Novel Qualitative Structure-Activity Relationships for the Antinociceptive Actions of H₂ Antagonists, H₃ Antagonists and Derivatives


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ABSTRACT

Recent studies have shown that cimetidine, burimamide and improgan (also known as SKF92374, a cimetidine congener lacking H₂ antagonist activity) induce antinociception after intracerebroventricular administration in rodents. Because these substances closely resemble the structure of histamine (a known mediator of some endogenous analgesic responses), yet no role for known histamine receptors has been found in the analgesic actions of these agents, the structure-activity relationships for the antinociceptive effects of 21 compounds chemically related to H₂ and H₃ antagonists were investigated in this study. Antinociceptive activity was assessed on the hot-plate and tail-flick tests after intracerebroventricular administration in rats. Eleven compounds induced time-dependent (10-min peak) and dose-dependent antinociceptive activity with no observable behavioral impairment. ED₅₀ values, estimated by nonlinear regression, were highly correlated across nociceptive assays (r² = 0.98, n = 11). Antinociceptive potencies varied more than 6-fold (80–464 nmol), but were not correlated with activity on H₁, H₂ or H₃ receptors. Although highly potent H₃ antagonists such as thioperamide lacked antinociceptive activity, homologs of burimamide and thioperamide containing N-aromatic substituents retained H₃ antagonist activity and also showed potent, effective analgesia. A literature review of the pharmacology of these agents did not find a basis for their antinociceptive effects. Taken with previous findings, the present results suggest: 1) these compounds act on the brain to activate powerful analgesic responses that are independent of known histamine receptors, 2) the structure-activity profile of these agents is novel and 3) brain-penetrating derivatives of these compounds could be clinically useful analgesics.

Several studies have established that the neuromodulator HA induces antinociception when directly administered into the CNS (Lamberti et al., 1996; Parolaro et al., 1989; Bhattacharya and Parmar, 1985; Onodera and Ogura, 1983; Glick and Crane, 1978). Although the pharmacology of this response is complex, both H₁ and H₂ antagonists have been reported to inhibit HA-induced antinociception (Thoburn et al., 1994; Parolaro et al., 1989; Netti et al., 1988; Bhattacharya and Parmar, 1985). However, other H₂ antagonists (cimetidine and ranitidine) induce antinociception in the absence of exogenous HA when administered directly into the brain (Li et al., 1996; Leza et al., 1990; Oluymí and Hart, 1991; Netti et al., 1984, 1988).

In previous work from one of our laboratories (Li et al., 1996), cimetidine-induced antinociception was characterized by studying improgan, a chemical congener of cimetidine that lacks H₂ antagonist activity (see table 1 for structure). Improgan, formerly known as SKF92374 (Li et al., 1996), induced a highly effective, reversible, dose-related and time-related inhibition of both supraspinally mediated (hot plate) and intraspinally mediated (tail flick or tail immersion) nociceptive responses in rats (Li et al., 1996) and mice (Li et al., 1997a) after ivt administration. The compound had a similar profile in rats when studied with a mechanical nociceptive test (Li et al., 1997a). Additional behavioral testing in rats showed that a large dose of improgan lacked effects on spontaneous locomotor activity (implying the absence of stimulant or depressant actions) and on an accelerated rotord test (implying the absence of motor impairment, Li et al., 1997a). These results suggest that improgan-like compounds have selective analgesic properties after ivt administration. While the present work was in progress, the antinociceptive activity of burimamide, another closely related compound with both H₂ and H₃ blocking properties (table 1), was reported after ivt administration in mice (Lamberti et al., 1996).

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ABBREVIATIONS: CNS, central nervous system; HA, histamine; ivt, intracerebroventricular; SAR, structure-activity relationship.
TABLE 1
Structures of HA, H$_2$ antagonists, H$_3$ antagonists and derivatives

