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IMETIT AND N-METHYL DERIVATIVES. THE TRANSITION FROM POTENT AGONIST TO ANTAGONIST AT HISTAMINE H_3 RECEPTORS.[1]

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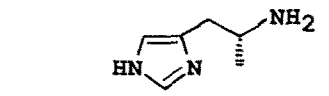
Abstract: Imetit {S-[2-(imidazol-4-yl)ethyl]isothiourea} is a potent H_3-agonist in vitro (on rat brain cortical slices; EC_{50} = 1 nM) and in vivo (ED_{50} ~ 1 mg/kg per os in mice). N-Methylimetit is also an agonist (EC_{50} = 15 nM) but dimethyl and trimethyl derivatives are antagonists (K_i = 50 - 500 nM).

Histamine receptors have been classified into three main subtypes designated H_1, H_2 and H_3.[2,3,4] The most recently described is the H_3 receptor at which histamine acts to modulate its own synthesis and release. Very potent and pharmacologically specific compounds have been described for use in characterising the pharmacology of the H_3 receptor[5] e.g. the agonist (R)α-methylhistamine (**1**) and the antagonist thioperamide (**2**).

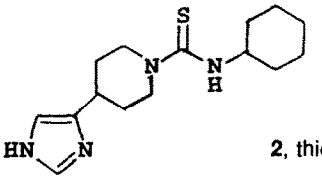
A recent report[6] of two other extremely potent agonists, S-[2-(imidazol-4-yl)ethyl]isothiourea and 4-(imidazol-4-yl)butyramidine prompts us to report our work relating to the former compound which we have called imetit.[7]

Previous studies[4] have shown that some H_2-receptor ligands such as impromidine (**3**) and burimamide (**4**) also possess high affinity for the H_3 receptor. These observations prompted us to study the activity at cerebral H_3 receptors of several compounds which had been at the origin of the design of H_2-receptor antagonists.[10] Among these was S-[2-(imidazol-4-yl)ethyl]isothiourea which had been characterised previously as a weak partial H_2-receptor agonist.[10] As indicated by formula **5** (Table 1), imetit resembles histamine by being an imidazole derivative substituted at position 4(5) by a short chain of two carbon atoms. In place of the ammonium group of histamine imetit possesses an isothiouronium moiety.

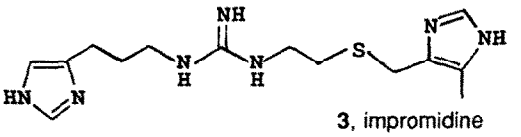
Imetit was tested for its effect on [^{3}H]histamine release from slices of rat cerebral cortex which had been preincubated for 30 min with L-[^{3}H]histidine (0.3 μM) using the procedure of Arrang et al.[4] The spontaneous efflux (2 mM K^+) of [^{3}H]histamine from rat cerebral cortex slices into the medium represented 6.0 ± 0.5% of the total [^{3}H]histamine. After a 2 min incubation with 30 mM K^+, the release represented 13.9 ± 0.7% of the total



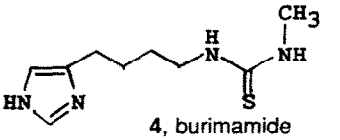
1, (R)α-methylhistamine



2, thioperamide



3, impromidine

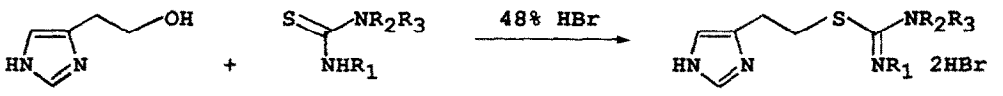


4, burimamide

[^{3}H]histamine over the spontaneous efflux. Histamine and imetit inhibited the tritiated amine release with the same maximal effect (55 ± 4%). The concentrations required for half-maximal inhibition of release were (EC_{50}) 62 ± 10, 4.0 ± 0.9 and 1.0 ± 0.3 nM for histamine, (R)α-methylhistamine and imetit respectively. Thus in this preparation imetit has approximately 62 times the potency of histamine and 4 times that of (R)α-methylhistamine. Imetit, given orally to mice, induced a long-lasting decrease in the brain level of the histamine catabolite, N-*tele*-methylhistamine, with an ED_{50} value of approximately 1 mg/kg (6 μmol/kg) which is about 4 times lower than that of (R)α-methylhistamine.

