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*DPI – 3290 (I) : A MIXED OPIOID AGONIST WITH POTENT ANTINOCICEPTIVE ACTIVITY

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*DPI-3290 is currently being co-developed with Organon Corporation as Org 41793

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Running Title: Pharmacology of DPI-3290

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Abbreviations: MVD, Mouse Vas Deferens; GPI, Guinea Pig Ileum; TIPP, (Tyr-Tic-Phe-Phe); nor-BNI, (nor-binaltorphimine); CTOP, (cyclic [D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2]); DPDPE, (cyclic [D-Pen^2,D-Pen^5]enkephalin); MPE, maximal percent effect; NLX, naloxone; NTI, naltrindole; DPI-3290, (+) 3-((α-R)-α-((2S,5R)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-N-(3-fluorophenyl)-N-methylbenzamide; U69593, (5α,7α,8α)-(-)-N-methyl-N-(7-(1-pyrrolidinyl)-1-oxaspiro(4.5) dec-8-yl)burzeneacetamide; DAMGO, D-Ala, MePhe, Gly(ol) enkephalin

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Abstract

Compound DPI-3290, ((+) 3-((α-R)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl-N-(3-fluorophenyl)-N-methylbenzamide), is one of a series of novel centrally acting agents with potent antinociceptive activity that binds specifically and with high affinity to opioid receptors. In saturation equilibrium binding studies performed at 25°C using membranes from rat brain or guinea pig cerebellum, the K_i values measured for DPI-3290 at *delta* (δ), *mu* (μ), and *kappa* (κ) opioid receptors were 0.18 ± 0.02 nM, 0.46 ± 0.05 nM, and 0.62 ± 0.09 nM, respectively. In vas deferens isolated from laboratory mice, DPI-3290 decreased electrically induced tension development in a concentration-dependent manner with corresponding IC_{50} values of 1.0 ± 0.3 nM, 6.2 ± 2.0 nM, and 25.0 ± 3.3 nM at δ, μ, and κ receptors, respectively. The activity of DPI-3290 in isolated vas deferens tissue was approximately 20,000, 175.8, and 1,500.0 times more efficacious than morphine, and 492, 2.5, and 35 times more efficacious than fentanyl at δ, μ, and κ receptors, respectively. In ileal strips isolated from guinea pigs, DPI-3290 inhibited tension development with a corresponding IC_{50} value of 3.4 ± 1.6 nM at μ opioid receptors and 6.7 ± 1.6 nM at κ opioid receptors. Intravenous administration of 0.05±0.007 mg/kg of DPI-3290 produced a 50% antinociceptive response in rats. The antinociceptive properties of DPI-3290 were blocked by naloxone (0.5mg/kg, sc). When compared to morphine, this study demonstrated that DPI-3290 is more potent and elicited a similar magnitude of antinociceptive activity in the rat, actions mediated by its mixed opioid receptor agonist activity. The marked antinociceptive activity of DPI-3290 will likely

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provide a means for relieving severe pain in patients that require analgesic treatment.

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Nucleotide sequences for three distinct pertussis toxin-sensitive heterotrimeric GTP binding protein coupled opioid receptors have been reported with approximately 65% homology existing between their amino acids (Chen et al., 1993a; Chen et al., 1993b, Kieffer et al, 1992). These distinct opioid receptors termed *mu*, *delta* and *kappa*, are the binding sites for the endogenous peptide molecules endorphins, enkephalins, and dynorphins that have been shown to elicit a variety of pharmacologic actions (Chang, 1984). The most notable of these opioid receptor-mediated actions is analgesia.

