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Antinociceptive Activity of Derivatives of Improgran and Burimamide

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HOUGH, L. B., J. W. NALWALK, R. LEURS, W. M. P. B. MENGE AND H. TIMMERMAN. *Antinociceptive activity of derivatives of improgan and burimamide.* PHARMACOL BIOCHEM BEHAV **65**(1) 61–66, 2000.—Improgan, a compound related to H_2 and H_3 antagonists, induces antinociception in rodents after intraventricular administration. Characteristics of improgan and its congeners include: (a) morphine-like antinociception on thermal and mechanical tests in two species; (b) no impairment of motor coordination or locomotor activity; (c) evidence for a novel, nonopioid mechanism that is independent of known histamine receptors; (d) lack of tolerance with daily dosing; and (e) unique structure–activity relationships (SARs). Presently, the antinociceptive activity of several new derivatives of improgan was investigated in rats. Among compounds similar to burimamide, VUF4577 (possessing a two-carbon side chain) and VUF4582 (an *N*-phenyl derivative of VUF4577) induced complete, dose- and time-dependent antinociception on the hot-plate and tail-flick tests with no behavioral side effects. These compounds (with ED_{50} values of 71–117 nmol) were approximately twice as potent as burimamide itself (a four-carbon derivative). Two other derivatives in which the thiourea group (C=S, known to cause human toxicity) was replaced by either nitroethene ($C{=}CH{-}NO_2$, VUF5405) or urea (C=O, VUF5407) also showed effective, potent antinociception on both assays. The latter compound is the most potent improgan-like drug discovered to date (ED_{50} = 71 nmol). Furthermore, positional isomers of antinociceptive compounds either lacked activity (VUF5394) or induced toxicity (VUF5393), revealing a high degree of pharmacological specificity. Although the mechanism of improgan antinociception remains unknown, the present results show promise for the further development of safe, effective, and potent pain-relieving compounds. © 1999 Elsevier Science Inc.

Antinociception Analgesia Histamine Improgan

PREVIOUS work from our laboratories has described the antinociceptive activity of improgan, a chemical congener of the H_2 antagonist cimetidine that lacks H_2 antagonist activity (see Table 1 for structure). Improgan, formerly known as SKF92374 (12), 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 (12) and mice (13) after intraventricular (IVT) administration. The compound had a similar profile in rats when studied with a mechanical nociceptive test (13). Additional behavioral testing showed that improgan lacked effects on spontaneous locomotor activity (implying the absence of stimulant or depressant actions) and on an accelerated rotorod test (implying the absence of motor impairment) (13). The antinociceptive activity of burimamide, another closely related compound with both H_2 and H_3 blocking properties (Table 1), has also been reported after IVT administration in mice (11). Pharmacological studies have established that the antinociceptive activities of improgan and burimamide are not due to actions on opiate receptors, on H_1, H_2, or H_3 receptors, or on histamine metabolism (9,11,12,14). Thus, these compounds, which are chemically related to histamine antagonists, induce analgesia after CNS administration, but act by an unknown mechanism.

More recently, the SAR for the antinociceptive actions of 21 compounds related to improgan and burimamide were studied (9). Antinociceptive potencies varied over nearly sixfold, and were not correlated with activity on H_1, H_2, or H_3 receptors. Furthermore, a novel structure–activity profile for these agents was delineated. For example, the highly potent

