass spectrum (EI) m/e 280 (M^+), 181 ($Im(CH_2)_4NCS)^+$, 141 ($C_6H_{11}NCS)^+$, 123, 109, 95, 81 ($Im(CH_2)_n)^+$ where n = 4, 3, 2, 1 respectively, 67 $(Im)^+$, 56 $((CH_2)_4)^+$, 45 $(CO_2H)^+$.

Anal. Calcd for $C_{14}H_{24}N_4S.0.85(CO_2H)_2.0.03^iPrOH$:

C, 52.86; H, 7.29; N, 15.62.
Found: C, 52.99; H, 7.23; N, 15.24.

S-Methyl-N-cyclohexyl-N'-(3-(imidazol -4-yl)propyl)isothiourea dihydroiodide hemihydrate

(21) (sample YK336C/UCL1134-C_2)

N-(N'-Cyclohexyl-thiocarbamoyl)-3(imidazol-4-yl) propylamine **(19)**, sample YK192B, (0.150g, 0.56mmol) obtained previously, was dissolved in absolute ethanol (1ml). The colourless solution was cooled with an ice/water bath. HI aqueous (54-56%, 1.7g, 0.73mmol) was added dropwise to give an orange solution. Et_2O (20ml) was added, causing a yellow oil to appear. The mixture was evaporated to dryness, azeotroping with Et_2O and dried at room temperature at 0.5torr/3 hours, to yield the hydroiodide salt as a yellow hygroscopic solid, YK336A (0.262g, 119%, probably due to excess HI. MeI (0.11g, 0.8mmol) was added to a yellow solution of YK336A (0.262g, max. 0.56mmol) in MeOH (2ml) and the mixture was refluxed for 5 hours. Solvent was evaporated, followed by drying at 40°C/0.13torr/2 hours, to yield a yellow solid, YK336B (0.288g, 0.54mmol, 96%). YK336B (0.272g, 0.51mmol) was dissolved in ^iPrOH (3ml), reducing filtrate volume to 1ml. Trituration with Et_2O caused a brown oil to appear. The ether was decanted, washing with more ether. The brown oil was evaporated to dryness and dried further over P_2O_5 at 60°C/0.3torr/3 hours to obtain

an extremely hygroscopic yellow solid, YK336C (0.222g, 0.41mmol, 80%). While further drying YK336C over P_2O_5 at 0.3torr/4 hours/40-80°C, it melted but survived to give brown crystalline product (0.179g).

YK336C was obtained as an extremely hygroscopic brown crystalline solid : mp not obtained due to excessive hygroscopicity.

Solubility, sol (H_2O, MeOH, DMSO), insol (Et_2O).

TLC R_f 0.62 (silica, NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 98.55% at 13.13min (Lichrosorb RP Select B 7μm 4+250x4mm, 1ml/min, UV 240nm 0.05aufs, A/B (85/15 to 50/50 over 20min) where A is water with 0.1% trifluoroacetic acid and 0.5g/500ml hexanesulphonic acid (Na salt) and B is acetonitrile with 0.1% trifluoroacetic acid and 5% water).

UV (MeOH) λ_{max} 219nm (ε 41,800; logε 4.62).

IR (KBr) 3403 (s(br), OH str, H bonded OH), 3006 (s, CH str, C=C-H), 2851, 2929 (s, CH str, $>CH_2$, $-CH_3$), 2355 (m, NH str), 1601 (s, NH bend), 1522 (s, C=N str), 1446 (m, CH def), 1285 (m, OH bend), 807 (m, CH oopd, $R_2C=CHR$) cm^{-1}.

^1H NMR (400MHz, DMSO) δ 8.90-8.77 (v br d, 1H, Im-2), 8.45-8.35 (v br s, 1H, NH), 7.44-7.37 (v br s, 1H, Im-5), 3.60 (br s, 1H, CH-N), 3.54-3.18 (m, >20H, obscured by HOD, Im-CH_2, CH_2N, HOD), 2.67 (s, 3H, S-CH_3), 1.99-1.03 (m, 12H, Im-$CH_2C\underline{H}_2$, remaining cyclohexyl CH_2's), trace impurity at δ3.17.

Mass spectrum (FAB) m/e 281 (M^+), 265 (M^+-Me), 109 $(Im(CH_2)_3)^+$, 124 $(Im(CH_2)_3NH)^+$, 168 $(Im(CH_2)_3NHCS)^+$, 156 ($C_6H_{12}N{=}CSMe)^+$.

Anal. Calcd for $C_{14}H_{24}N_4S.2HI.0.5H_2O$:

C, 30.84; H, 4.99; N, 10.27; I, 46.55; S, 5.88.

Found : C, 30.82; H, 4.59; N, 10.02; I, 46.53; S, 5.93.

N,N'-Dimethyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dihydrobromide[21].

(22) (sample YK302C/UCL1124)

A solution of 2-(hydroxyethyl)imidazol-4-yl **(50)** (sample WT76/3156) (0.500g, 4.46mmol) and 1,3-dimethyl-2-thiourea (0.536g, 5.14mmol) in HBr aqueous (48%, 5ml, 44.2mmol) was heated under reflux for 26 hours. The orange reaction mixture was evaporated, azeotroping with ^{i}PrOH, then Et_2O. Repeated stirring with Et_2O, decanting and then drying at 0.3torr/5 hours/room temperature, yielded a sticky orange solid (1.776g). This was crystallized from ^{i}PrOH/EtOH (1/1, 50ml), reduced in volume (15ml). Two crops of cream solid were obtained, YK302A (0.998g), mp 202-203°C, and YK302C (0.103g). Combined yield : 1.101g, 3.06mmol, 66%. YK302A and YK302B (1.096g, 2.93mmol) were recrystallized together from ^{i}PrOH/EtOH (2/1, 60ml), reducing the filtrate volume (50ml). A cream crystalline solid YK302C (0.819g) was obtained, together with a second crop of cream solid YK302D (0.123g), mp 189-190°C.

YK302C was obtained as a cream crystalline solid : mp 203-204°C (from ^{i}PrOH/EtOH, 2/1; reported[21]. mp 203-204°C).

Solubility, sol (H_2O), sp sol (EtOH), insol (Et_2O, EtOAc, $CHCl_3$).

TLC R_f 0.41 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 98.78% at 9.78min (Lichrosorb RP Select B 7µm 4+250x4mm, 1ml/min, UV 215nm 0.5aufs, A/B (9/1) where A is water with 0.5% acetonitrile and 0.1% trifluoroacetic acid and 0.005M hexanesulphonic acid (Na salt) and B is acetonitrile with 5% water and 0.1% trifluoroacetic acid).

UV (MeOH) λ_{max} 214nm (ε 15,000; logε 4.18).

IR (KBr) 3001 (s, NH str, amino salt), 2919, 2869 (s, CH str, CH_2, CH_3), 2831 (m, CH str, N-CH_3), 1615 , 1522 (s, NH bend, C=N str, C=C str), 1467, 1430 (m CH def), 1305, 1152 (m, C-N str, aromatic secondary amine, aliphatic amine), 840 (m, CH oopd, R_2C=CRH), 730 (w, CH_2 rock) cm^{-1}.

^1H NMR (200MHz, D_2O, referenced to ^tBuOH at δ1.28) δ 8.69 (s, 1H, Im-2), 7.42 (s, 1H, Im-5), 3.52 (t, J = 6.91, 6.94Hz, 2H, CH_2S), 3.24, (t, J = 6.67, 6.98Hz, 2H, $ImCH_2$), 3.04 (d, J = 17.77Hz, 6H, $NHC\underline{H}_3$, $NC\underline{H}_3$).

Mass spectrum (FAB) m/e 199 (M^++1), 127 $(Im(CH_2)_2S)^+$, 95 $(Im(CH_2)_2)^+$, 71 $(MeN{=}C{-}NHMe)^+$.

Anal. Calcd for $C_8H_{14}N_4S.2HBr$:

C, 26.68; H, 4.48; N, 15.56; Br, 44.38; S, 8.90.

Found : C, 26.76; H, 4.50; N, 15.31; Br, 44.06; S, 8.83.

N-N'-Diethyl-S-(2-(imidazol-4-yl)ethyl)isothiourea trioxalate

(23) (sample YK365E/UCL1211-J_2)

2-(Hydroxyethyl)imidazol-4-yl **(50)** (sample WT76/3156) (0.500g, 4.46mmol) was dissolved in aqueous HBr (48%, 5ml, 4.2mmol). N,N'-diethylthiourea (0.65g, 4.91mmol) was added and the mixture refluxed for 89 hours. It was attempted to obtain a solid HBr salt by azeotroping and precipitating with Et_2O. Removal of solvent gave the crude HBr salt as an orange oil (1.81g, 105% yield due to solvent retention). This was converted to base by treatment with aqueous $NaHCO_3$ (to pH9) followed by extraction with $CHCl_3$ (4 x 10ml) and drying ($MgSO_4$). The crude base obtained YK366A (0.142g) was converted to the oxalate salt by treatment with oxalic acid (2.5 molar equivalents) in EtOH and precipitation with Et_2O, to give the oxalate salt YK366B

(0.116g). The aqueous layer was reduced to dryness under vacum and extracted with ^{i}PrOH (60ml) to give crude base as a white oil (1.10g). This was converted to oxalate by treatment with oxalic acid (2 molar equivalents) in ^{i}PrOH. Precipitation with Et_2O gave oxalates YK365A and YK365B (0.92g and 0.42g respectively, overall oxalate yield including YK366B, 66%). YK365A was recrystallized from ^{i}PrOH to give YK365C and YK365D (0.176g, mp 155-156°C and 0.690g respectively).TLC showed impurities so YK365C and YK365D (total 0.363g) were purified by semi-preparative HPLC (Lichrosorb RP Select B 4+250x10mm column, UV 220nm detector, 4ml/min flow, A/B is 100/0 going to 60/40 over 20 minutes, where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with and 5% water)) to give the trioxalate (0.303g, semi-preparative HPLC yield 83%) as a brown oil. This was dried (0.3torr/room temperature/2 hours), dissolved in cold iPrOH (10ml) and filtered by gravity. Solvent was removed and the oil dried (0.5torr/room temperature/20 hours) to give YK365E (0.164g).

YK365E was obtained as a yellow oil.

Solubility, sol (H_2O, MeOH), insol (Et_2O).

TLC R_f 0.49 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 100% at 9.18 minutes (Lichrosorb RP Select B 7μm 250x4mm, 1ml/min, UV 220nm 0.05aufs, A/B 95/5) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 5% water and 0.1% trifluoroacetic acid).

UV (MeOH) λ_{max} 216nm (ε 17,100; logε 4.23).