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>$Ar$</th>
<th>$Y$</th>
<th>$X$</th>
<th>$NH$- $R$</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>HA</td>
<td>Agonist</td>
<td>Imidazol-4-yl</td>
<td>$\text{CH}_2$-</td>
<td>$\text{H}$</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>Cimetidine</td>
<td>H$_2$ Antagonist</td>
<td>4-Methylimidazol-5-yl</td>
<td>$-\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Methyl}$</td>
<td>$d</td>
<td>$</td>
</tr>
<tr>
<td>Improgan</td>
<td>Control$^a$</td>
<td>Imidazol-4-yl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Methyl}$</td>
<td>f</td>
<td></td>
</tr>
<tr>
<td>VUF8299</td>
<td>Control$^b$</td>
<td>Phenyl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Methyl}$</td>
<td>d, f</td>
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</tr>
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<td>Metiamide</td>
<td>H$_3$ Antagonist</td>
<td>4-Methylimidazol-5-yl</td>
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<td>$\text{Methyl}$</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>Tiotidine</td>
<td>H$_3$ Antagonist</td>
<td>2-Guanidinio-thiazol-4-yl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Methyl}$</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>Ranitidine</td>
<td>H$_3$ Antagonist</td>
<td>2-(Dimethyl-aminomethyl)-furan-5-yl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Methyl}$</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>Zolantidine</td>
<td>H$_3$ Antagonist</td>
<td>3-(1-Piperidinylmethyl)phenyl</td>
<td>$\text{O-}{\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{2-Benzthiazole}$</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>SKF95299</td>
<td>H$_3$ Antagonist (weak)</td>
<td>3-(1-Piperidinylmethyl)phenyl</td>
<td>$\text{O-}{\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Phenyl}$</td>
<td>g</td>
<td></td>
</tr>
<tr>
<td>Burimamide</td>
<td>H$_3$ Antagonist$^c$</td>
<td>Imidazol-4-yl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Methyl}$</td>
<td>$d, h$</td>
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</tr>
<tr>
<td>Norburimamide</td>
<td>H$_3$ Antagonist</td>
<td>Imidazol-4-yl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Methyl}$</td>
<td>h</td>
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<td>VUF4740</td>
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<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Phenyl}$</td>
<td>h</td>
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</tr>
<tr>
<td>VUF4685</td>
<td>H$_3$ Antagonist</td>
<td>Imidazol-4-yl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Phenylthyl}$</td>
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<td>VUF4741</td>
<td>H$_3$ Antagonist</td>
<td>Imidazol-4-yl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Cyclohexyl}$</td>
<td>h</td>
<td></td>
</tr>
<tr>
<td>VUF4686</td>
<td>H$_3$ Antagonist</td>
<td>Imidazol-4-yl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Cyclohexyl}$</td>
<td>h</td>
<td></td>
</tr>
<tr>
<td>VUF4687</td>
<td>H$_3$ Antagonist</td>
<td>Imidazol-4-yl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{Phenylidyl}^b$</td>
<td>h</td>
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<tr>
<td>Thioperamide</td>
<td>H$_3$ Antagonist</td>
<td>Imidazol-4-yl</td>
<td>$1,4$-phenylidyl$^b$</td>
<td>$\text{Methyl}$</td>
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<tr>
<td>VUF5261</td>
<td>H$_3$ Antagonist</td>
<td>Imidazol-4-yl</td>
<td>$1,4$-phenylidyl$^b$</td>
<td>$\text{Methyl}$</td>
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<tr>
<td>VUF5262</td>
<td>H$_3$ Antagonist</td>
<td>Imidazol-4-yl</td>
<td>$1,4$-phenylidyl$^b$</td>
<td>$\text{Methyl}$</td>
<td>This paper</td>
<td></td>
</tr>
<tr>
<td>Clobenpropit</td>
<td>H$_3$ Antagonist</td>
<td>Imidazol-4-yl</td>
<td>$\text{CH}_2\text{S-}{\text{CH}_2\text{S-}\text{NH-}{\text{H-}{\text{CO-N-CN}}}}$</td>
<td>$\text{4-Chlorobenzyl}$</td>
<td>h</td>
<td></td>
</tr>
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</table>

The pharmacological classification and chemical structures of drugs assessed for antinociceptive activity in the present study.

$^a$ Chemical congeners of cimetidine virtually devoid of H$_2$ antagonist activity. 
$^b$ Burimamide is also a weak H$_3$ antagonist. 
$^c$ Structure of 1,4-phenylidyl bridging group is: 

References: $^a$ Cooper et al., 1990; $^b$ Li et al., 1996; $^c$ Sterk et al., 1987; $^d$ Gogas et al., 1989; $^e$ Leurs et al., 1995b.

The fact that several (but not all) H$_2$ and H$_3$ antagonists induce antinociception after CNS administration implies the possible existence of a novel class of analgesic agents. The close similarity in structure among cimetidine, improgan and burimamide (table 1) further suggests that these compounds could be acting by a similar (but unknown) mechanism. With respect to improgan, both in vitro (Li et al., 1996) and in vivo (Li et al., 1997a,b) studies with agonists and antagonists suggest that the effects are not caused by an action on opiate receptors, on H$_1$, H$_2$ or H$_3$ receptors or on HA metabolism. With respect to burimamide, detailed dose-response studies in mice suggest that neither H$_2$ nor H$_3$ receptors mediate burimamide-induced antinociception (Lamberti et al., 1996). An H$_3$-related antinociceptive mechanism is also excluded by findings showing that: 1) improgan-induced antinociception is not opposed by the H$_3$ agonist (R)-α-methylhistamine (Li et al., 1997b); 2) improgan and cimetidine are both very weak H$_3$ antagonists (Li et al., 1996); and 3) burimamide-like effects (i.e., full activity against thermal tail-flick responses) are not produced by potent, selective H$_3$ antagonists such as thioperamide (Lamberti et al., 1996; Li et al., 1996). With respect to the third point, however, the possibility of subtypes of H$_3$ receptors should not be overlooked (Leurs et al., 1996; West et al., 1990). The suggestion (Lamberti et al., 1996) that burimamide antinociceptive action might be related to its weak H$_1$ antagonist properties seems unlikely because selective H$_1$ antagonists are inactive on the tail-flick test (Dews and Graham, 1946; Li et al., 1997b).