H_3 agonists and antagonists are of particular interest for studying histamine function in the brain but at present the compounds available do not readily penetrate into the CNS. The chemical structure of imetit did not suggest that it would be able to cross the blood-brain barrier any more successfully than histamine, which is itself known to cross poorly.[11] Hydrogen bonding is believed to play a key role in reducing the ability of compounds to penetrate into the CNS[12] and the isothiourea group has two hydrogen-acceptor and three hydrogen-donor possibilities. It is of great interest, therefore, to explore the possibility of reducing the hydrogen-donor capability by replacing N-H with N-methyl.

Methyl groups were introduced at the N atoms of the isothiourea moiety via the synthetic route shown in the Scheme in which 4-(2-hydroxyethyl)imidazole was treated with the appropriate N-substituted thiourea in 48% hydrobromic acid under reflux for 26-80 hours. Compounds were isolated as dihydrobromide salts and characterised by mp, nmr, mass spectra, hplc and CHNBrS analyses (Table 1) and tested for their effect on histamine release from rat brain cortical slices, either as agonists (EC_{50}) or antagonists (Ki).



It was found that one methyl substituent could be accommodated on the isothiourea group to provide an agonist (compound **6**) which, although less active than imetit, still possessed four times the potency of histamine.[13]

An additional methyl group, either on the same or on the other nitrogen atom (**9** and **7** respectively), however, gave an antagonist, compound **7** being ten times more potent than **9**. Joining the two methyl groups together, as in an imidazoline ring (**8**), also gave an antagonist but of lower affinity than **7**. A third methyl group (**10**) also reduced affinity. Compound **6** (N-methylimetit) was tested in vivo; when given orally to mice it produced a decrease in the brain level of N-*tele*-methylhistamine, and had an ED_{50} of approximately 25 mg/kg (136 μmol/kg).

To date, all reported potent agonists and antagonists at H_3 receptors are imidazole derivatives[15-17] so, presumably, for the agonists imetit (**5**) and its N-methyl congener (**6**) the imidazole ring of these compounds must simulate the imidazole ring of histamine at the receptor. The isothiourea group is basic (similar pKa to that of aliphatic amines i.e. 9–10)[18] and would be protonated at physiological pH so that the cationic isothiouronium moiety could mimic the alkylammonium cation of histamine.[10, 19]

Table 1. Compounds and test results

No	R_1	R_2	R_3	m.p.* °C	cryst. solvent	formula	EC_{50} nM	Ki nM
5	H	H	H	219-220	EtOH:Et_2O(9:1)	$C_6H_{10}N_4S$,2HBr	1.0±0.3	
6	CH_3	H	H	182-183	EtOH:^{i}PrOH(1:1)	$C_7H_{12}N_4S$,2HBr	15±3	
7	CH_3	CH_3	H	203-204	EtOH.^{i}PrOH(1:2)	$C_8H_{14}N_4S$,2HBr		51±22
8	CH_2—	CH_2	H	219-221	EtOH	$C_8H_{12}N_4S$,2.4HBr		260±50
9	H	CH_3	CH_3	188-190	EtOH:^{i}PrOH(1:20)	$C_8H_{14}N_4S$,2HBr		ca 500
10	CH_3	CH_3	CH_3	147-149	^{i}PrOH	$C_9H_{16}N_4S$,2HBr		≥ 500
	(R)α-methylhistamine						4.0±0.9	
	histamine						62±10	

* Lit.[14] m.p. **5** (210-212); **6** (180-181); **7** (203-204); **8** (227-229 for 2HBr).

The isothiouronium sulphur atom in **5** is in a similar position in the side chain to the ammonium nitrogen atom of histamine and could be considered as an isostere. Like the latter, it would carry a partial positive charge but, in contrast, it could only act as a hydrogen-bond acceptor, whereas ammonium is a hydrogen-bond donor. Therefore this sulphur atom cannot act as a good functional replacement for nitrogen.