IR (Nujol) 3600-2400 (s(br), OH str, NH str), 2954, 2917, 2849 (s, CH str, Nuj), 1674 (s, C=O str, C=N str), 1615 (s, NH bend), 1448 (m, CH def, Nuj), 1383 (m, CH_3 sym def, Nuj), 1262, 1341, 1383 (m, OH bend), 1202 (s, C-S str), 1131 (s, C-O str), 798, 832 (s, CH oopd, R_2C=CHR), 720 (s, CH_2 rock) cm^{-1}.

^{1}H NMR (400MHz, CD_3OD) δ 8.77 (s, 1H, Im-2), 7.44 (s, 1H, Im-5), 3.57 (t, J = 7.05Hz, 2H, $C\underline{H}_2S$), 3.53-3.51 (m, 2H, $NC\underline{H}_2$), 3.43-3.37 (m, 2H, $NHC\underline{H}_2$), 3.31 (m, 1H, NH, or impurity), 3.18 (t, J = 7.02Hz, 2H, $ImC\underline{H}_2$), 1.32-1.24 (m, 6H, $2CH_3$), 1.15 (d, J = 6.17Hz, 0.18H, $2CH_3$ of ^{i}PrOH).

^{13}C NMR (100MHz, CD_3OD) δ 166.88/162.91 (S$\underline{C}$/$\underline{C}$O), 135.46 (Im-2), 132.53 (Im-5), 118.19 (Im-4), 41.68/40.39 (N$\underline{C}H_2$/NH$\underline{C}H_2$), 31.50/25.22 (Im$\underline{C}H_2$/$\underline{C}H_2$S), 15.04/13.31 ($NCH_2\underline{C}H_3$/$NHCH_2\underline{C}H_3$).

Mass spectrum (FAB) m/e 227 $(M+1)^+$, 128 $(Im\text{-}CH_2CH_2\text{-}SH)^+$, 95 $(Im\text{-}CH_2CH_2)^+$, 45 $(COOH)^+$.

Anal. Calcd for $C_{10}H_{18}N_4S.3(CO_2H)_2.0.03^iPrOH$:

C, 38.79; H, 4.90; N, 11.25; S, 6.43.

Found: C, 38.72; H, 5.11; N, 11.63; S, 6.58.

N-N'-Di-n-propyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dioxalate

(24) (sample YK403D/UCL1192-J_2)

2-(Hydroxyethyl)imidazol-4-yl **(50)** (sample WT76/3156) (0.500g, 4.46mmol) and N,N'-Di-n-propyl thiourea (0.715g, 4.46mmol) in aqueous HBr (48%, 5ml, 44.2mmol) was refluxed for 88 hours. Solvent was removed under vacuum and the crude yellow oil (2.16g, 117% due to solvent retention) was converted to base by treatment with $NaHCO_3$ (to pH9) followed by extraction with CH_2Cl_2 (4 x 10ml) and drying ($MgSO_4$). The crude base YK403B (0.82g, 72% overall yield) was dissolved in ^{i}PrOH and converted to the oxalate salt by treatment with oxalic acid (2.2 molar equivalents) in ^{i}PrOH. Solvent was removed, and the white sticky residue left in Et_2O in the fridge over 4 days. The solid was filtered from the ether to give YK403C (1.025g, 50% overall

crude oxalate yield). This was recrystallized from ^{i}PrOH/EtOH (5:1) to give YK403D and YK403E (0.455g and 0.488g respectively, 92% recrystallization yield, 46% overall yield after 1st recrystallization).

YK403D was obtained as a white crystalline solid : mp 155-156°C (from ^{i}PrOH/EtOH, 5:1).

Solubility, sol (H_2O, MeOH), sp sol (^{i}PrOH), insol (Et_2O).

TLC R_f 0.30 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 96.8% at 12.6 minutes and 2.8% at 5.6 minutes (Lichrosorb RP Select B 7μm 250x4mm, 1ml/min, UV 215nm 0.05aufs, A/B 90/10) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 5% water).

UV (MeOH) λ_{max} 215nm (ε 16,000; logε 4.22).

IR (KBr) 3434 (s, NH str), 2933, 2899, 2872 (m, CH str), 1616, 1403 (s, CO_2^- antisym and sym str), 1534 (w, NH bend), 1464 (m, CH def), 854 (w, CH oopd, R_2C=CHR), 721 (m, CH_2 rock) cm^{-1}.

^{1}H NMR (400MHz, CD_3OD) δ 8.63 (s, 1H, Im-2), 7.37 (s, 1H, Im-5), 3.56 (t, J = 7.30, 7.24Hz, 2H, CH_2S), 3.43-3.31 (m, 4H, 2C$\underline{H}_2$CH_2CH_3), 3.16 (t, J = 7.30Hz, 2H, CH_2), 1.68-1.64 (m, 4H, 2C$\underline{H}_2$CH_3), 0.97 (t(br), J = 8.92, 7.65Hz, 6H, 2CH_3).

^{13}C NMR (100MHz, CD_3OD) δ 167.32/166.25 (S$\underline{C}$/$\underline{C}$O), 135.70 (Im-2), 133.01 (Im-5), 117.94 (Im-4), 48.03/46.91 ((N$\underline{C}H_2$/NH$\underline{C}H_2$, obscured by CD_3OD), 31.74/25.64 (Im$\underline{C}H_2$/$\underline{C}H_2$S), 23.95/22.26 (NC$H_2\underline{C}H_2$/NHC$H_2\underline{C}H_2$), 11.51/11.29 (N$(CH_2)_2\underline{C}H_3$/NH$(CH_2)_2\underline{C}H_3$), impurities at δ 30.70 and 29.06.

Mass spectrum (FAB) m/e 255 $(M+1)^+$, 154 $(Im\text{-}CH_2CH_2\text{-}SCNH)^+$, 127 $(Im\text{-}CH_2CH_2\text{-}S)^+$, 95 $(Im\text{-}CH_2CH_2)^+$.

Anal. Calcd for $C_{12}H_{22}N_4S.2.25(CO_2H)_2$: C, 43.37; H, 5.84; N, 12.26; S, 7.02.

Found : C, 42.95; H, 5.70; N, 12.79; S, 6.92.

N-N'-Di-n-butyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dioxalate

(25) (sample YK369C/UCL1176-J_2)

2-(Hydroxyethyl)imidazol-4-yl **(50)** (sample WT76/3156) (0.500g, 4.46mmol) was dissolved in aqueous HBr (48%, 5ml, 44.2mmol). N,N'-Di-n-butyl thiourea (0.92g, 4.88mmol) was added and the mixture refluxed for 89 hours. Solvent was removed under vacuum, to yield a crude orange oil (2.14g, 108% crude yield due to solvent retention). This was converted to base by treatment with $NaHCO_3$ (to pH9) followed by extraction with $CHCl_3$ (4 x 10ml) and drying ($MgSO_4$). The crude base was converted to the oxalate salt, by dissolving in absolute ethanol and treating with oxalic acid (2.5 molar equivalents) in absolute ethanol. Solvent was removed under vacuum and the residue was azeotroped with Et_2O to give the crude oxalate YK370B (1.91g, 93% overall yield). This was recrystallized from ^iPrOH to give YK369A (mp 110-113°C, 0.56g, 30% yield from 1st crop of 1st recrystallization) and YK369B (1.37g). YK369A was recrystallized again from ^iPrOH to give YK369C (178mg, 32% yield from 1st crop of 2nd recrystallization) and YK369D (68mg).

YK369C was obtained as a white crystalline solid : mp 112-114°C (from ^iPrOH).

Solubility, sol (H_2O, MeOH), sp sol (^iPrOH), insol (Et_2O).

TLC R_f 0.48 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 100% at 9.47min (Lichrosorb RP Select B 7μm 250x4mm, 1ml/min, UV 254nm 0.1aufs, A/B 75/25) where A is water with 0.1% trifluoroacetic acid and 0.5g/100ml hexane sulphonic acid(Na salt) and B is acetonitrile with 5% water).

UV (MeOH) λ_{max} 215nm (ε 17,000; logε 4.23).

IR (KBr) 3441, 3402 (s, NH str), 2958, 2870 (s, CH str), 1611, 1526 (s, w, NH bend), 1611, 1403 (s, m, CO_2^- antisym and sym str), 1459 (m, CH def), 827 (w, CH oopd, $R_2C{=}CHR$), 720 (s, CH_2 rock) cm^{-1}.

^1H NMR (400MHz, CD_3OD) δ 8.71 (s, 1H, Im-2), 7.41 (s, 1H, Im-5), 3.58 (t, J = 7.21, 7.25Hz, 2H, CH_2), 3.43 (d(br), J = 26.16Hz, 4H, $2C\underline{H}_2CH_2CH_2CH_3$), 3.18 (t, J = 7.14, 7.21Hz, 2H, CH_2), 1.63 (d(br), 4H, $2C\underline{H}_2CH_2CH_3$), 1.39 (s(br), 4H, $2C\underline{H}_2CH_3$), 0.96 (t, J = 7.20, 7.33Hz, 6H, $2CH_3$).

^{13}C NMR (100MHz, CD_3OD) δ 167.08/166.59 (S$\underline{C}$/$\underline{C}$O), 135.55 (Im-2), 132.53 (Im-5), 118.15 (Im-4), 46.45/45.20 ($N\underline{C}H_2/NH\underline{C}H_2$), 32.70/30.92 ($NCH_2\underline{C}H_2/NHCH_2\underline{C}H_2$), 31.63/25.45 ($Im\underline{C}H_2/\underline{C}H_2S$), 21.04/20.79 ($N(CH_2)_2\underline{C}H_2/NH(CH_2)_2\underline{C}H_2$), 14.06/14.01 ($N(CH_2)_3\underline{C}H_3/NH(CH_2)_3\underline{C}H_3$).

Mass spectrum (FAB) m/e 283 $(M+1)^+$, 189 $(S{=}C(NHBu)_2{+}H)^+$, 155 $(C(NBu)_2)^+$, 127 $(ImCH_2CH_2S)^+$, 95 $(ImCH_2CH_2)^+$.

Anal. Calcd for $C_{14}H_{26}N_4S.2C_2H_2O_4$: C, 46.75; H, 6.54; N, 12.11; S, 6.93.
Found : C, 46.41; H, 6.56; N, 11.96; S, 7.14.

N-Methyl-S-(2-(imidazol-4-yl)ethyl)isothiourea dihydrobromide[21].