Further study of the mechanism of antinociceptive action of improgan-like compounds is warranted because these agents bear obvious structural resemblance to that of HA (table 1), a substance strongly implicated in analgesic mechanisms, and known HA receptors do not seem to participate in the antinociceptive effects of these agents. To perform a detailed pharmacological investigation of the latter hypothesis, the antinociceptive effects of 21 structurally related agents have been investigated in this study. The results show compelling evidence for a novel pharmacological activity of these compounds. The discovery that certain derivatives of burimamide are potent, effective antinociceptive agents is also reported.

Methods

Animals. Male Sprague-Dawley rats (Taconic Farms, Inc., Germantown, NY), weighing 210 to 320 g at the time of testing, were maintained on a reverse 12-hr light/dark cycle (lights on 7:00 P.M., lights off 7:00 A.M.) and used for nociceptive testing. The reverse cycle has been widely used for nociceptive testing in this and other laboratories, because rodents are nocturnal (Gogas et al., 1989; Li et al., 1996, 1997a,b). Adult male Dunkin-Hartley guinea pigs (350–450 g, Harlan CPB, Zeist, The Netherlands) were used for in vitro assays of H$_3$ activity. All experiments were reviewed and approved by the appropriate Institutional Animal Care and Use Committees.

Drugs and solutions. Compounds assessed for antinociceptive activity are in table 1. (HA was not included in the present study.) Cimetidine, metiamide, improgan (SKF92374), burimamide, norburimamide (bases) and zolantidine dimaleate were kindly provided by Dr. Robin Ganellin, formerly of SmithKline Beecham, Herts, U.K. Thioperamide maleate was purchased from RBI (Natick, MA). Tioti-
dine base was kindly provided by Dr. David McCurdy, formerly of Stuart Pharmaceuticals (Wilmington, DE). Ranitidine dihydrochloride was kindly provided by Dr. D.E. Bays (Gloxo Group Res. Ltd., Ware, Herts, UK). Except for VUF5261 and VUF5262, VUF-prefixed compounds and R-α-methylhistamine dihydrochloride were available from laboratory stock. These include clofenpropit (also known as VUF9153, Van der Goot et al., 1992), VUF8298 (Sterk et al., 1987), VUF8299 (Sterk et al., 1987) and the remaining burimamide derivatives (Vollinga et al., 1995).

VUF5261 and VUF5262 (free bases) were synthesized from 4-(imidazol-4(S)-yl)piperidine according to Arrang et al. (1987) except that the corresponding isothiocyanates were used instead of cyclohexyl isothiocyanate (VUF5261: 67% yield, m.p. = 193.6°C; analysis: C = 53.42 [calc = 53.54]; H = 7.23 [calc = 7.19]; N = 24.87 [calc = 24.98]; VUF5262: 22% yield, m.p. = 127.1°C; analysis: C = 62.90 [calc = 62.91]; H = 6.26 [calc = 6.33]; N = 19.64 [calc = 19.56]). SKF95299 was synthesized by a modification of literature procedures (Fujiwara, 1983; Young et al., 1988). Reductive amination of 3-hydroxybenzaldehyde with piperidine and sodium cyanoborohydride gave 3-[4-(piperidin-1-ylmethyl)phenoxy]propylamine by treatment with aniline acetic acid followed by treatment with 3-bromopropanol/potassium carbonate. Subsequent treatment with benzaldehyde with piperidine and sodium cyanoborohydride gave burimamide (1987) except that the third latency before the drug treatment was recorded. After wash-out, antagonists were preincubated for 15 min during stimulation, and the H3 agonist R-α-methylhistamine was recorded. After wash-out, antagonists were preincubated 15 min during stimulation, and the H3 agonist dose-response curve was redetermined. Antagonists were studied at three concentrations, ranging from 3 nM to 1 μM. All fits converged with statistically significant (P < .05) regression parameters. For each parameter (ED50 and n), mean and S.E.M. values of the regression were obtained.

Assay of H3 antagonist activity. VUF5261 and VUF5262 were assessed for H3 antagonist activity on the guinea pig isolated jejunal, as described previously (Vollinga et al., 1992; Leurs et al., 1996). The intestine was removed rapidly and kept in oxygenated (95% O2-5% CO2) Krebs’ buffer (composition in mM: NaCl, 118; KCl, 5.6; CaCl2, 2.5; MgSO4, 1.18; NaH2PO4, 1.28; NaHCO3, 25; and glucose, 5.5). Jejunal segments (2 cm) were equilibrated at 37°C for 60 min, then stimulated maximally (15 V, 0.1 Hz, 0.5 msec duration), and isotonic contractions were recorded. After 30 min of stimulation, a cumulative dose-response curve for the H3 agonist R-α-methylhistamine was recorded. After wash-out, antagonists were preincubated 15 min during stimulation, and the H3 agonist dose-response curve was redetermined. Antagonists were studied at three concentrations, ranging from 3 nM to 1 μM. Four preparations were used for each compound.