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TABLE 1
STRUCTURES OF COMPOUNDS RELATED TO IMPROGAN Ar-Y-X-NH-R

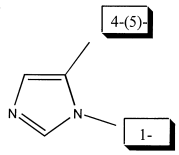
Drug	Class	Ar‡	Y	X	R	References
Histamine	Agonist	Imidazol-4-(5)-y1	—$(CH_2)_2$—	—	H	(9)
Improgan	Control*	Imidazol-4-(5)-y1	—$(CH_2)_3$NH—	—C(=N—CN)—	methyl	(9;12)
Cimetidine	H_2 Antagonist	4-Methylimidazol-5-y1	—CH_2—S— $(CH_2)_2$—NH—	—C(=N—CN)—	methyl	(9;12)
Burimamide	H_3 Antagonist†	Imidazol-4-(5)-y1	—$(CH_2)_4$—NH—	—C(=S)—	methyl	(9;11)
Norburimamide	H_3 Antagonist	Imidazol-4-(5)-y1	—$(CH_2)_3$—NH—	—C(=S)—	methyl	(9)
VUF4577	H_3 Antagonist	Imidazol-4-(5)-y1	—$(CH_2)_2$—NH—	—C(=S)—	methyl	(17)
VUF4582	H_3 Antagonist	Imidazol-4-(5)-y1	—$(CH_2)_2$—NH—	—C(=S)—	phenyl	(17)
VUF5405	unknown	Imidazol-4-(5)-y1	—$(CH_2)_4$—NH—	—C(=CH— NO_2)—	methyl	–
VUF5407	unknown	Imidazol-4-(5)-y1	—$(CH_2)_4$—NH—	—C(=O)—	phenyl	–
VUF5393	unknown	Imidazol-1-yl	—$(CH_2)_3$—NH—	—C(=CH— NO_2)—	methyl	–
VUF5394	unknown	Imidazol-1-y1	—$(CH_2)_3$—NH—	—C(=S)—	methyl	–

The pharmacological classification and chemical structures of compounds related to improgan and burimamide are shown. Drugs shown in bold have been previously reported to have antinociceptive activity; others were tested presently.

*Chemical congener of cimetidine virtually devoid of H_2 antagonist activity.

†Burimamide is also a weak H_2 antagonist.

‡Due to tautomerism, monosubstituted imidazoles have the following numbering system:



H_3 antagonist thioperamide lacked antinociceptive activity, but closely related congeners showed both H_3 antagonist activity and antinociception. However, the structure–activity profile of this family of compounds is incomplete. Presently, we have further investigated the actions of compounds related to improgan and burimamide, and report potent antinociceptive activity of several new derivatives.

METHOD

Animals

Male Sprague–Dawley rats (Taconic Farms, Inc., Germantown, NY), weighing 200–320 g at the time of testing, were maintained on a reverse 12 L:12 D cycle (lights on 1900, light off 0700) and used for nociceptive testing. 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. VUF4577 (oxalate) and VUF4582 (oxalate) were available from laboratory stock (18). Others were synthesized as described below. Salts were dissolved in isotonic saline. Bases were dissolved in HCl (1.0–1.2 N), titrated to a pH between 5.5–6.5, and diluted with saline. Vehicle injections consisted of either saline, or neutralized, diluted HCl. All doses are given as equivalent to the base form of the drug.

Chemical Synthesis

VUF 5405 (base) was synthesized according to (8) in two steps starting from 4-(4-aminobutyl)-1H-imidazole dihydrobromide (18) and 1,1-bis(methylthio)-2-nitroethylene followed by methylamine and recrystallized from water [m.p. = 206.5–207.5; analysis: C = 50.03 (calc. 50.20), H = 7.14 (calc. 7.16), N = 29.38 (calc. 29.27)]. VUF 5407 (base) was synthesized according to (6) starting from 4-(4-aminobutyl)-1H-imidazole dihydrobromide (17) and phenyl isocyanate. The product was purified by chromatography (SiO_2, ethyl acetate/methanol) and recrystallized from water [m.p. = 139.4–140.2; analysis: C = 64.99 (calc. 65.09), H = 7.13 (calc. 7.02), N = 21.60 (calc. 21.69)]. VUF 5393 (base) was prepared in a procedure analogous to VUF 5405 starting from 1-(3-aminopropyl)-imidazole and recrystallized from methanol/ether [m.p. = 167.9–179.1; analysis: C = 48.04 (calc. 47.99), H = 6.71 (calc. 6.71), N = 30.87 (calc. 31.09)]. VUF 5394 (base) was prepared from 1-(3-aminopropyl)-imidazole and methyl isothiocyanate as described (17) [m.p. = 108.2–109.8; analysis: C = 48.32 (calc. 48.46), H = 7.01 (calc. 7.12), N = 28.32 (calc. 28.25)].

Surgery for Microinjections

The microinjection apparatus, consisting of a chronically implanted guide cannula along with a stylet and an injection cannula, has been previously described in detail (4). Rats were anesthetized with methohexital (50 mg/kg) and supplemented with methoxyflurane. Unilateral guide cannulas were stereotaxically implanted into the brain and anchored to the skull with three stainless steel screws and dental cement. After surgery, animals were individually housed with food and water freely available for 1 week before testing. Guide cannulas were implanted such that injections were made into the left lateral ventricle. Coordinates [in mm from bregma; (15)] for the guide cannulas were: AP −0.8, ML +1.5, DV −3.3, 0° angle. Injection cannulas were made to extend 1 mm ventrally beyond the tip of the guides. Each animal was only used for a single experiment.