(26) (sample YK298D/UCL1123)

A solution of 2-(hydroxyethyl)imidazol-4-yl **(50)** (sample WT76/3156) (0.500g, 4.46mmol) and 1-methyl-2-thiourea (0.402g, 4.46mmol) in HBr aqueous (48%, 5ml, 44.2mmol) was heated under reflux for 26 hours. The pale yellow reaction mixture was evaporated to dryness under reduced pressure, azeotroping with ^{i}PrOH, then Et_2O. Repeated stirring in Et_2O, decanting, and then removing solvent at 0.3torr/5 hours/room temperature, resulted in a sticky off-white solid (2.003g). This was dissolved in ^{i}PrOH and treated with excess Et_2O. An off-white hygroscopic solid was filtered off, YK298A (1.47g, 4.25mmol, 95%). YK298A (1.47g, 4.25mmol) was recrystallized from ^{i}PrOH/EtOH (1/1, 50ml), the volume of the filtrate being reduced (5ml) and kept in the freezer overnight. An off-white solid YK298B (1.031g, 2.98mmol, 70%), mpt. 178-180°C was obtained. YK298B (1.031g, 2.98mmol) was recrystallized from ^{i}PrOH/EtOH (1/1, 100ml), the filtrate volume being reduced (3ml) and kept in the fridge overnight. An off-white crystalline solid YK298D (0.896g) was obtained, together with a second crop YK298E (60mg).

YK298D was obtained as an off-white crystalline solid : mp 182-183°C (from ^{i}PrOH/EtOH abs, 1/1; reported[21]. mp 180-181°C).

Solubility, sol (H_2O), sp sol (EtOH), insol (Et_2O, EtOAc, $CHCl_3$).

TLC R_f 0.39 (silica, NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 100% at 7.51min (Lichrosorb RP Select B 7µm 4+250x4mm, 1ml/min, UV 215nm 0.2 aufs, A/B (9/1) where A is water with 0.5% acetonitrile and 0.1% trifluoroacetic acid and 0.005M hexanesulphonic acid and B is acetonitrile wthe 5% water and 0.1% trifluoroacetic acid).

UV (MeOH) λ_{max} 210nm (ε 12,600; logε 4.10).

IR (KBr) 3255 (s, NH str, amine, imine), 3073 (s, NH str, amine salt), 2987, 2829, 2724 (s, CH str, CH_2, CH_3, N-CH_3), 1647 (s, C=N str, C=C str), 1443, 1432 (m, CH def), 1308, 1160 (s, C-N str, aromatic secondary amine, aliphatic amine), 826 (m, CH oopd, R_2C=CRH), 725 (w, CH_2 rock) cm^{-1}.

^1H NMR (200MHz, D_2O, referenced to ^tBuOH at δ1.28) δ 8.70 (s, 1H, Im-2), 7.41 (s, 1H, Im-5), 3.50 (t, J = 6.92, 6.49Hz, 2H, CH_2), 3.23 (t, J = 6.67, 6.73Hz, 2H, CH_2), 3.00 (s, 3H, Me).

Mass spectrum (FAB) m/e 185 $(M+H)^+$, 127 $(Im(CH_2)_2S)^+$, 95 $(Im(CH_2)_2)^+$.

Anal. Calcd for $C_7H_{12}N_4S.2HBr$: C, 24.29; H, 4.08; N, 16.19; Br, 46.18; S, 9.26.
Found : C, 24.63; H, 4.14; N, 15.86; Br, 45.91; S, 9.20.

2-(2-(imidazol-4-yl)ethyl)-thioimidazoline dihydrobromide[21].

(27) (sample YK314F/UCL1133)

A solution of 2-(hydroxyethyl)imidazol-4-yl **(50)** (sample WT76/3156) (0.500g, 4.46mmol) and 2-imidazolidinethione (0.524g, 5.13mmol) in HBr aqueous (48%, 5ml, 44.2mmol) was heated under reflux for 17 hours. The reaction mixture was evaporated to dryness under reduced pressure, azeotroping with ^iPrOH, to yield a pale orange solid, YK314A (1.65g, 4.22mmol, 95%), mp 190-200°C. This was recrystallized from EtOH (120ml), the filtrate volume being reduced (20ml). Two crops were obtained, a white solid YK314B (0.539g), mpt. 217-219°C, and a smelly orange solid YK314C (0.461g), mpt. 180-200°C. Combined yield : 1.0g, 61%. YK314B (0.470g, 1.2mmol) was recrystallized from EtOH (100ml) the filtrate volume being reduced (30ml). Two crops were obtained, a white crystalline solid YK314D (0.254g), mp 216-217°C, and

an off-white solid YK314E (0.131g), mp 215-216°C. Combined yield : 0.385g, 82%. YK314D was dried to remove water at 100°C/0.5torr/4 hours over P_2O_5 after an unsatisfactory micoanalysis result, to give YK314F (0.222g).

YK314F was obtained as a white crystalline solid : mp 219-221°C (from EtOH; reported[21]. mp 227-229°C for the dihydrobromide).

Solubility, sol (H_2O), sp sol (EtOH), insol (Et_2O).

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

HPLC 100% at 5.71minutes (Lichrosorb RP Select B 7μm 4+250x4mm, 1.5ml/min, UV 220nm 0.1aufs, A/B (9/1) where A is water with 0.1% trifluoroacetic acid and 0.5g/500ml hexanesulphonic acid (Na salt) and B is acetonitrile with 5% water and 0.1% trifluoroacetic acid).

UV (MeOH) λ_{max} 216nm (ε 14,800; logε 4.17).

IR (KBr) 3044 (s, CH str, C=C-H), 2899 (s, CH str, CH_2, CH_3), 2604 (m, NH str), 1616 (s, NH bend, C=N str, C=C str), 1544 (s, C=N str, >C=N conjugated), 1443 (m, CH def), 1381 (m, CH_3 symmetrical def), 802 (m, CH oopd, R_2C=CHR), 764, 705 (CH_2 rock) cm^{-1}.

^1H NMR (200MHz, DMSO) δ 9.00 (s, 1H, Im-2), 7.54 (s, 1H, Im-5), 3.88 (s, 4H, imidazoline), 3.55 (t, J = 7.05, 6.99Hz, 2H, CH_2S) 3.08 (t, J = 6.99, 3.84Hz, 2H, CH_2), impurity at δ1.9 (s).

Mass spectrum (FAB) m/e 197 $(M+H)^+$, 127 $(ImCH_2CH_2)^+$, 95 $(ImCH_2CH_2S)^+$, 69 $(imidazoline)^+$.

Anal. Calcd for $C_8H_{12}N_4S.2.4HBr$: C, 24.61; H, 3.72; N,14.35; Br, 49.11.
Found : C, 24.56; H, 3.85; N, 14.94; Br, 48.81.

N,N,N'-Trimethyl-S-(2-(imidazol-4-yl)ethyl)isothiourea

dihydrobromide

(28) (sample YK332F/UCL1140-B_2)

2-(Hydroxyethyl)imidazol-4-yl (50) (sample WT76/3156) (0.500g, 4.45mmol) was dissolved in HBr aqueous (48%, 5ml, 44.2mmol). Trimethylthiourea (0.580g, 4.91 mmol) was added, rinsing with water. The mixture was refluxed for 83 hours. The reaction mixture was evaporated to dryness under reduced pressure, repeatedly stirred with Et_2O, decanted, and solvent removed at 0.3torr/3 hours/room temperature to give a very viscous orange oil, YK332A (1.804g). This was crystallized from ^{i}PrOH/EtOH abs. (1/1, 40ml), the filtrate being reduced (5ml). Two crops were obtained, an off-white solid YK332B (0.22g), mpt. 135-138°C, and YK332C (1.443g). Combined crude yield : 1.66g, 100%. YK 332C (1.443g) was recrystallized from ^{i}PrOH (5ml) to give a sticky off-white solid YK332D (97mg) and an orange oily solid (approx. 1.48g, solvent retention). YK332B (0.213g) was recrystallized from ^{i}PrOH (5ml), the filtrate volume reduced (2ml). A white crystalline solid YK332F (0.128g), was obtained, together with a second crop of sticky solid YK333A (63mg). Combined yield : 0.191g, 90%.

YK332F was obtained as a white crystalline solid : mp 147-149°C (from ^{i}PrOH).

Solubility, sol (H_2O,MeOH), insol (Et_2O).

TLC R_f 0.54 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 99.12% at 14.47min (Lichrosorb RP Select B 7μm 4+250x4mm, 1ml/min, UV 220nm 0.05aufs, A/B (91/9) where A is water with 0.1% trifluoroacetic acid and 0.5g/500ml hexanesulphonic acid (Na salt) and B is acetonitrile with 5% water and 0.1% trifluoroacetic acid).

UV (MeOH) λ_{max} 233nm (ε 9,200; logε 3.96), broad.

IR (KBr) 3034 (m, CH str, C=C-H), 2934, 2902, 2872 (s, CH str, CH_2, CH_3), 2780 (m, CH str, N-CH_3, N-CH_2), 2646 (m(br), NH str), 1608 (s, NH bend, C=N str, C=C str), 1500 (m, C=N str, >C=N conjugated), 1470 (m, CH def), 1373 (m, CH_3 symmetrical def), 817 (w, CH oopd, R_2C=CHR), 756 (CH_2 rock) cm^{-1}.

^1H NMR (200MHz, DMSO) δ 8.97 (br s, 1H, Im-2), 7.57 (br s, 1H, Im-5), 2.60-3.60 (m, 13H, CH_2CH_2, NCH_3, $N(CH_3)_2$), impurity at δ1.9 (s).

^{13}C NMR (DMSO) δ 166.91 (SCN), 134.38 (Im-2), 130.68 (Im-5), 116.86 (Im-4), 33.63, 32.35, 24.93 (Im-$\underline{C}H_2\underline{C}H_2$, 3 Me).

Mass spectrum (FAB) m/e 213 $(M+H)^+$, 127 $(ImCH_2CH_2S)^+$, 95 $(ImCH_2CH_2)^+$, 85 $(Me_2N$-C=NMe)+.

Anal. Calcd for $C_9H_{16}N_4S.2HBr$: C, 28.89; H, 4.85; N, 14.98; Br, 42.71; S, 8.57.
Found : C, 28.55; H, 4.76; N, 14.80; Br, 42.04; S, 8.36.

N-Cyclohexyl-S-(2-(imidazol-4-yl)ethyl)isothiourea trifluoroacetate

(29) (sample YK392A/UCL1209-J_2)

***Synthesis of cyclohexylisothiourea*: (29/I)** : Cyclohexylisothiocyanate (2.40g, 16.99mmol) was dissolved in $CHCl_3$ (50ml), cooled in an ice/water bath and stirred magnetically. NH_3 gas was bubbled through via a Drikold/acetone condenser for approximately 5 minutes, when gas escaping from the top of the condenser turned Universal indicator paper blue. The solution was stirred at room temperature overnight. Solvent was removed under vacuum, $CHCl_3$ (5ml) was added and shiny white crystals

were filtered off, YK389A (1.74g, 65% yield). Mp 164-166°C (reported[64]. 162°C), TLC R_f 0.77 (silica; NH_4OH-MeOH-EtOAc, 1:1:5); mass spectrum (EI) m/e 158 $(M)^+$.