Results

Base-line and vehicle antinociceptive scores. As documented previously (Li et al., 1996, 1997a, b), animals receiving ivt injections of saline vehicle showed no changes in nociceptive threshold on either test at any of the test times (not shown). In the present study, %MPE values for saline-injected animals were 5.0 ± 2.7 and −0.59 ± 2.7 for hot-plate and tail-flick tests, respectively (10 min, mean ± S.E.M., n = 6).

Overview of antinociceptive results. Of 21 compounds studied, 11 agents induced time- and dose-dependent antinociceptive activity on both the hot-plate and tail-flick tests. Figure 1 shows the time course of antinociceptive activity on the hot-plate test for selected doses of many of the compounds. Results were similar for the 5- and 10-min groups.
The highest doses tested induced maximum (i.e., 0.98, n evoked responses suggestive of toxicity (three compounds (metiamide, tiotidine and VUF4740) behavior or motor impairment. However, large doses of ED50 values were highly correlated across the tests (excellent agreement between hot-plate and tail-flick scores; end). Hot-plate ED50 values varied by approximately 6-fold both the hot-plate (figs. 2–5) and tail-flick tests (fig. 6, leg-

Fig. 1. Time course of hot-plate antinociceptive responses after ivt administration of selected doses (µg, in parentheses) of H3 antagonists and burimamide congeners (A, top) and other derivatives of burimamide and thioperamide (B, bottom). Animals were tested for base-line responses, received ivt injections (5 µl) and were retested 5, 10 and 30 min later (absissa). Antinociceptive responses are shown (ordinate, %MPE, mean ± S.E.M., n = 4–8).

ED50 for these agents (table 2). ED50 values were not estimated for VUF4741 (fig. 3) or VUF5262 (fig. 5), which gave highly variable responses that were not dose-dependent. Responses to the highest dose of VUF4686 tested (60 µg) were smaller than the effects of the lower doses. Although the two lower doses showed dose-dependent responses (fig. 4), an ED50 was not estimated (the 30-µg dose yielded an ED75 effect, fig. 4, table 2).

Antinociceptive activity of selected H3 antagonists (fig. 2 and table 2). Among H3 antagonists and chemical congeners, ranitidine and burimamide showed the highest antinociceptive potency (ED50 < 200 nmol); metiamide, tiotidine, cimetidine and improgan (devoid of H3 activity) showed considerably lower activity (ED50 > 250 nmol). Zolantidine and its analog SKF95299 were inactive at the highest dose tested (100 µg, table 2). VUF8299 (a cimetidine congener lacking H3 activity, Sterk et al., 1987) was inactive at 100 µg. VUF8298 (an effective H3 antagonist which is the 2-pyridyl homolog of cimetidine) was inactive at 150 µg. Data for cimetidine and improgan were reported previously (Li et al., 1996).

Antinociceptive activity of burimamide derivatives. Several structural analogs of burimamide were studied. Variations in the length of burimamide’s carbon side-chain (–(CH2)Y–) showed the analgesic potency of norburimamide (Y = 3) to be similar to that of burimamide (Y = 4). However, the longer chain analog VUF4740 (Y = 6) was approximately twice as potent as the other compounds (fig. 3, table 2). Variations in burimamide’s N-terminal substituents (where R = CH3 in burimamide) produced several high-potency antinociceptive agents (ED50 = 70–80 nmol): VUF4685 (R = phenyl), VUF4686 (R = benzyl) and VUF4687 (R = phenyl-ethyl, fig. 4). The N-cyclohexyl congener of burimamide (VUF4684, fig. 4) was only weakly active as an antinociceptive agent, with only about one third of the activity of the aromatically substituted congeners (VUF4685, VUF4686 and VUF4687, table 2).

Antinociceptive activity of clobenpropit, thioperam-

Toxicity and dose dependence. For most compounds (figs. 2–5), the highest doses tested induced maximum (i.e., cut-off) antinociception on both tests, with no observable behavioral or motor impairment. However, large doses of three compounds (metiamide, tiotidine and VUF4740) evoked responses suggestive of toxicity (e.g., abnormal posture, jumping, biting or vocalizing), and no antinociceptive data are reported for these treatment groups. Lower doses of metiamide and VUF4740 had no such effects and gave dose-dependent antinociceptive responses, permitting estimates of
The pA2 values were derived from Schild plots with slopes of estimated dose-response parameters and number of subjects. Otitidine, VUF8298 and VUF8299 showed negligible activity. See table 2 for described in figure 1 and under “Materials and Methods.” Results are 10 analogs after ivt administration. Injection and testing were performed as

Antinociceptive dose-response curves for H2 antagonists after ivt administration

Fig. 2. Antinociceptive dose-response curves for H2 antagonists and analogs after ivt administration. Injection and testing were performed as described in figure 1 and under “Materials and Methods.” Results are 10 min data from the hot-plate test. For compounds that were active, fitted dose-response curves estimated by nonlinear regression are shown. Tiotidine, VUF8298 and VUF8299 showed negligible activity. See table 2 for estimated dose-response parameters and number of subjects.