Opioids produce their therapeutic effects by acting at the same receptors as the endogenous opioid peptides (Erspamer et al., 1989). They alter synaptic transmission by modulating the presynaptic release of neurotransmitters such as acetylcholine, norepinephrine, serotonin, dopamine and substance P (Hagelberg et al., 2002). Changes in receptor-operated potassium currents, adenylate cyclase activity and intracellular free ionized calcium concentrations all have been reported to contribute to these changes in synaptic transmission (Murthy and Makhlouf, 1996, Fan and Crain, 1995). To date, hyperpolarization of membrane potential and the corresponding effects on voltage sensitive calcium channels (Tang, 1994) together represent the most plausible mechanisms for explaining the actions of opioid receptor active ligands in blunting the cellular excitability of neurons. Whatever the specific mechanism, by modulating the release of these neurotransmitters opioids inhibit responses to painful stimuli.

Opioid receptors are widely distributed throughout the central and peripheral nervous system and play a fundamental neuromodulatory role in the perception of pain (Quock et al., 1999). Today, most clinically available opioid drugs act by targeting the *mu* subclass of the opioid receptor for

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mediating the relief of moderate-to-severe pain, reflecting their similarity to morphine (Clotz et al.,1991; Holder et al., 1995; Inturrisi et al., 1991). These clinically available *mu* opioid receptor drugs, of which morphine and fentanyl remain the most widely used, also produce respiratory depression at therapeutic doses. The rostrodorsal surface of the pons, the nucleus tractus solitarius and the nucleus ambiguous are specific sites linked to respiratory depression stemming from the use of opioids (Hurle et al., 1985; Morin-Surun et al., 1984). Studies have compared the ratio of analgesia to respiratory depression in a series of morphine like opioids. These studies have demonstrated that when equivalent analgesic doses are compared, the degree of respiratory depression is similar in magnitude to that measured with morphine.

Recently, the analgesic activity of several non-peptide *delta* opioid receptor agonists has been described (Chang *et al*., 1993; Comer et al., 1993; Dykstra et al., 1989; Lee et al., 1993; Portoghese, 1991). Using these ligands, there is now experimental evidence to support the hypothesis that a compound with combined *mu* and *delta* opioid receptor agonist activities would have potential clinical advantages over drugs acting predominantly at the *mu* opioid receptor (O'Neill et al., 1997; Su et al., 1998). To this end, we have synthesized a series of piperazinyl methylbenzamide analogs in an attempt to define a compound that: 1) possess both *mu* and *delta* receptor affinity and intrinsic activity; 2) has strong analgesic activity; 3) has a long duration of action; 4) does not cause excessive respiratory depression. Several compounds of this series appear to fulfill these criteria. Herein we report the radioligand binding affinity (K_i) at *mu*, *delta*, and *kappa* opioid receptors, the intrinsic activity in MVD and guinea pig ileum, and the antinociceptive properties in rats with one such agent, DPI-3290 {chemical

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name, (+) 3-((α-R)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl-N-(3-fluorophenyl)-N-methylbenzamide}.

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Methods

Membrane preparation for radioligand binding. The brains from male albino Sprague-Dawley rats were obtained from Pel Freez Biologicals (Rogers, AR) and cerebellum from male albino guinea pigs from Accurate Chemical and Scientific Corporation (Westbury, NY). The tissue was rinsed with ice-cold 50mM Tris-HCl buffer (pH 7.4, 25^{o}C) containing the following protease inhibitors: 50ug/ml soybean trypson inhibitor, 0.1 mM phenylmethylsulfonyl floride (PMSF) and 1 mM ethylenedinitrilotetraacetic acid (EDTA), 10µg/mL Leupeptin, 200µg/ml Bacitracin, and 0.5 µg/mL Aprotinin. Brains were minced with scissors and homogenized in 5 to 10 volumes/g wet wt. in ice-cold 50mM Tris buffer containing protease inhibitors. The homogenate was prepared using a motor-driven glass-Teflon homogenizer (nominal clearance, 0.13-0.18 mm). The homogenate was centrifuged at 6,000 x g for 15 min at 4^{o}C, and the resulting supernatant centrifuged at 41,000 x g for 30 min. This membrane pellet was resuspended in 10 volumes/g wet wt of 10mM Tris-sucrose buffer and sonicated with a Polytron tissue grinder (10 sec, low speed). The homogenate was centrifuged at 41,000 x g for 30 min at 4^{o}C. The resulting membrane pellet was resuspended in 50 mM Tris buffer with protease inhibitors at a final protein concentration that ranged from 40µg/ml to 50µg/ml. This membrane fraction was frozen under liquid N_2 and stored at –80^{o}C prior to use in radioligand binding studies. Protein determination was determined by the method of Bradford (1976).