Alternative synthesis of cyclohexylthiourea[64]. **(29/I)** : To a solution of ammonium thiocyanate (3.8g, 0.05mol) in dry acetone (25ml, over K_2CO_3) was added benzoyl chloride (7.0g, 0.05mol) dropwise with stirring and the mixture refluxed for 30 minutes. A solution of cyclohexylamine (4.96g, 0.05mol) in dry acetone was added with stirring at such a rate that the solution refluxed gently. The reaction mixture was poured into cold water (100ml) and heated with aqueous NaOH (10%, 50ml). On cooling to room temperature a white solid seperated out of the yellow solution. This was filtered off, YK362F (6.90g). Following the workup of the filtrate as given in the reference (acidifying with HCl to pH2, followed by making just alkaline with NH_4OH to pH8, and recrystallizing the solid obtained from EtOH) gave a very low yield of product, YK362C (26mg). However, extraction of YK362F with hot $CHCl_3$ (2 x 50ml), reducing in volume under vacuum, and filtering the solid thus obtained, gave cyclohexylthiourea as a white shiny solid, YK362G (3.59g,45%). Mp 165-167°C (reported[64]. 162°C), TLC R_f 0.83 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

Synthesis of N-Cyclohexyl-S-(2-(imidazol-4-yl)ethyl)isothiourea trifluoroacetate **(29)** : 2-(Hydroxyethyl)imidazol-4-yl **(50)** (sample WT76/3156) (0.500g, 4.46mmol) was dissolved in aqueous HBr (48%, 5ml, 44.2mmol). Cyclohexylthiourea, YK389A (0.643g, 4.06mmol) was added and the mixture was refluxed for 70 hours. Solvent was removed under vacuum, and the residue azeotroped with Et_2O to give the crude HBr salt as an orange viscous oil (1.98g, 118% yield due to solvent retention). This was converted to base by treatment with aqueous $NaHCO_3$ (to pH9), extraction with $CHCl_3$ (3 x 25ml) and drying ($MgSO_4$). Crude base (1.08g, 105% yield due to solvent retention) was converted to the oxalate by treatment with oxalic acid (2.5 molar equivalents) and precipitation with Et_2O to give crude oxalate as a creme solid YK393C (1.36g, 78% yield). This was recrystallized from ^iPrOH to give YK393E and YK393F (0.246g and 0.52g respectively). YK393E was shown to be 93%

pure by HPLC and was recrystallized further from ^{i}PrOH/EtOH (2/1) to give YK393G and YK393H (52mg and 157mg respectively). TLC showed imputities, so YK393G and YK393H (total 209mg) were purified by semi-preparative HPLC (Lichrosorb RP Select B 250x10mm column, UV 220nm detector, 4ml/min flow, A/B (88/12), where A is water and 0.1% trifluoroacetic acid and B is acetonitrile and 0.1% trifluoroacetic acid and 5% water) to give the product as a yellow oil (270mg). This was dissolved in ^{i}PrOH (25ml) and filtered by gravity. Removal of solvent under vacuum and drying (0.1torr/room temperature/9 hours) gave YK392A (0.168g, 63% semi-preparative HPLC yield).

YK392A was obtained as a brown solid : mp not obtained due to excessive hygroscopicity.

Solubility, sol (H_2O,MeOH), insol (Et_2O).

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

HPLC 100% at 5.85min (Lichrosorb RP Select B 7μm 250x4mm, 1ml/min, UV 220nm 0.1aufs, A/B (85/15) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 5% water and 0.1% trifluoroacetic acid).

UV (MeOH) λ_{max} 212nm (ε 14,700; logε 4.17).

IR (KBr) 3600-2600 (s(br), OH str, CH str, NH str), 2933, 2857 (s, CH str), 1676 (s, NH bend, C=O str, C=N str), 1431 (m, CH def), 1353 (w, OH bend), 1294, 1137 (s, CF str, CS str, CO str), 722 (m, CH_2 rock) cm^{-1}.

^{1}H NMR (400MHz, CD_3OD) δ 8.84 (s, 1H, Im-2), 7.43 (s, 1H, Im-5), 3.92 (septet, J = 6.15Hz, 0.2H, CH of ^{i}PrOH), 3.61-3.55 (m, 1H, NC<u>H</u>), 3.51 (t, J = 7.08, 7.02Hz, 2H, C<u>H</u>$_2$S), 3.16 (t, J = 6.80, 7.30Hz, 2H, C<u>H</u>$_2$), 1.95-1.67 , 1.41-1.18 (m, 10H, C_6<u>H</u>$_{10}$), 1.15 (d, J = 6.15Hz, 1.2H, 2CH_3 of ^{i}PrOH).

^{13}C NMR (CD_3OD) δ 166.29 (CS), 135.38 (Im-2), 132.34 (Im-5), 118.22 (Im-4), 55.36 (Cyclohexyl-1), 32.66 (Cyclohexyl-2), 31.17/26.07 (Im-CH_2/CH_2S), 25.76 (Cyclohexyl-3), 25.44 (Cyclohexyl-4).

Mass spectrum (FAB) m/e 253 $(M+H)^+$, 154 $(Im(CH_2)_2\text{-}SCN)^+$, 127 $(Im(CH_2)_2S)^+$, 95 $(Im(CH_2)_2)^+$.

Anal. Calcd for $C_{12}H_{20}N_4S.2.5CF_3CO_2H.0.2^iPrOH$:
C, 38.47; H, 4.42; N, 10.20; S, 5.84.
Found : C, 38.51; H, 4.27; N, 10.38; S, 5.93.

S-(2-(2-Pyridinyl)ethyl)isothiourea dihydrobromide[19., 21., 69.]

(30) (sample YK373C/UCL1151)

A mixture of commercially obtained 2-(2-hydroxyethyl)pyridine (1.074g, 8.72mmol), thiourea (0.67g, 8.80mmol) and HBr aqueous (48%, 5ml, 44.2mmol) was refluxed for 95 hours. The reaction mixture was evaporated to dryness under reduced pressure, azeotroping with ^iPrOH, the Et_2O to give a crude white sticky solid (3.328g). This was crystallized from ^iPrOH/EtOH/MeOH (1/2/3,100ml), reducing the filtrate volume (50ml). Two crops were obtained, a white solid YK373A (1.567g), mp 224°-226°C, and a yellow sticky solid YK373B (1.914g). Crude yield : 3.48g, 116% probably due to solvent retention. YK373A (1.567g) was recrystallized from EtOH/MeOH (3/1, 100ml). Two crops were obtained, a white crystalline solid YK373C (1.11g) and a white solid YK373D (0.361g). Combined yield : 1.471g, 94% recrystallization yield, 50% overall yield.

YK373C was obtained as a white crystalline solid : mp 232-234°C (from EtOH abs/MeOH,3/1; reported[21.] mp 229-230°C).

Solubility, sol (H_2O, MeOH), insol (Et_2O).

TLC R_f 0.38 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 100% at 8.39 minutes (Kromasil C18 5μm 250x4.6mm, 1ml/min, UV 254nm 0.2aufs, A/B (100/0 over the first 10 minutes, then changing to 1/1 over the next 20 minutes) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 5% water and 0.1% trifluoroacetic acid).

UV (MeOH) λ_{max} 208, 258 nm (ε 10,500, 4,000; logε 4.02, 3.60).

IR (KBr) 3299-3014 (s(br), NH str, CH str, Pyr), 2855 (s, CH str, CH_2), 1639, 1615, 1523 (s, s, m, NH bend, C=N str, C=C str), 1465, 1436 (s, CH def), 1338, 1317 (m, C-N str), 966, 954 (m, CH oopd, Pyr), 783 (s, CH oopb, Pyr), 717 (m, CH_2 rock) cm^{-1}.

^1H NMR (400MHz, CD_3OD) δ 8.83 (d, J = 5.87Hz, 1H, Pyr-6), 8.62 (m, 1H, Pyr-5), 8.13 (d, J = 7.86Hz, 1H, Pyr-3), 8.02 (m, 1H, Pyr-4), 3.72 (t, J = 6.87, 7.51Hz, 2H, $C\underline{H}_2S$), 3.54 (t, J = 7.30, 7.02Hz, 2H, $C\underline{H}_2$).

^{13}C NMR (100MHz, CD_3OD) δ 171.54 (CS), 154.75 (Pyr-2), 148.31 (Pyr-6), 143.04 (Pyr-5), 129.13 (Pyr-3), 127.10 (Pyr-4), 33.71/30.29 (Pyr$\underline{C}H_2$/$\underline{C}H_2$S).

Mass spectrum (FAB) m/e 182 $(M+H)^+$, 138 $(PyrCH_2CH_2S)^+$, 107 $(PyrCH_2CH_3)^+$, 92 $(PyrCH_2)^+$, 78 $(Pyr)^+$.

Anal. Calcd for $C_8H_{11}N_3S.2HBr$: C, 28.01; H, 3.82; N, 12.25; Br, 46.58; S, 9.34.
Found : C, 28.14; H, 3.78; N, 12.04; Br, 46.33; S, 9.51.

S-(3-(2-Pyridinyl)propyl)isothiourea dihydrobromide

(31) (sample YK377C/UCL1152)

A mixture of commercially obtained 3-(2-pyridinyl)-1-propanol (1.047g, 7.63mmol), thiourea (0.59g, 7.75mmol) and HBr aqueous (48%, 5ml, 44.2mmol) was refluxed for 88 hours. The reaction mixture was evaporated to dryness under reduced pressure, azeotroping with ^{i}PrOH, the Et_2O. The crude off-white oily solid was crystallized from ^{i}PrOH/EtOH (1/2) giving a crop of off-white solid YK377A (2.055g), mpt. 155°-157°C, and a second crop of yellow oily pungent solid YK377B (0.674g). Combined yield : 2.72g, 100%. YK377A (2.055g) was recrystallized from ^{i}PrOH/EtOH abs. (1/2, 50ml). Two crops were obtained, a white crystalline solid YK377C (1.802g) and a sticky off-white solid YK377D (0.138g). Combined yield : 1.94g, 94% recrystallization yield, 71% overall yield.

YK377C was obtained as a white crystalline solid : mp 156-158°C (from ^{i}PrOH/EtOH abs, 1/2).