**TABLE 2**

Antinociceptive (hot-plate) potencies of H2 and H3 antagonists after ivt administration

Antinociceptive ED50 values and pharmacological activity of the present compounds are summarized. ED50 values were estimated from hot-plate data; nearly identical results were found with the tail-flick test (fig. 6). The active compounds are numbered for referencing in figure 6.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug no.</th>
<th>Drug Name</th>
<th>Doses Fitted</th>
<th>Subjects Fitted</th>
<th>Fitted ED50 (nmol ± S.E.M.)</th>
<th>Fitted SLOPE ± S.E.M.</th>
<th>H2 Ks</th>
<th>H2 Kd</th>
<th>H3 Ks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most potent (ED50 = 70–110 nmol)</td>
<td>1 VUF4686</td>
<td>–</td>
<td>7⁰</td>
<td>30⁰</td>
<td>104.5⁰</td>
<td>–</td>
<td>0.079⁰</td>
<td></td>
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<tr>
<td>Moderately potent (ED50 &gt; 250 nmol)</td>
<td>5 Ranitidine</td>
<td>3</td>
<td>15</td>
<td>34.3 ± 5.0</td>
<td>109.3 ± 15.9</td>
<td>3.6 ± 2.9</td>
<td>&gt;100 '</td>
<td>0.063' 50'</td>
<td></td>
</tr>
<tr>
<td>Least potent (ED50 &gt; 250 nmol)</td>
<td>9 VUF4684</td>
<td>3</td>
<td>15</td>
<td>79.9 ± 5.9</td>
<td>285.1 ± 21.0</td>
<td>5.4 ± 1.6</td>
<td>–</td>
<td>0.080⁰</td>
<td></td>
</tr>
</tbody>
</table>

* For this compound, value given is estimated ED50.

Omitted higher doses because of toxicity.

No antinociceptive effect of this dose.

Some differences in antinociceptive efficacy may exist, however, because not all of the compounds achieved 100% scores (e.g., VUF4684, VUF4686, VUF4741). In most cases, a large degree of antinociception was obtained without observable motor or behavioral impairment. Although motor or balance tests were not performed in the present study, previous experiments found that near-maximal antinociceptive doses of impropogan do not change locomotor activity or rotorod performance (Li et al., 1997a). The reduction in nociceptive responses without impairment of motor function implies that these compounds act on the brain to reduce pain perception (i.e., produce analgesia).

The antinociception produced by these compounds was characterized in studies of time course (fig. 1) and dose (figs. 2–5). The former results, showing peak effects 5 to 10 min after ivt administration with a return to near base-line latencies at 30 min, demonstrate the reversibility of the drug effects. The dose-response relationships found for most of the compounds permitted estimates of in vivo antinociceptive potency. The reliability of these estimates is strengthened by the excellent agreement between ED50 values obtained from two independent nociceptive tests (fig. 6) and by the size of the estimated S.E.M. values (usually 10–20% of the corresponding ED50, table 2). For the tail-flick assays, S.E.M. values (fig. 6, legend) and slope values (not shown) were larger than for the corresponding hot-plate estimates (table 2). Although most potency estimates were based on three or more doses, ED50 values for VUF4740 were derived from two doses; these values should be interpreted with some caution.

**H2 antagonist activity of VUF5261 and VUF5262.** As found previously (Leurs et al., 1996), Rα-methylhistamine induced dose-dependent inhibition of the neurogenic contractions of the guinea pig jejunum (pD2 = 7.9 ± 0.2, n = 8, not shown). In this preparation, both VUF5261 and VUF5262 behaved as competitive H2 antagonists, with pA2 values of 7.4 ± 0.1 and 8.7 ± 0.1, respectively (mean ± S.E.M., n = 4). The pA2 values were derived from Schild plots with slopes of 1.14 ± 0.14 and 0.85 ± 0.19, respectively, not significantly different from unity.

**Discussion**

The present results show that several compounds possessing H2 antagonist and/or H3 antagonist activity induce dose-related antinociception after ivt administration in rats. Under the conditions used (i.e., high temperature thermal stimuli), the cut-off or near cut-off latencies produced by most of the compounds show highly effective antinociception.
The results suggest that the present compounds can be classified into three potency groups (table 2). ED50 values for the most potent group (70–110 nmol, table 3) show that these compounds have about one third of the analgesic potency of ivt morphine in rats (Appelbaum and Holtzman, 1985; Yeung and Rudy, 1980). The slope parameters of the presently obtained dose-response curves (usually 3–5) are somewhat steep; analysis of published morphine dose-response curves (Appelbaum and Holtzman, 1985; Yeung and Rudy, 1980) by the same methods used in this study yielded slope values of about 1.5, with confidence intervals between 0 and 3. The significance of these slope values is unclear.

A critical assumption in the analysis of the present findings is that the active compounds share a common antinociceptive mechanism. Although this seems likely based on the SARs found, confirmation of this assumption awaits the discovery of agents capable of selectively inhibiting the activity of these agents.