Nociceptive Testing

Two nociceptive tests were used—the radiant heat tail-flick test (5), and the hot-plate test (7). For the tail-flick test, the radiant heat source was set such that baseline latencies were generally between 3 and 4 s, with a 15-s cutoff. The heat source was not adjusted for individual animals. The ventral surface of tail (2–5 cm from the tip) was exposed to radiant heat, and the latency for tail movement was recorded. For the hot-plate test, animals were placed on a 52° surface, and the latency to a hind paw lift or lick was recorded, with a maximal

exposure of 60 s. Baseline latencies were 8–14 s. Three to seven hours into the dark portion of the diurnal cycle, animals were tested for baseline nociception (one hot-plate test followed by three tail-flick tests). Animals were then gently secured by wrapping with a laboratory pad, the stylet was removed, and the injection cannula was inserted. Drugs were injected manually (a total volume of 5 μl over 5 min). Successful injection was assured by following movement of an air bubble in the tubing between the syringe and the cannula and by the absence of leakage. One minute after the end of the infusion, the injection cannula was removed and the stylet replaced. Animals were retested with single hot-plate and tail-flick tests at 5, 10, and 30 min after the replacement of the stylet. Each tail-flick test was performed 1 min after a hot-plate test. At the end of each experiment, animals received IP pentobarbital (50 mg/kg) and IVT (5 μl) injections of India Ink. Successful IVT injections were verified by observing the proper distribution of ink throughout the ventricular system. Data from animals whose injections were outside the lateral ventricle or who had unsuccessful injections were excluded.

Analysis of Antinociceptive Data

Antinociceptive scores for each animal were calculated as percent of maximum possible effect (%MPE), where

$$\%\text{MPE} = \frac{\text{drug latency} - \text{baseline latency}}{\text{cutoff latency} - \text{baseline latency}} \times 100$$

For the tail flick test, the third latency before the drug treatment was used as the baseline score, because the first two scores are higher than the subsequent latencies when no drug is given. Results for each treatment group are given as mean %MPE ± SEM. Data for all doses of each drug were fitted by use of iterative nonlinear regression methods (Graphpad Prism, San Diego, CA) to the following equation:

$$E = 100 - \frac{100}{\left(1 + \frac{D}{\text{ED}_{50}}\right)^n}$$

where E is observed analgesic effect (% MPE), D is the dose of drug injected (μg), n is the slope function of the dose–response curve, and ED_{50} is the dose of drug inducing a 50% of maximum effect (μg). All fits converged with statistically significant ($p < 0.05$) regression parameters. For each parameter (ED_{50} and n), mean, SEM and 95% confidence intervals of the regression were obtained.

Assay of H_2 and H_3 Antagonist Activity

H_2 activity was assessed in binding experiments with labeled iodoaminopotentidine on the rat H_2 receptor expressed in CHO cells as described (16). H_3 binding was performed in rat cerebral cortical membranes with ^{125}I-iodophenpropit (10). Competition curves were generated to estimate pKi values in three independent experiments performed in triplicate.

RESULTS

Baseline and Vehicle Antinociceptive Scores

As documented previously (9,12–14), animals receiving IVT injections of saline vehicle showed no changes in nociceptive threshold on either test at any time (not shown).

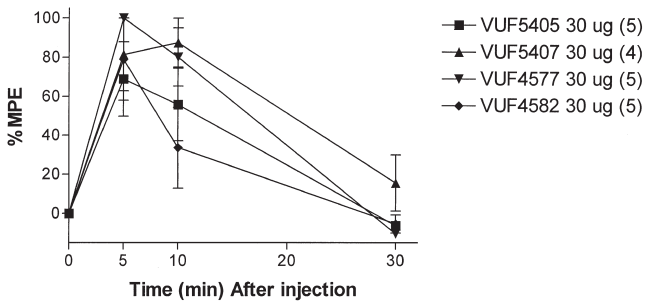


FIG. 1. Time course of antinociceptive responses after IVT administration of improgan derivatives (30 μg). Animals were tested for baseline responses, received an IVT injection (5 μl) and were retested 5, 10, and 30 min later (abscissa). Antinociceptive responses are shown (ordinate, hot plate %MPE, mean ± SEM) for the number of subjects in parentheses. See Table 1 for structures of the compounds tested.