Solubility, sol (H_2O, MeOH), insol (Et_2O).

TLC R_f 0.40 and a very faint trace at 0.86 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 100% at 17.09min (Kromasil C18 5µm 250x4.6mm, 1ml/min, UV 254nm 0.1aufs, A/B (100/0 over the first 10min, then changing to 1/1 over the next 20min) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 5% water and 0.1% trifluoroacetic acid).

UV (MeOH) λ_{max} 208, 259 nm (ε 9,900, 3,600; logε 4.00, 3.55).

IR (KBr) 3244-3043 (s(br), NH str), 2938, 2861 (s, CH str), 1631, 1612 (s, NH bend, C=N str), 1466, 1443 (m, CH def) 1319 (m, C-N str), 971, 940 (m, CH oopd, Pyr), 782 (s, CH oopb, Pyr), 715 (m, CH_2 rock) cm^{-1}.

^{1}H NMR (400MHz, CD_3OD, trace impurity at δ1.15) δ 8.77 (d, J = 5.90Hz, 1H, Pyr-6), 8.58 (m, 1H, Pyr-5), 8.07 (d, J = 8.14Hz, 1H, Pyr-3), 7.97 (m, 1H, Pyr-4), 3.36-3.26 (m, Pyr$C\underline{H}_2$, $C\underline{H}_2S$, obscured by CD_3OD), 2.25 (quintet, J = 7.67Hz, 2H, $PyrCH_2CH_2$).

^{13}C NMR (100MHz, CD_3OD) δ 172.33 (CS), 157.03 (Pyr-6), 148.33 (Pyr-2), 142.64 (Pyr-5), 128.77 (Pyr-3), 126.51 (Pyr-4), 33.05/30.96 (Pyr$\underline{C}H_2$/$\underline{C}H_2S$), 29.20 ($PyrCH_2\underline{C}H_2$).

Mass spectrum (FAB) m/e 196 $(M+H)^+$, 120 $(PyrCH_2CH_2CH_2)^+$, 106 $(PyrCH_2CH_2)^+$, 78 $(Pyr)^+$.

Anal. Calcd for $C_9H_{13}N_3S.2HBr$: C, 30.27; H, 4.23; N, 11.77; Br, 44.75; S, 8.98.
Found : C, 30.47; H, 4.20; N, 11.46; Br, 44.80; S, 9.10.

N,N'-Di-n-butyl-S-(ethyl)isothiourea oxalate

(32) (YO16A/UCL1213-J)

Ethanol (0.431g, 9.36mmol), aqueous HBr (48%, 6ml, 52.4mmol) and di-N,N'-n-butyl isothiourea (1.740g, 9.24mmol) were refluxed for 5 days. More ethanol (3ml, 51.5mmol) was added and refluxing continued for a further day. It was attempted to obtain the HBr salt as a solid by removing solvent under vacuum, freeze/drying, azeotroping, and precipitating with Et_2O. The crude oil YO13A (2.526g, 92% overall crude HBr salt yield) was converted to base by treatment with aqueous $NaHCO_3$ (to pH9) and extraction with CH_2Cl_2 and drying ($MgSO_4$), to give YO15A (1.809g, 90% overall crude base yield). This was converted to the oxalate by treatment with oxalic acid (1.2 molar equivalents) in ^{i}PrOH, removal of solvent under vacuum and precipitating

with Et_2O. The hygroscopic solid filtered off was washed with Et_2O to give YO15B (1.118g, 40% overall crude oxalate yield). It was attempted to recrystallize this from ^iPrOH, but no crystals were obtained, so the ^iPrOH was removed under vacuum and the product precipitated with Et_2O, the Et_2O decanted carefully, and the crystals (mp 67-70°C) dried (0.7torr/room temperature/3 hours) to give YO16A (0.490g, 44% yield from crude oxalate).

YO16A was obtained as a white crystalline solid : mp 74-75°C (precipitated from Et_2O).

Solubility, sol (H_2O, MeOH), sp sol (^iPrOH), insol (Et_2O).

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

HPLC 100% at 15.98 minutes (Lichrosorb RP Select B, 250x4mm, 1ml/min, UV 220nm, A/B (90/10) where A is water with 0.01% triethylamine and B is methanol with 0.01% triethylamine).

UV (MeOH) λ_{max} 217nm (ε 10,700 ; logε 4.03).

IR (KBr) 3435, 3135 (s(br), OH str, NH str), 2957, 2927, 2869 (m, CH str), 1718, 1700 (m, C=O str, saturated acid), 1609, 1519 (m, NH bend), 1609, 1402 (s, m, CO_2^- anti and sym str), 1456 (m, CH def), 720 (s, CH_2 rock) cm^{-1}.

^1H NMR (400MHz, CD_3OD) δ 4.92 (s, H_2O), 3.49 (m(br), 2H, NCH_2), 3.39 (m(br), 2H, $NHC\underline{H}_2$), 3.22 (q, J_{av} = 7.30Hz, 2H, SCH_2), 1.64 (quintet (br), J_{av} = 7.0Hz, 4H, $2NCH_2C\underline{H}_2$), 1.39 (t, J = 7.27Hz, m, 7H, $C\underline{H}_3CH_2S$, $2C\underline{H}_2CH_3$), 0.97 (t, J = 7.30Hz, 6H, $2CH_3$).

^{13}C NMR (100MHz, CD_3OD) δ 168.5/167.0 (CS/CO), 46.0/45.0 (NCH_2/$NHCH_2$), 32.5/31.0 ($CH_2S/\underline{C}H_2CH_2CH_3$), 27.0/20.5 ($\underline{C}H_3CH_2S/\underline{C}H_2CH_3$), 14.0 ($CH_3$).

Mass spectrum (FAB) m/e 216 $(M+H)^+$, 201 $(M^+-Me)^+$, 187 $(M^+-Et)^+$, 155 $(M^+-EtS)^+$.

Anal. Calcd for $C_{11}H_{24}N_2S.(CO_2H)_2.0.75H_2O$:

C, 48.81; H, 8.66; N, 8.76; S, 10.02.

Found : C, 48.97; H, 8.33; N, 8.83; S, 9.95.

O-(3-Pyridin-2-yl-propyl)isourea dihydrochloride

(33) (sample YK423B)

2-(3-Hydroxy-propyl)-pyridine (0.500g, 3.64mmol) and cyanamide (0.306g, 7.29mmol) was stirred in sodium dried benzene (50ml) saturated with dry HCl (until the fumes turned Universal indicator paper red) at room temperature, following the general method of preparation of isoureas.[45.,50.] After 6 days, more cyanamide (0.076g, 1.82mmol) was added, and stirring continued for a further 14 days. A white solid (1.43g, mp 116-119°C) was filtered off and after drying in a vacuum desiccator, was recrystallized from ^{i}PrOH (80ml) to give YK423B (0.421g, 46% overall yield) and a filtrate reduced to dryness under vacuum to give YK423C.

YK423B was obtained as a white solid : mp 135-137°C (from ^{i}PrOH).

Solubility, sol (H_2O, MeOH), insol (Et_2O).

TLC R_f 0.52 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC 97.6% at 8.4 minutes (Lichrosorb RP Select B, 250x4mm, 1ml/min, UV 254nm, A/B (100/0 for 10 minutes, going to 50/50 over the next 20 minutes) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 0.1% trifluoroacetic acid and 5% water).

UV (MeOH) λ_{max} 207nm and 259nm (ε 2,800, logε 3.45 and ε 3,500, logε 3.54).

IR (KBr) 3000-3600 (s, NH str, and pyridine ring CH str), 2880-2940 (m, CH str), 1684 (s, C=N str), 1614, 1519 (s, NH bend and pyridine ring C=C str), 1464 (s, CH def), 1157 (s, CO str), 782 (s, CH oopb) cm^{-1}.

^1H NMR (400MHz, CD_3OD) δ 8.78 (d, J = 5.57Hz, Pyridine-6), 8.59 (t, J = 7.94Hz, Pyridine-5), 8.06 (d, J = 8.08Hz, Pyridine-3), 7.98 (t, J = 6.24Hz, Pyridine-4), 4.42 (t, J = 6.04Hz, CH_2O), 3.24-3.34 (m, CH_2, obscured by CH_3OH), 2.37-2.30 (m, CH_2).

^{13}C NMR (100MHz, CD_3OD) δ 163.73 (O-C), 157.26 (Pyr-6), 148.40 (Pyr-5), 142.61 (Pyr-2), 128.67 (Pyr-3), 126.53 (Pyr-4), 71.03 (CH_2O), 30.64 (Pyr-CH_2), 28.66 (Pyr-$CH_2\underline{C}H_2$).

Mass spectrum (FAB) m/e 180 $(M+H)^+$; 136 $(Pyr\text{-}(CH_2)_3O)^+$; 120, 106, 92, $(Pyr\text{-}(CH_2)_n)^+$ where n = 1,2,3; 78 $(Pyr)^+$.

Anal. Calcd for $C_9H_{13}N_3O$, 2HCl : C, 42.87; H, 6.00; N, 16.67; Cl, 28.18.
Found : C, 42.48; H, 5.87; N, 16.63; Cl, 28.65.

O-(2-Pyridin-2-yl-ethyl)isourea (51) (sample YK418C)

2-(2-Hydroxyethyl)pyridine (0.500g, 4.06mmol) and cyanamide (0.342g, 8.12mmol) was stirred in sodium dried benzene (50ml) saturated with dry HCl (until the fumes turned Universal indicator paper red) at room temperature. After 6 days, more cyanamide (0.17g, 4.06mmol) was added, more HCl bubbled through the mixture, and stirring continued for a further 14 days. The benzene was decanted to give a crude off-white solid (1.45g). This was recrystallized from ^iPrOH to give YK419B as an off-white solid (0.860g, mp 138-139°C). YK419B was recrystallized from ^iPrOH to give YK419D

(0.574g, mp 143-144°C). Analytical HPLC showed 94.8% purity (with a retention time of 11.2 minutes) which was considered insufficient, so YK419D was further recrystallized from ^{i}PrOH to give YK419F (0.327g, mp 143-144°C), which in turn was only 81% pure by analytical HPLC (Lichrosorb RP Select B, 250x4mm, 1ml/min, UV 220nm, A/B (99/1) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 0.1% trifluoroacetic acid and 5% water, retention time of 10.14 minutes). Preparative HPLC (Lichrosorb RP Select B, 250x10mm, 4ml/min, UV 220nm, A/B (95/5) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 0.1% trifluoroacetic acid and 5% water) was performed on YK419F (0.244g) to give YK418A (26mg), YK418B (20mg) and YK418C (270mg, with a retention time of 10.0 minutes).