The analgesic potencies of the present compounds are important for understanding the mechanism of action of these agents. In general, antinociceptive doses of these drugs are larger than those needed to block H2 or H3 receptors. For example, ivt ranitidine inhibited footshock-induced antinociception in rats (an H2 effect) with an IC50 of 6.3 nmol (Gogas and Hough, 1989); this dose is approximately 17-fold lower than the hot-plate antinociceptive ED50 (table 2). Although the brain concentrations achieved by the present treatments are unknown, they can be estimated. If a Kd concentration of ranitidine (63 nM on the H2 receptor, table 2) is achieved at brain receptors by an IC50 ivt dose (6.3 nmol), then it can be crudely estimated that the antinociceptive activity of ivt ranitidine is achieved at brain concentrations in the range of 1 μM (17 × 63 nM). It is not known whether the presently active compounds are behaving as receptor agonists or antagonists. However, the antinociceptive activity of these drugs seems unlikely to be caused by either action on H1, H2 or H3 receptors. An H3-agonist mechanism can be excluded because selective H3 antagonists like pyrilamine do not show antinociceptive activity on the tail-flick test (Dews and Graham, 1946; Li et al., 1997b), and at 100 μM, cimetidine, ranitidine, burimamide and improgan are either inactive or weakly active on the guinea pig ileum H3 response (table 2). An H3 agonist action is ruled out by results showing the inability of a large dose of pyrilamine to inhibit improgan...
In vitro pharmacology of cimetidine, burimamide and derivatives

TABLE 3

In vitro pharmacology of cimetidine, burimamide and derivatives

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug</th>
<th>Active Concentration</th>
<th>Order of Potency</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA-activated regulation of 5-HT uptake</td>
<td>tiot</td>
<td>0.2 nM</td>
<td>tiot-&gt;ran-&gt;cim-&gt;norbur</td>
<td>“atypical” HA receptor</td>
<td>c</td>
</tr>
<tr>
<td>3H-cimetidine binding</td>
<td>cim</td>
<td>0.2 µM</td>
<td>cim-&gt;bur-&gt;ran</td>
<td>Ran and tiot (1 mM) inactive</td>
<td>d</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;á&lt;/sub&gt; receptor</td>
<td>cim</td>
<td>0.1–100 µM</td>
<td>cim-&gt;met-&gt;ran</td>
<td>Ran much less active</td>
<td>e</td>
</tr>
<tr>
<td>Imidazoline (I&lt;sub&gt;i&lt;/sub&gt;) sites</td>
<td>cim</td>
<td>0.1 µM</td>
<td>cim-&gt;ran, tiot</td>
<td>Ran and tiot inactive (100 µM)</td>
<td>f,g</td>
</tr>
<tr>
<td>Imidazoline (I&lt;sub&gt;j&lt;/sub&gt;) sites</td>
<td>cim</td>
<td>&gt;1 mM</td>
<td>Very high concentrations</td>
<td>g</td>
<td></td>
</tr>
<tr>
<td>Alpha-1 receptor</td>
<td>bur</td>
<td>2 µM</td>
<td>bur-&gt;cim-&gt;met</td>
<td>Relevance to analgesia unclear</td>
<td>h</td>
</tr>
<tr>
<td>Alpha-2A receptor</td>
<td>cim</td>
<td>4 µM</td>
<td>Cim analgesia not clonidine-like</td>
<td>i,j</td>
<td></td>
</tr>
<tr>
<td>Alpha-2B receptor</td>
<td>cim</td>
<td>100 µM</td>
<td>High concentrations</td>
<td>j,l</td>
<td></td>
</tr>
<tr>
<td>Androgen receptor</td>
<td>cim</td>
<td>8 µM</td>
<td>cim-&gt;met-&gt;tiot</td>
<td>Tiotidine inactive</td>
<td>k</td>
</tr>
<tr>
<td>HA-activated Ca influx (human eosinophils)</td>
<td>cim</td>
<td>13 µM</td>
<td>thio-&gt;bur-&gt;cim</td>
<td>Novel H&lt;sub&gt;3&lt;/sub&gt; subtype?</td>
<td>k</td>
</tr>
<tr>
<td>Mu opiate receptor</td>
<td>cim</td>
<td>16–100 µM</td>
<td>–</td>
<td>Unconfirmed in unpublished studies</td>
<td>l,m</td>
</tr>
<tr>
<td>Diamine oxidase (DAO)</td>
<td>bur</td>
<td>20 µM</td>
<td>bur-&gt;met, cim</td>
<td>Bur 20–&lt;sup&gt;+&lt;/sup&gt; more active</td>
<td>n</td>
</tr>
<tr>
<td>TXA&lt;sub&gt;2&lt;/sub&gt; synthetase</td>
<td>bur</td>
<td>25 µM</td>
<td>bur-&gt;met, cim</td>
<td>Relevance to analgesia unclear</td>
<td>o</td>
</tr>
<tr>
<td>Benzodiazepine binding</td>
<td>cim</td>
<td>70 µM</td>
<td>cim-&gt;met</td>
<td>Met 50–&lt;sup&gt;+&lt;/sup&gt; less active</td>
<td>l</td>
</tr>
<tr>
<td>Muscarinic receptor</td>
<td>cim</td>
<td>100–300 µM</td>
<td>cim-&gt;tio-&gt;ran</td>
<td>Very high concentrations</td>
<td>p</td>
</tr>
<tr>
<td>HA methyltransferase (HMT)</td>
<td>bur</td>
<td>160–280 µM</td>
<td>bur-&gt;met, cim</td>
<td>Bur 10–&lt;sup&gt;+&lt;/sup&gt; more active, high</td>
<td>q</td>
</tr>
<tr>
<td>Beta receptor</td>
<td>cim</td>
<td>200 µM</td>
<td>cim-&gt;met, bur</td>
<td>Very high concentrations</td>
<td>p</td>
</tr>
<tr>
<td>HA (“Hic”) inhibition of platelet aggregation</td>
<td>cim</td>
<td>0.1–1 µM</td>
<td>cim-&gt;ran</td>
<td>Very high concentrations</td>
<td>r</td>
</tr>
<tr>
<td>Arachidonate cyclo-oxygenase</td>
<td>bur</td>
<td>2.3 mM</td>
<td>–</td>
<td>Very high concentrations</td>
<td>o</td>
</tr>
</tbody>
</table>