Time Course of Antinociception

Four compounds tested presently (VUF4577, VUF4582, VUF5405, and VUF5407) showed good antinociceptive activity on both the hot-plate and tail-flick tests after IVT administration. No unusual behavioral or motor effects were noted after administration of these agents. The time course of action of these compounds (30 μg) is shown in Fig. 1 (hot-plate test). Peak activity was observed 5–10 min after administration, with little or no response 30 min later, demonstrating the reversibility of these effects. Tail-flick responses (not shown) were similar to effects seen on the hot-plate test.

Antinociceptive Potencies

All four compounds showed dose-dependent antinociception on both tests (Fig. 2), with no observable behavioral or motor effects. In contrast, VUF5394 was inactive at 60 and 100 μg at 5 min (Fig. 2) and at later times (not shown). Administration of VUF5393 at 30, 60, and 100 μg induced dose-dependent signs of toxicity, including "explosive motor behavior," and nociceptive testing was not performed with this compound. The hot-plate dose–response data of Fig. 2 were fitted to estimate antinociceptive potencics (Table 2). The four compounds showed ED_{50} values with overlapping confidence intervals (18.4–24.9 μg, 71–117 nmol). Fitted slope parameters of the hot-plate dose–response curves ranged from 4.0–4.5 (not shown). Comparisons of the hot-plate activities of the four new improgan derivatives with those of previously reported compounds (Table 2) show that the new agents are approximately two-, three-, and fourfold more potent than burimamide, improgan, and cimetidine, respectively. All of the compounds are significantly more potent than burimamide, as judged by the lack of overlap in the ED_{50} confidence intervals (Table 2).

H_2 and H_3 Antagonist Activity (Table 2)

All of the compounds tested were either inactive or poorly active (VUF5405) on the H_2 receptor. They ranged from inactive (VUF5393) to moderately active (VUF5407) on the H_3 receptor.