YK418C was obtained as a pale yellow crystalline solid : mp 70(80-105)°C (unrecrystallized from preparative HPLC, probably solvents present).

^{1}H NMR (200MHz, CD_3OD) δ 8.67–8.80 (m, 1H, Pyr-6), 8.27–8.44 (m, 1H, Pyr-5), 7.73–7.96 (m, 2H, Pyr-3,4), 4.42 (t, J = 6.08Hz, 2H, CH_2), 3.51 (t, J = 6.03, 5.72Hz, 2H, CH_2).

Mass spectrum (FAB) m/e 166 $(M+H)^+$; 228 (impurity); 122 $(Pyr\text{-}(CH_2)_2OH)^+$; 106 $(Pyr\text{-}CH_2CH_3)^+$; 93 $(Pyr\text{-}CH_3)^+$; 78 $(Pyr)^+$.

O-(2-Imidazol-4-yl-ethyl)isourea (52) (sample YK406C)

2-(Hydroxyethyl)imidazol-4-yl (**50**) (sample WT76/3156) (0.500g, 4.46mmol) and cyanamide (0.375g, 8.92mmol) was stirred in sodium dried benzene (50ml) saturated with dry HCl (until the fumes turned Universal indicator paper red) at room temperature. After 4 days, more cyanamide (0.19g, 4.46mmol) was added, and stirring continued for a further 14 days. The benzene was decanted and the sticky white solid (1.55g)

recrystallized from ^{i}PrOH/MeOH (2/1, 100ml) and triturated with Et_2O to give an off-white solid YK407B (1.088g) which was recrystallized from ^{i}PrOH/EtOH (2/1, 150ml) to give a white solid YK407D (0.612g, mp 143-144°C) and YK407E (0.387g). YK407D was shown to contain the product by MS(FAB), but only 65% pure by analytical HPLC. Preparative HPLC (Lichrosorb RP Select B, 250x25mm, UV 220nm, A/B (95/5) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 0.1% trifluoroacetic acid and 5% water) was performed on YK407D to obtain YK406A and other fractions YK406B (retention time span 4.7 to 8.2 minutes). MS(FAB) showed the product to be in YK406B, which was then passed through another preparative HPLC column (Lichrosorb RP Select B, 250x10mm, 4ml/min, UV 230nm, A/B (95/5) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 0.1% trifluoroacetic acid and 5% water) to give YK406C (505mg, with a retention time of 7.60 minutes) and YK406D (63mg).

YK406C was obtained as a pale yellow solid : mp 70(80-105)°C (unrecrystallized from preparative HPLC, probably solvents present).

^{1}H NMR (200MHz, CD_3OD) δ 8.88 (s, 1H, Im-2), 7.49 (s, 1H, Im-5), 4.56 (t, J = 6.10Hz, 2H, CH_2), 3.35-3.23 (m, CH_2, obscured by MeOH).

Mass spectrum (FAB) m/e 155 $(M+H)^+$; 111 $(Im\text{-}(CH_2)_2O)^+$; 95 $(Im\text{-}CH_2CH_2)^+$; 81 $(Im\text{-}CH_2)^+$; 68 $(Im)^+$.

N,N-di-methyl-O-(2-imidazol-4-yl-ethyl)isourea

(53) (sample YK414A)

2-(Hydroxyethyl)imidazol-4-yl (**50**) (sample WT76/3156) (0.500g, 4.46mmol) and di-methylcyanamide (0.630g, 8.99mmol) was stirred in sodium dried benzene (50ml)

saturated with dry HCl (until the fumes turned Universal indicator paper red) at room temperature for 7 days. The benzene was decanted and dry CH_2Cl_2 (50ml) was added, together with a further portion of di-methylcyanamide (0.630g, 8.99mmol) and the mixture refluxed for 15 hours. The reaction mixture was reduced to dryness under reduced pressure, azeotroping with Et_2O. The crude was passed through a preparative HPLC column (Lichrosorb RP Select B, 250x10mm, UV 220nm, A/B (99/1) where A is water with 0.1% trifluoroacetic acid and B is acetonitrile with 0.1% trifluoroacetic acid and 5% water) to give YK414A (132mg, with a retention time of 3.54 to 4.89 minutes) and other fractions YK414B(172mg) and YK414C (110mg). A small sample of YK414A was passed through another preparative HPLC column (Lichrosorb RP Select B, 250x10mm, 5ml/min, UV 215nm, A/B (95/5) where A is water with 0.01% triethylamine and B is acetonitrile with 0.01% triethylamine) to give YK414D (26mg, with a retention time of 8.0 minutes) which was shown to be the starting alcohol by ^1H NMR (200MHz) and MS(FAB).

YK414A was obtained as a yellow oil.

^1H NMR (200MHz, CD_3OD) δ 8.81 (s, 1H, Im-2), 7.36 (s, 1H, Im-5), 4.08 (s, impurity), 3.82 (t, J = 6.03Hz, 2H, CH_2), 3.31 (s, 2H) and 3.09 (m, 2H) should integrate to 6H (and not 4H) altogether if the signal is due to $N(Me)_2$ (the peak at δ3.31 is likely to be a MeOH solvent peak anyway), 2.92 (t, J = 5.89Hz, 2H, $\underline{CH_2}$). The spectrum indicates that the compound is decomposing to the starting material alcohol.

Mass spectrum (FAB) m/e 183 $(M+H)^+$; 218 (trace impurity); 154 (M+2H-2Me)+; 113 $(Im\text{-}(CH_2)_2OH + H)^+$; 95 $(Im\text{-}CH_2CH_2)^+$; 81 $(Im\text{-}CH_2)^+$; 68 $(Im + H)^+$.

MULTISTEP SYNTHESIS OF 2-(4-PIPERIDINYL)-PYRIDINE (47)

See Scheme 3.2.

1-Benzyl-4-(2-pyridinyl)-piperidin-4-ol dihydrochloride[61]. (16)

(samples YK223A, YK173A (base), YK135C)

The reaction using commercially available 1-benzyl-4-piperidone, 2-bromopyridine and n-butyl lithium in Et_2O to give **(16)** (sample YK111F/UCL1033, given earlier) (combined yield after column 73%) was repeated on a 0.24mol scale, this time extracting with $CHCl_3$ (5 x 50ml) immediately after neutralizing the reaction mixture with $NaHCO_3$ to give the crude base as a red oil (56.45g, 109% crude yield due to solvent retention). The column chromatography was also omitted and the crude purified by formation of the dihydrochloride with ethanolic HCl, a cream solid (10.11g, 15% overall yield) and subsequent recrystallization from EtOH abs (600ml) to give off-white solids YK223A, B and C (8.44g, 83% combined recrystallization yield).

YK223A was obtained as an off-white solid : mp 240-242°C (from EtOH abs; compare with YK111F mp 254-255°C).

TLC R_f 0.48 (silica; $CHCl_3$-MeOH, 8:1).

^1H NMR (200MHz, D_2O) of YK223C compared well with that of sampleYK111F given earlier.

Note The reaction was also carried out on a 0.1mol scale to give the base as a crude red/brown oil, YK173A (26.4g, 123% due to solvent retention) and on a 0.05mol scale to give the dihydrochloride as a hygroscopic orange solid, YK135C (12.52g, 73%).

1-Benzyl-4-(2-pyridinyl)-1,2,5,6-tetrahydropyridine dihydrochloride

(44)

(samples YK262A, YK270A , YK322B)

1-Benzyl-4-(2-pyridinyl)-piperidin-4-ol dihydrochloride **(16)**, sample YK223B (0.11g, 0.32mmol) was added in small portion to $SOCl_2$ (10ml) which was stirred magnetically and cooled using an ice/water bath. The colourless solution was stirred at room temperature for 1 hour and left for 2 days, excluding moisture by fitting a $CaCl_2$ drying tube. Solvent was evaporated under reduced pressure, azeotroping with Et_2O, drying at 70°C/0.03torr/3 hours to give YK262A (0.101g, 0.31mmol, 98% crude yield)

YK262A was obtained as a hygroscopic off-white solid : mp 153°C (effervescence, glassy at 135°C).

TLC R_f 0.89 (silica; $CHCl_3$-MeOH, 3:1).

^1H NMR (400MHz, DMSO) δ 8.60 (d, 1H, Pyr-6), 7.95 (m, 1H, Pyr-5), 7.36-7.78 (m, 7H, Pyr-3,4, Ph), 6.74 (s(br), 1H, C=C<u>H</u>), 4.43 (m, 2H, C<u>H</u>$_2$Ph), 3.7-4.3 (v br s, H_2O), 2.60-3.85 (m, 6H, C<u>H</u>$_2$C<u>H</u>$_2$NC<u>H</u>$_2$).

Mass spectrum (EI) m/e 250 (M^+), 172 (M-Pyr)$^+$, 159 (M-CH_2Pyr)$^+$, 106 (PhCH_2NH)$^+$, 91 (PhCH_2)$^+$, 78 (Pyr)$^+$.

Anal. Calcd for $C_{17}H_{18}N_2$.2HCl : C, 63.16; H, 6.24; N, 8.67.
Found : C, 62.91; H, 6.42; N, 8.37.

Note The experiment was also carried out on a 0.0155mol scale using YK223A in $SOCl_2$ (50ml), stirring at room temperature for 45 minutes and standing over 2 days. The dihydrochloride YK270A was a hygroscopic yellow solid (4.94g, 0.015mol, 99% crude yield). The same experiment was repeated on a 0.0157mol scale using YK222A in $SOCl_2$ (50ml), stirring for 22 hours, to give a mustard colour hygroscopic solid YK322B (5.41g, 106% due to solvent retention).

1-Benzyl-4-(2-pyridinyl)-piperidine **(45)**

(samples YK354B, YK342A (dihydrochloride))

1-Benzyl-4-(2-pyridinyl)-1,2,5,6-tetrahydropyridine dihydrochloride **(44)**, sample YK322B (2.47g, 7.24mmol) was dissolved in EtOH abs (50ml). Pd/C (10%, 1.047g) was added, and the mixture shaken in a Parr apparatus at room temperature, with H_2 (60psi, 4bar) for a total of 69 hours. During this time, the catalyst was replaced and acetic acid added twice (0.5g, 2ml and 0.3g, 3ml respectively). The reaction mixture was filtered, evaporated to dryness under reduced pressure, stirred with Et_2O and decanted several times, to give the dihydrocloride as a green solid YK354A (1.57g, 4.6mmol, 64%). This was dissolved in water (5ml), basified with $NaHCO_3$ (pH2 to 9) with much frothing, extracted with CH_2Cl_2 (10 x 5ml), dried ($MgSO_4$). Evaporation of solvent gave the base as YK354B (0.922g, 3.65mmol, 50% overall yield).