a) Drug abbreviations: cim, cimetidine; tiot, tiotidine; bur, burimamide; ran, ranitidine; thio, thioperamide.

b) IC<sub>50</sub> or K<sub>a</sub> value for the compound named. A range of concentrations indicates findings from more than one study. Where a receptor is given as the target, the compounds acted as antagonists.

References:
ization of improgan antinociception prompted further investigation of the $H_3$ analgesia hypothesis. Although virtually inactive at $H_1$ and $H_2$ sites, this compound behaved as an $H_3$ antagonist in the micromolar range; cimetidine was slightly less potent on both analgesia and at the $H_3$ receptor (Li et al., 1996; table 2). However, ivt coadministration of the $H_3$ agonist (R)-$\alpha$-methylhistamine failed to reduce improgan antinociception, lending no support to the $H_3$ antagonist hypothesis (Li et al., 1997b). Furthermore, coadministration of $H_3$ antagonists and improgan produced no evidence for an $H_3$ agonist analgesic mechanism (Li et al., 1997b). It was also considered possible that blockade of brain $H_3$ receptors could induce analgesia, but that thioperamide’s effects on other receptors (e.g., 5-HT$\alpha$, Leurs et al., 1995a) might prevent expression of this response. If this were the case, however, thioperamide also should have reduced improgan antinociception, an effect not observed (Li et al., 1997b).

The present experiments provide considerable additional data for evaluation of the $H_3$ analgesia hypothesis. Figure 7 shows the relationship between antinociceptive potency and $H_3$ receptor activity for 11 compounds. The data show that the $H_3$ receptor does not contribute to the antinociceptive responses measured presently. The correlation fails not only because of ranitidine (a compound with moderate antinociceptive potency and very low $H_3$ activity, data point on bottom right of fig. 7), but because of the lack of analgesic activity of potent $H_3$ antagonists like thioperamide, GT-2016 and clobenpropit (none of which are included in fig. 7). Also note that the N-cyclohexyl congener of burimamide (VUF4684) is considerably less potent than burimamide as an analgesic, a pattern distinct from the respective $H_3$ activities of these agents (table 2). The scales of the axes of figure 7 also rule out a meaningful role for the $H_3$ receptor, because the “most potent” and “least potent” groups (table 2) differ by only 5- to 6-fold on analgesic activity, but vary by 300- to 1000-fold on $H_3$ receptor activity (table 2, fig. 7). Although the existence of $H_3$ receptor subtypes seems likely (Leurs et al., 1996; West et al., 1990), the possibility that one such subtype contributes to analgesic mechanisms would require much additional work to be verified. Thus, the present results confirm the hypothesis that $H_1$, $H_2$ and $H_3$ receptors seem to be excluded as the mechanism of antinociceptive action for the compounds studied presently.