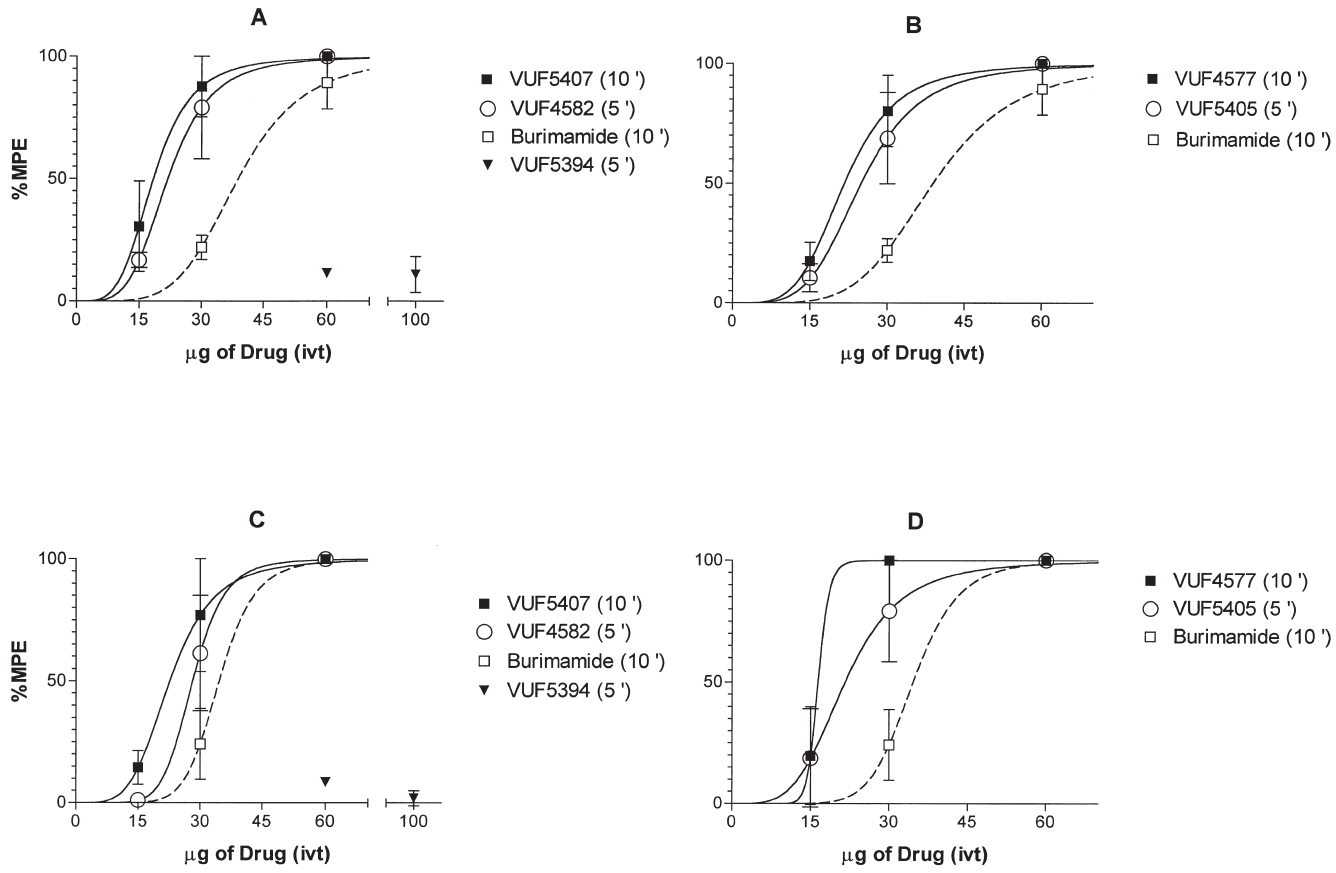


FIG. 2. Antinociceptive dose–response curves for improgan congeners after IVT administration. Injection and testing were performed as described in Fig. 1 and in the Method section. Results from the hot-plate (A, B, top) and tail-flick (C, D, bottom) tests are shown for the compounds and times given. See Table 2 for ED_{50} values and number of subjects. Burimamide results were previously reported (9), and have been included for comparison.

DISCUSSION

Previous work has suggested that improgan-like antinociceptive agents belong to a new pharmacological family of pain-relieving drugs. Although the mechanism of action of these compounds remains unknown, the present work reports the discovery of several new members of this family with potent activity. In addition, the SAR for these compounds has been broadened and refined.

TABLE 2

PHARMACOLOGICAL ACTIVITIES OF IMPROGAN DERIVATIVES

Drug	No. of Subjects Tested	Fitted ED_{50} (HP, μg) [95% C.I.]	Fitted ED_{50} (HP, nmol)	H_2 pKi	H_3 pKi
Improgan*	25	68.0 [56.8–79.2]	330	3.47	5.27
Cimetidine*	21	116.2 [69.8–163.0]	463	6.10	4.48
Burimamide*	21	38.9 [31.6–46.2]	183	5.10	7.02
Norburimamide*	18	43.1 [30.9–55.2]	217	3.94	6.42
VUF4577	14	21.6 [16.6–26.6]	117	<4	5.40 ± 0.03
VUF4582	15	21.9 [15.8–28.0]	89	<4	5.10 ± 0.03
VUF5405	15	24.9 [18.6–31.1]	104	5.00 ± 0.07	6.00 ± 0.07
VUF5407	11	18.4 [12.7–24.1]	71	<4	6.80 ± 0.09
VUF5393	3	toxic	—	<4	<4
VUF5394	5	>>100	>>504	<4	6.01 ± 0.15

Antinociceptive ED_{50} values and pharmacological activity of the present compounds are summarized. ED_{50} values (mean and 95% confidence intervals) were estimated from hot plate data; similar results were found with the tail flick test (Fig. 2).

*Data for these compounds were previously reported (9), and are included for comparison.