YK354B was obtained as a crude brown solid.

TLC R_f 0.61 (silica; $CHCl_3$-MeOH-NH_4OH, 5:1:trace).

^1H NMR (200MHz, $CDCl_3$) δ 8.51 (br s, 1H. Pyr-6), 7.63 (t, J = 7.0Hz, 1H, Pyr-5), 7.50-7.05 (m, 7H, Pyr-3,4, Ph), 3.76 (s, 2H, CH_2Ph), 3.17 (br d, J = 14.0Hz, 2H,

Pip-2 (CH eq), Pip-6 (CH eq)), 2.82 (br m, 1H, Pyr-CH), 2.37 (br m, 2H, Pip-2 (CH axial), Pip-6 (CH axial)), 2.04 (br s, 4H, Pip-3 (CH_2), Pip-5 (CH_2)).

Mass spectrum (EI) m/e 252 (M^+), 161 (Pyr-Pip)$^+$, 106 (Ph-CH_2-NH)$^+$, 91(Ph-CH_2)$^+$, 78 (Pyr)$^+$.

Note The experiment was also carried out on a 1.84mmol scale in MeOH (30ml), with Pd/C (10%, 0.30g) in a Berghof apparatus (magnetic stirring) at room temperature and H_2 (7bar, 101.5psi). Reaction was observed to be complete up to this stage by tlc after 69 hours, but reaction conditions were sustained for a total of 136 hours. After filtering off the catalyst, solvent was evaporated under reduced pressure, azeotroping with Et_2O, to give the dihydrochloride salt as a green oil, YK342A (0.59g, 1.74mmol, 95%).

1-Ethoxycarbonyl-4(2-pyridinyl)piperidine (46)

(samples YK358B, YK397A)

1-Benzyl-4-(2-pyridinyl)-piperidine (**45**), sample YK346B (which is sample YK342A recovered as starting material from an unsuccessful attempt to debenzylate with Na/NH_3) (0.363g, 1.44mmol) was suspended in Na dried benzene (10ml). Chloroethyl formate (0.44g, 4.08mmol) was added together with MeOH (1ml) to dissolve the YK346B, and the mixture refluxed following the general procedure[70], for a total of 60 hours. After the initial 20 hours the reaction mixture was evaporated to dryness under reduced pressure and the crude examined by ^1H NMR and MS, indicating partial reaction. Neat $ClCO_2Et$ (10ml) was replaced as solvent until the reaction was complete. The reaction mixture was evaporated to dryness under reduced pressure, azeotroping with glacial acetic acid, then Et_2O to obtain YK358B (0.358g, max 1.36mmol, 112% crude yield, due to solvent retention).

Crude YK358B was obtained as a brown foamy solid.

TLC R_f 0.94 with traces at 0.78 and 0 (silica; $CHCl_3$-MeOH-NH_4OH, 5:1:trace).

^1H NMR (200MHz, CD_3OD) was crude but indicated presence of a pyridine ring δ7-9, no benzyl group, and peaks due to the Et group at δ1.25 and δ1.96.

Mass spectrum (EI) m/e 234 (M^+), 219 $(M-CH_3)^+$, 189 $(M-OEt)^+$, 161 $(Pyr-Pip)^+$, 156 $(M-Pyr)^+$, 120 $(Pyr-Pip-CHCH_2NH)^+$, 106 $(Pyr-CH=CH_2+1)^+$, 91 $(Pyr-CH)^+$, 78 $(Pyr)^+$.

4(2-Pyridinyl)piperidine[26.,27.] **(47)**

(samples YK384A, YK397C (dioxalate))

A mixture of 1-ethoxycarbonyl-4(2-pyridinyl)piperdine **(46)**, sample YK358B (0.358g, max 1.36mmol), HBr aqueous (48%, 3 x weight equivalent) and glacial acetic acid (11 x weight equivalent) was refluxed for 3 hours following the general procedure.[70.] On cooling to room temperature, ice was added to the reaction mixture made alkaline pH1 to pH14 with NaOH dil), extracted with CH_2Cl_2 (5 x 20ml), dried ($MgSO_4$). Evaporation to dryness yielded the base as a crude brown oil, YK385A (0.208g, 1.28mmol, 94% crude yield). Presence of the product was verified by Tlc, ^1H Nmr and MS. Silica gel column chromatography of the crude, using $CHCl_3$-MeOH (5:1) as eluants proved successful in the sense that contaminating starting material was eluted off, while the product remained at the top of the column. The top band was therefore extracted with $CHCl_3$/MeOH (1/1, 250ml) with heating, and the white solid (30mg) obtained, extracted with ^iPrOH (2 x 30ml), to give a colourless oil YK385C (11mg), identified by TLC and ^1H NMR. More product was extracted from the column using MeOH with 2% NH_4OH

(250ml), to give a brown oil YK385D (23mg). Combined yield from column (34mg, 0.21mmol, 16%). YK385D (23mg, 0.14mmol) was dissolved in EtOH abs (1ml) and treated with oxalic acid (0.028g, 0.312mmol) in EtOH abs (1ml). Addition of Et_2O, scratching, decanting repeatedly, resulted in the crude dioxalate (42mg, 40%). Recrystallization from ^{i}PrOH/EtOH abs (1/1) yielded the dioxalate YK384A (10mg, 24%)

YK384A was obtained as a brown solid : mp 191-192°C (no mp was reported by Bowden[26.,27.]).

TLC R_f 0.23 (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

Mass spectrum (EI) m/e 162 (M^+), 134 (Pyr-Pip-CHNH)$^+$, 120 (Pyr-Pip-$CHCH_2NH)^+$, 106 (Pyr-$CH{=}CH_2$ +1)$^+$, 93 (Pip-2H)$^+$, (Pyr-$CH_3)^+$, 77 (Pyr)$^+$.

^{1}H NMR (400MHz, CD_3OD) δ 8.53 (m, 1H, Pyr-6), 7.84 (t, J = 7.73Hz, 1H, Pyr-5), 7.41 (d, J = 7.89Hz, 1H, Pyr-3), 7.33 (m, 1H, Pyr-4), 3.55-3.45 (m, ~3H, Pyr-CH, Pip-2(CH eq), Pip-6(CH eq), 3.19-3.10 (m, ~3H, NH, Pip-2(CH ax), Pip-6(CH ax), 2.15 -2.03 (m, ~4H, Pip-3(CH_2), Pip-5(CH_2), 1.2 (^{i}PrOH and EtOH present.

Note The experiment was repeated on a 3.28mmol scale using YK354B, refluxing for 20 hours in a neat $ClCO_2Et$, and then refluxing the crude with HBr/Acetic acid for 3 hours, making alkaline with NaOH, extracting with CH_2Cl_2, drying ($MgSO_4$), treating with activated charcoal, to obtain crude base as a yellow oil. This was converted to the dioxalate salt, using 2.6 molar equivalents of oxalic acid in ^{i}PrOH/EtOH, to give a crude yellow solid, YK397C (0.49g, 3.57mmol, 82%).

MULTISTEP SYNTHESIS OF 3(IMIDAZOL-4-YL)PROPYLAMINE (41)

See Scheme 3.1.

Imidazol-4-yl propenamide (39)

(samples YK232A, YK254B, YK266A)

Commercially obtained trans-urocanic acid **(37)** (1.03g, 7.45mmol) was added in small portions to $SOCl_2$ (10ml, distilled). The apricot coloured suspension was refluxed for 1hour, solvent distilled off, and the material dried at room temperature/0.3torr/3 hours to give the acyl chloride as and apricot coloured solid **(38)**, sample YK230A (1.40g, 97% crude yield).

NH_4OH (specific gravity 0.88, 20ml) was added in small portions to **(38)**, sample YK230A (1.40g, 7.25mmol) in a cold water bath, and the mixture stirred at room temperature for 30 minutes. The brown reaction mixture was evaporated to dryness under reduced pressure, to give a light brown solid (2.02g, product with NH_4Cl). This was dissolved in H_2O (15ml) and basified with $NaHCO_3$ (pH 6 to 8), extracted with $CHCl_3$ (3 x 10ml), evaporated to dryness under reduced pressure, to give a brown solid (2.38g, product base with starting material and inorganic material). This was absorbed onto silica and passed through a silica column, using $CHCl_3$/MeOH (1/1) as eluant, to give the product **(39)**, sample YK232A (0.329g, 33% overall yield after column).

YK232A was obtained as a yellow solid : mp 198-200°C dec.

Solubility, sol (DMSO), v sp sol ($CHCl_3$, THF), insol (Et_2O).

TLC R_f 0.62 (silica; $CHCl_3$-MeOH, 1:1).

^{1}H NMR (200MHz, DMSO; contains impurities at δ6.88 and δ7.46) δ 7.68 (s, 1H, Im-2), 7.38 (s, 1H, Im-5), 7.27 (d, J = 16.0Hz, 1H, C<u>H</u>=CH), 6.50 (d, J = 16.0Hz, 1H, CH=C<u>H</u>).

Mass spectrum (EI) m/e 137 (M^+), 120 M-NH_2)$^+$, 109 (Im-CH=CH-CH_3+H)$^+$, 93 (Im-CH=CH)$^+$, 81 (Im-CH_2)$^+$, 68 (Im)$^+$.

Note The experiment was repeated on a 0.0724mol scale, to give the acylchloride YK254A (13.81g, 99% crude yield). This was converted to the amide using $CHCl_3$ (100ml) saturated with NH_3, cooled with an ice/water bath and stirring for 30 minutes. The reaction mixture was extracted with ^{i}PrOH (100ml) at room temperature, to give the product as a yellow solid YK254B (4.51g, 127% crude yield, contaminated with both starting material and NH_4Cl).

Conversion of acylchloride hydrochloride salt to amide was also caried out on a 0.044mol scale, using YK254A, and treating with $CHCl_3$/NH_3 saturated, to give crude product YK266A (10.99g, approx 40% NH_4Cl) on evaporation to dryness.

<u>Imidazol-4-yl-propanamide</u> (40)

(samples YK294D, YK294C, YK310B)

Imidazol-4-yl propenamide **(39)**, sample YK266A (1.03g, 7.5mmol) was dissolved in water (10ml). Na/Hg amalgam (2.3%, 30.70g, 30.7mmol of Na) was added using a glass spatula. Stirring at room temperature for 3 hours caused slight exothermicity, and colour changes from yellow to green to grey. The reaction mixture was decanted from Hg, filtered through celite, and the water freeze-dried off overnight to yield and off-white solid (2.19g). This was passed through a silica gel column, using MeOH as the eluant. The first fraction obtained yielded a white solid YK294B (0.405g). YK294B

(0.316g) was extracted with ^{i}PrOH/EtOH abs (1/1, 5ml, warm). The filtrate was reduced to 2ml and a solid YK294D filtered off (64mg). The second fraction yielded a pale yellow solid YK294C (0.726g). Combined overall yield from column : 1.13g, 108%, silica contamination.