Because the actions of burimamide-like compounds are not limited to $H_4$ receptors, the additional pharmacology of these agents must be considered in the search for the mechanism(s) of antinociceptive action (table 3). Some effects of these drugs (e.g., antagonism at muscarinic, $beta$ adrenergic, benzodiazepine, HMT and “Hic” sites) occur only at high micromolar or low millimolar concentrations, which are probably not relevant to the present results. For other potential sites of action (e.g., labeled cimetidine binding, histaminergic regulation of 5-HT uptake, androgen receptors or imidazoline $I_1$ sites), potencies of these drugs may be relevant, but the inactivity of ranitidine and/or tiotidine in these systems argues against the significance of these sites for analgesic responses (table 3). An unusual HA response in eosinophils is inhibited by appropriate doses of burimamide and cimetidine, but thioperamide was very active in this system (table 3), in contrast to the present results. Other potential targets are not excluded by pharmacological criteria. For example, the burimamide-induced inhibition of thromboxane $A_2$ synthetase (25 $\mu$M, table 3) might be achieved by the present ivt doses, but this effect would not be expected to induce analgesia. Inhibition of arachidonate cyclooxygenase, which does cause analgesia, was not observed below millimolar concentrations of these drugs (table 3). Older reports that cimetidine acts weakly at $mu$ opioid receptors (table 3) may have possible relevance to the present results. However, unpublished studies in our laboratory show slight and no inhibition of $mu$ opioid receptor binding by cimetidine and improgan, respectively, at 100 $\mu$M. Furthermore, because improgan antinociception was unaffected by large doses of naltrexone, opioid analgesic mechanisms are unlikely to account for the antinociception produced by these compounds (Li et al., 1997b). Also noteworthy may be the reported inhibition of diamine oxidase (DAO) by burimamide (IC$_{50}$ = 20 $\mu$M); metiamide and cimetidine were about 20-fold less active (Bieganski et al., 1980). Although only small amounts of DAO are present in the brain, and this enzyme is not thought to contribute substantially to brain HA metabolism, the product of this reaction, imidazole acetic acid, is pharmacologically active (Ernsberger et al., 1995; Thomas and Prell, 1995). Thus, this mechanism may be of further interest.

The present results could be related to brain catecholamine systems. Burimamide, metiamide and cimetidine (50–250 $\mu$g ivt) decreased rat hypothalamic norepinephrine levels with an order of potency similar to that found presently on analgesia (Nowak, 1980; Nowak et al., 1978). These effects may be related to the ability of burimamide to activate the peripheral sympathetic system after large systemic doses (Brimblecomb et al., 1976). An action on central $alpha$-2 receptors may also require further investigation, because information is limited, and the reported potencies of cimetidine on two $alpha$-2 subtypes are somewhat discrepant (table 3). Although blockade of $alpha$-2 receptors might account for the ability of these compounds to deplete brain norepinephrine levels, this effect would not be expected to cause analgesia, because $alpha$-2 agonists, not $alpha$-2 antagonists induce analgesia (Tasker and Melzack, 1989). Centrally adminis-

![Fig. 7. Relationship between antinociceptive potency and $H_3$ antagonist affinity of 11 compounds. The antinociceptive ED$_{50}$ value (hot plate, abscissa, $-log_{_10}$ mol, ± transformed S.E.M.) for each drug is plotted against the $pA_2$ value (ordinate, $-log_{_10} K_d$ ± S.E.M.) of that compound for the $H_3$ receptor. No correlation is evident (also note differences in axis scales). See table 2 for details.](image-url)
tered metiamide reverses ivt clonidine-induced hypotension (Karppanen, 1981), but this effect is thought to occur by blockade of imidazole (I$_1$), not alpha-2 sites (Ernsberger et al., 1995). However, ranitidine’s inactivity at I$_1$ sites seems to exclude them as an analgesic target (table 3). Other poten-
tially significant adrenergic sites include alpha-1 receptors, which are blocked by burimamide in the low micromolar range (table 3).

Spinal noradrenergic systems participate in pain-relieving mechanisms, and the alpha-2 receptor agonists clonidine and dextemetomidine are thought to act at supraspinal and intraspinal sites to induce antinociceptive responses (Tasker and Melzack, 1989; Idanpaan-Heikkila et al., 1994). Because of overlapping structural and pharmacological profiles, the possibility that the presently observed antinociception res-
ults from a clonidine-like (i.e., alpha-2 agonist) effect requires further study. However, the following observations suggest that impropog antinociception is not mediated by a clonidine-like mech-
nism: 1) studies in progress in our laboratory show that impropog antinociception is not antagonized by yohimbine treatment at reverse clonidine analgesia (B. Y. Li and L. B. Hough, unpublished); and 2) unlike clonidine analgesia (which is accompanied by sedation and depression of locomotor activity, e.g., Smythe and Pappas, 1989), near-maximal impropog antinociception was observed without inhibition of spontaneous locomotor activity (Li et al., 1997a).

Despite the pharmacological considerations above, the mechanism of impropog antinociception remains unknown. However, the present results have revealed new information on the structural requirements for the analgesic activity of this class of compounds. For compounds closely related to cimetidine and impropog (see table 1), there seems to be an imidazole requirement, because VUF89289 (a pyridyl analog) and VUF89299 (a phenyl analog) were both inactive (table 2). However, imidazoles are not an absolute requirement for activity, because ranitidine (a substituted furan) was active. Netti et al. (1988) reported that ivt ranitidine induced analgesia, but no data were shown. With respect to other H$_2$ antagonists, the inactivity of zolantidine and SKF95299 is un-
explained, the same pattern was seen with another N-phenyl homolog of thioperamide, seems to support this hypothesis. The lack of analgesic activity of VUF5262, the N-phenyl homol of thioperamide, shows that, unlike the case for burimamide, the open-chain and piperidine-bridging groups are not equivalent. That is, substitution of the N-methyl group with N-phenyl increased analgesic potency for burim-
amide, but not for the thioperamide analog VUF5261. Furt-
her work is required to discover the mechanism of action of these compounds and to develop congeners that could be used for the clinical relief of pain.

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ical synthesis.

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