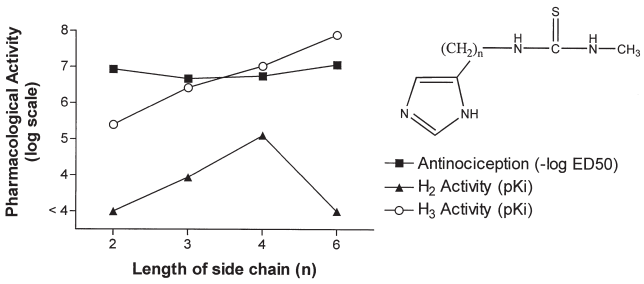


FIG. 3. Relationship between antinociceptive activity and HA receptor activity among burimamide derivatives. Antinociceptive activity (squares, -log molar hot-plate ED_{50}, Table 2) is plotted as a function of side-chain length for VUF4577, norburimamide, and burimamide (n = 2–4, respectively) and VUF4740 [n = 6]. pKi values for H_2 (triangles) and H_3 (circles) activity are also taken from Table 2 and literature (9,17).

Burimamide Derivatives

The present results show that VUF4577 and its *N*-phenyl congener (VUF4582) are potent antinociceptive agents (Fig. 2) that lack significant activity on either H_2 or H_3 receptors (Table 2). The H_3 activity of these compounds, presently assessed by binding methods, agrees closely with previous bioassay results (17). Previous work has also shown that burimamide-like drugs lack meaningful activity on H_1 receptors (17). Thus, the antinociceptive activities of VUF4577 and VUF4582 further support the conclusion that these compounds are not acting by known histaminergic mechanisms. Figure 3 shows that the relationships among burimamide derivatives for antinociceptive activity, H_2 activity, and H_3 activity are easily distinguished. For example, VUF4577 (containing a two-carbon side chain, Table 1), has the same antinociceptive potency as VUF4740 [the six-carbon congener studied previously (9)], yet the H_3 affinities of these agents differ by over 100-fold. VUF4577 and VUF4740 are approximately twice as potent as derivatives with the intermediate-sized chains (norburimamide and burimamide, with three and four carbons, respectively, Table 2). Nevertheless, compared with the H_2 and H_3 receptor profiles, antinociceptive activity is retained on a log scale over a broad range of chain lengths (Fig. 3).

Positional Isomers of Improgan-Like Compounds

Although variations in chain length among burimamide derivatives do not abolish antinociceptive activity, changes in the aromatic nucleus of these compounds have dramatic effects on biological activity. Present (Table 2) and previous results (9) show that antinociceptive activity is retained in many imidazole-containing agents when side chains are attached through the 4-(5)-carbon (Table 1). However, positional isomers attached through the imidazole nitrogen (N-1, e.g., VUF5394) showed no activity (cf. norburimamide). Previous studies showed that substitution of the imidazole nucleus in cimetidine with either phenyl or 2-pyridinyl moieties also resulted in loss of activity (9). Thus, a high degree of specificity is required among imidazole-containing antinociceptive agents, although a requirement for imidazole is not absolute, because ranitidine, an amino-furan, also shows antinociceptive activity (9).

Requirements for Polar Groups in Improgan-Like Agents

SAR studies of H_2 antagonists (from which improgan was derived) found that several "polar groups" (X in Table 1) can be substituted for the thiourea (C=S) group in burimamide (3). These include cyanoguanidine (C=N—CN, e.g., cimetidine), nitroethenylguanidine (C=CH—NO_2, e.g., ranitidine), and others. Present results confirm that the nitroethenylguanidine group also supports antinociceptive activity. Thus, VUF5405, the nitroethene equivalent of burimamide (Table 1), was not only active, but nearly twice as potent as the latter. VUF5405 has approximately the same potency as ranitidine, a furan-containing nitroethene derivative (9). The present results also show that urea (C=O) can be substituted for thiourea to retain antinociceptive activity. Thus, VUF5407 showed dose- and time-dependent activity on both nociceptive assays (Fig. 2). VUF5407 is the first urea-containing improgan derivative tested, and is the most potent antinociceptive agent in this class discovered to date (Table 2). It has approximately one-third of the analgesic activity of IVT morphine (1). Because VUF5407 (ED_{50} = 71 nmol) possesses antinociceptive potency comparable to its thiourea equivalent [VUF4685, ED_{50} = 81 nmol (9), urea-containing improgan derivatives show promise for further development. These drugs are also preferable to thiourea-containing derivatives, which showed human toxicity in early clinical trials (2). Based on structural similarities to burimamide and ranitidine, the present compounds are not likely to enter the CNS after systemic dosing. However, brain-penetrating congeners of these drugs are in development, which may be clinically useful for the relief of pain.

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