Presumably the isothiouronium $-NH_2^+$ groups have to simulate the histamine $-NH_3^+$; they are, however, further away in space relative to the imidazole ring. Imetit would have to adopt a folded conformation to mimic the relative spatial disposition of the imidazole and ammonium groups of histamine. Isothiouronium cations form strong interactions with oxyacid anions[20], and the isothiouronium moiety in imetit could conceivably interact with a carboxylate group at the receptor e.g. an aspartic acid residue as has been postulated for the interaction of the histamine $-NH_3^+$ at H_1 and H_2 receptors.[21-23]

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References and Notes

1 Communicated in part to the XXIInd Meeting of the European Histamine Research Society, Torremolinos, Spain, May 1992.

2 Ash, A.S.F.; Schild, H.O. *Br. J. Pharmacol. Chemother.* **1966**, *27*, 427.

3 Black, J.W.; Duncan, W.A.M.; Durant, G.J.; Ganellin, C.R.; Parsons, M.E. *Nature*, **1972**, *236*, 385.

4 Arrang, J.M.; Garbarg, M.; Schwartz, J.C. *Nature*, **1983**, *302*, 832

5 Arrang, J.M.; Garbarg, M.; Lancelot, J.C.; Lecomte, J.M.; Pollard, H.; Robba, M.; Schunack, W.; Schwartz, J.C. *Nature*, **1987**, *327*, 117.

6 Howson, W., Parsons, M.E.; Raval, P.; Swayne, G.T.G. *BioMed. Chem. Lett.* **1992,** *2*, 77.

7 This compound has been designated UCL 1095 (by us), SK&F 91105 (ref. 6) and VUF 8325 (ref. 8); to avoid this multiplicity of in-house numbers we proposed[9] the non-proprietary name imetit which is derived from the chemical name as follows: S-[2-(IMidazol-4-yl)ETthyl]IsoThiourea.

8 H. Timmerman; communicated to the 8th Noordwijkerhout-Camerino Symposium, Trends in Receptor Research, Camerino, September 1991, Abstr SCI.

9. Garbarg, M.; Arrang, J.M.; Rouleau, A.; Ligneau, X.; Dam Trung Tuong, M.; Schwartz, J.C., Ganellin, C.R.; *J. Pharmacol. Exp. Therap.*, **1992**, in press.

10 Ganellin, R. *J. Med. Chem.* **1981**, *24*, 913.

11 Schwartz, J. C., Arrang, J.M.; Garbarg, M.; Pollard, H.; Ruat, M. *Physiol. Rev.* **1991**, *71*, 51.

12 Young, R.C.; Mitchell, R.C.; Brown, T.H.; Ganellin, C.R.; Griffiths, R.; Jones, M.; Rana, K.K.; Saunders, D.; Smith, I.R.; Sore, N.E.; Wilks, T.J. *J. Med. Chem.* **1988** *31*, 656.

13 Schwartz, J.C.; Garbarg, M.; Arrang, J.M.; Ganellin, C.R.; Lecomte, J.M. Eur. Pat. 0458661 A, **1991**.

14 Black, J.W.; Durant, G.J.; Emmett, J.C.; Ganellin, C.R., Brit. Pat. 1296544, **1972**.

15 Cooper, D.G.; Young, R.C.; Durant, G.J.; Ganellin, C.R. in *Comprehensive Medicinal Chemistry*, Emmett JC, Ed.; Pergamon Press: Oxford, **1990;** Vol 3, pp 412-413

16 Lipp, R.; Stark, H.; Schunack, W. in *The Histamine Receptors*; Schwartz, J.C. and Haas, H.L., Eds.; Wiley Liss, New York, **1992;** pp 57-71

17 Leurs, R.; Van der Goot, H.; Timmerman, H. *Adv. Drug Res.* **1991**, *20*, 217.

18 Fastier, F.N. and Reid, C.S.W. *Brit. J. Pharmacol.* **1952**, *7*, 417.

19 Ganellin, C.R. *J. Appl. Chem. Biotechnol.* **1978**, *28*, 183.

20 Walker, J. *J. Chem. Soc.* **1949**, 1966; Kennard, O.; Walker J, *ibid*, **1963**, 5513

21 Gantz, I.; Schaffer, M.; Delvalle, J.; Logsdon, C.; Campbell, V.; Uhler, M.; Yamada, T. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 429.

22 Birdsall, N.J. *Trends in Pharmacol. Sci.* **1991**, *12*, 9.

23 Yamashita, M.; Fukui, H.; Sugama, K.; Horio, Y.; Ho, S.; Mizuguchi, H.; Wada, H. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 11515.