YK294D was obtained as a white solid : mp 169-171°C.

TLC R_f 0 (silica, NH_4OH-MeOH-EtOAc, 1:1:5).

IR (KBr) 3405, 3117 (NH str), 2949, 2931, 2848 (CH str), 1553 (C=O str), 1424 (CH def), 819 (CH oopd, R_2C=CHR).

^{1}H NMR (200MHz, DMSO) δ 7.43 (s, 1H, Im-2), 6.63 (s, 1H, Im-5), 2.66 (t, J = 7.54, 7.62Hz, 2H, Im-C$\underline{H_2}$), 2.23 (t J = 7.94, 6.98Hz, 2H, Im-$CH_2C\underline{H_2}$).

Mass spectrum (EI) m/e 140 $(M+1)^+$, 122 $(MH-NH_2)^+$, 95 $(ImCH_2CH_2)^+$, 81 $(ImCH_2)^+$, 68 $(Im)^+$.

Note The experiment was also carried out on a 0.017mol scale, using YK266A with Na/Hg (2.3%, 0.15mol Na) at room temperature for 6 hours. Instead of the column, the crude was filtered through a celite/charcoal/silica funnel, and then extracted with hot ^{i}PrOH to yield YK310B as a pale yellow solid (1.492g, 63%).

3-(Imidazol-4-yl)propylamine dipicrate[2., 3., 22., 23., 42., 58.] **(41)**

(samples YK318F, YK318D, YK306A)

Imidazol-4-yl-propanamide **(40)**, sample YK310B (1.49g, 0.0107mol) was dissolved in freshly distilled THF (100ml). $LiAlH_4$ (1.2g, 0.0316mol) was added, and the mixture stirred under N_2, refluxing for 19 hours. The $LiAlH_4$ was decomposed with supersat.

$NaHSO_4$, the pale yellow solid filtered off, washing with THF. After drying the THF ($MgSO_4$), evaporating to dryness under reduced pressure, and azeotroping with Et_2O, the crude base was obtained as a yellow oil (0.948g, 7.57mmol, 71% crude). This was treated with ethanolic HCl, and triturated with Et_2O, but no satisfactory solid was obtained. Evaporation of solvent gave a grey oil (0.905g). This was dissolved in water (100ml), and treated with aqueous picric acid (approx 75%, 100ml). The yellow solid obtained YK318A (0.282g) was recrystallized from EtOH (50ml), to obtain the dipicrate YK318D (95mg) dried at 60°C/0.5torr/4 hours. YK318D was dried further at 100°C/0.5torr/3 hours to give YK318F in an effort to remove to remove trace EtOH indicated by microanalysis. Filtrates from YK318A and D were reduced in volume (2ml), washed with Et_2O and decanted repeatedly to remove excess picric acid. Evaporation to dryness under vacuum (behind an explosion shield) yielded a yellow oil YK319A (0.713g). This gave a positive $AgNO_3$ test, indicating that the picric acid did not completely replace the HCl. HPLC indicated the presence of some 3(imidazol-4-yl) propylamine, but the majority of YK319A was a faster running compound; mass spectrometry showed peaks due to 3(imidazol-4-yl)propylamine, while ^1H NMR indicated 3(imidazol-4-yl)propylamine with an extra singlet at δ3.17 (2H).

YK318D and YK318F was obtained as a yellow solid : mp 221-223°C (from EtOH; reported 244-244.5°C[3]. from H_2O; reported 237-239°C[42]. from H_2O).

TLC R_f 0.23 and 0.47 yellow spot due to picric acid (silica; NH_4OH-MeOH-EtOAc, 1:1:5).

HPLC (YK318F) 94.59% (ignoring picric acid) at 8.00 minutes (Lichrosorb RP Select B 7μm 4+250x4mm, 1.5ml/min, UV 215nm 0.1aufs, A/B (95/5) where A is water with 0.1% trifluoroacetic acid and 0.5g/500ml hexanesulphonic acid (Na salt) and B is acetonitrile with 5% water and 0.1% trifluoroacetic acid).

^1H NMR (400MHz, DMSO, YK318D, trace EtOH present, and trace impurities at δ8.2(s) and δ8.3(s)) δ 9.03 (s, 1H, I[illegible]-2), 8.60 (s, approx 4H, picric acid, CH), 7.71

(br s, approx 2H, picric acid, OH), 7.46 (s, 1H, Im-5), 2.83 (m(br), 2H, $C\underline{H}_2NH_2$), 2.71 (t, J = 8.0Hz, 2H, $ImCH_2$), 1.86 (quintet, J = 8.0Hz, 2H, $ImCH_2CH_2$).

Mass spectrum (YK318D) (EI) m/e 229 (M^+, picric acid), 199 ((picric acid - NO)$^+$, ~~171 $((NO_2)_3\text{-}C_6H_2\text{-}(OH))^+$~~, 91 $(C_6H_2\text{-}OH)^+$, 125 (M^+), 108 $(ImCH_2CH{=}CH_2)^+$, 95 $(ImCH_2CH_2)^+$, 82 $(ImCH_2 + H)^+$, 68 $(Im)^+$.

Anal. Calcd for YK318D, $C_6H_{11}N_3.2C_6H_3N_3O_7.1C_2H_5OH$:
C, 38.16; H, 3.68; N, 20.02.
Found : C, 38.04; H, 3.10; N, 20.48.
Anal. Calcd for YK318F, $C_6H_{11}N_3.2C_6H_3N_3O_7.0.5C_2H_5OH$:
C, 37.63; H, 3.22; N, 20.79.
Found : C, 37.96; H, 2.85; N, 20.43.

Note The experiment was also carried out on a 0.29mmol scale using YK294D in THF (40ml), using $LiAlH_4$ (0.79mmol), refluxing for 16 hours to yield the base as a smelly colourless oil YK306A (36mg, 100% crude yield) identified by ^1H NMR (200MHz, DMSO). Treatment with ethanolic HCl gave an oily material.

CHAPTER SIX.

PHARMACOLOGICAL TESTING.

Pharmacological testing for H_3 histamine receptor 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 Four for results). 20 to 50mg of material were submitted for testing purposes. In vitro testing results were in terms of a K_i or EC_{50} value, depending on whether antagonist or agonist, or intermediate effects were observed. In vivo testing results were in terms of HA and τ-MeHA concentrations with time after dosage.

In vitro testing[12].

The bioassay developed by Prof. Schwartz and his group[7.,12.] is described in detail below. Briefly, rat cerbral cortex slices are incubated with [^{3}H]histidine, which leads 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 agonist) 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.[79.]

Slices (0.3mm thick) from cerbral 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/ml) were preincubated for 30 minutes at 37°C in the presence of 0.4μM [^{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. For this purpose, the basket was successively transferred to seven beakers containing 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, 250μl aliquots of the slice suspension (2.5-3.5 mg protein) were distributed into plastic microtubes kept at 37°C and containing, when required, 10μl of the solutions of the various drugs to be tested. Five minutes later, 250 μl of a modified Krebs-Ringer solution were added to give a final concentration of either 2mM or 30mM K^+. Exogenous unlabelled histamine (10^{-6}M) was added, when required, together with 30 mM K^+. Incubations were stopped 2 minutes later by rapid centrifugation, the pellets were homogenized in 200μl 0.01M HCl and [^{3}H]histamine present in the pellet and supernatant 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 analyzed for determination of EC_{50} values of agonists and 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 Prusoff[32.] :

$$K_i = IC_{50} / (1 + S / EC_{50})$$

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[57] with bovine serum albumin as standard.

In vivo testing[47]

The effect of a drug on histamine levels in the brain is measured in terms of its effect on HA turnover rate. Moreover, since transmethylation is the sole metabolic pathway in the brain, measurements of rates of accumulation of *tele*-methylhistamine (τ-MeHA) following inactivation of monoamine oxidase constitute useful indices of HA turnover. Simple radioimmunoassays (RIAs) have been developed by Garbarg et al[47] for HA and τ-MeHA which allow sensitive evaluation of these amine levels in small brain samples without prepurification.

Table 5.1. below illustrates the marked regional difference in both HA and τ-MeHA immunoreactivities, the hypothalamus containing the highest level of amine. The regional differences are less marked for τ-MeHA.

Table 5.1. Shows levels of HA and τ-MeHA in various regions of rat brain.

	HA (9ng/g)	τ-MeHA (ng/g)
Cerebral cortex	39.6 ± 5.2	40.0 ± 1.2
Striatum	38.5 ± 6.7	47.2 ± 3.6
Hippocampus	32.1 ± 5.0	29.1 ± 2.4
Hypothalamus	480 ± 44	157.0 ± 5.6

Values are means ± SEM obtained from six rats.

Effects of pharmacological treatments aimed at modifying the amine levels are shown in Table 5.2. below. Inhibition of HA synthesis by α-fluoromethylhistidine markedly reduce both HA and τ-MeHA levels in the cerebral cortex. In contrast, pargyline, a monoamine oxidase inhibitor, strongly increases the τ-MeHA level in this tissue. Thioperamide shows a large t-MeHA level increase at relatively low doses.

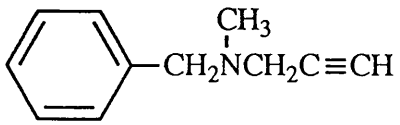
Table 5.2. Shows effects of pharmacological treatments on HA and τ-MeHA levels in rat cerebral cortex.

	HA (ng/g)	τ-MeHA (ng/g)
Controls	44.1 ± 3.3	32.7 ± 4.2
α-Fluoromethylhistidine (50mg/kg)	29.1 ± 3.0[a]	16.6 ± 2.2[a]
	(-36%)	(-50%)
Pargyline (75mg/kg)	54.4 ± 4.4	300 ± 20[a]
		(+817%)
Thioperamide	38.2 ± 5.8	91.5 ± 8.5[a]
		(+180%)

Animals were decapitated 4 hours after administration of α-fluoromethylhistidine (i.p.), 5 hours after pargyline (i.p.), and 1.5 hours after thioperamide (p.o.). Values are means ± SEM obtained from 5-9 rats.

[a] $p < 0.01$

The structure of the propargylamine pargyline is shown below. Structures of most of the other reagents may be found in Chapter One.



Pargyline

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