Radioligand binding. Membrane fractions were incubated with 0.1 nM [^{3}H] DPDPE (specific activity 50.6 Ci/mmol), 0.1 nM [^{3}H]DAMGO

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(specific activity 50.0 Ci/mmol) or 0.1nM [^{3}H]U69593 (specific activity 41.4 Ci/mmol) in 2 ml of 10 mM Tris-HCl buffer containing 5mM $MgCl_2$ and protease inhibitors. Incubation was carried out for 90 min at 25^{o}C, these conditions permitted the complete equilibration of the radioligand with its receptor. The reaction was terminated by rapid filtration through Whatman GF/C glass fiber filters using a cell harvester (model M-48R, Brandel Instruments, Gaithersberg, MD) followed by two 5-ml rinses with ice-cold 50mM Tris buffer. Specific binding was defined as that radioligand displaced by 1x10^{-6} M naloxone. Filters were counted by liquid scintillation spectrometry at an efficiency, determined by external standards, of 40 to 45%.

Vas deferens studies. Tension development in vas deferens was measured as described previously (Chang et al., 1999). Vas deferens were isolated from male CD-1 mice (Charles River, Raleigh, NC) weighing 20-25g following cervical dislocation. Muscles were suspended in individual organ baths containing Mg-free Krebs-Henseleit solution (37^{o}C, aerated with O_2-CO_2, 95:5) of the following composition (millimolar): NaCl, 117.5; KCl, 4.75; $CaCl_2$, 2.6; KH_2PO_4, 1.2; $NaHCO_3$, 24.5; and glucose, 11.

The vas deferens segments were positioned between platinum electrodes and connected to a Grass FTO3 isometric force transducer. Muscles were stimulated to contract by administering 400-msec pulse trains (1msec duration, supramaximal voltage, 10 Hz) with a Grass S88 stimulator; resting tension was 0.5g.

Ileum muscle studies. The ileum was isolated from male albino guinea pigs (Charles River, Raleigh, NC) weighing 300 – 500g and cut into 3 cm segments. Each segment was suspended in individual organ baths

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containing Krebs-Henseleit solution (37^{o}C, aerated with O_2-CO_2, 95:5) of the following composition (millimolar): NaCl, 117.5; KCl, 4.75; $CaCl_2$, 2.4; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 24.5; and glucose, 11. A resting tension of 1 g was applied to each tissue. Muscles were field stimulated to contract by administering 0.1-Hz pulses of 0.5 msec duration at supramaximal voltage to platinum electrodes with a Grass S88 stimulator.

To establish cumulative concentration-response relationships, compounds were added to organ baths and allowed to produce maximal response before addition of the next higher concentration. In experiments carried out to evaluate the action of a compound at a specific opioid receptor, the remaining opioid receptors were blocked by adding to the bath the appropriate antagonists, either *kappa* antagonist nor-BNI (15 x 10^{-9} M), *mu* antagonist CTOP (1 x 10^{-6} M), and/or *delta* antagonist TIPP (3 x 10^{-6} M) 10 min before the addition of test compound.

Blood gas and antinociceptive studies. Male albino Wistar Hannover rats (HDS Madison, WI) weighing 200-300g were anesthetized with 2% isoflurane in a 30% O_2 and 70% N_2O vehicle. The femoral artery and external jugular vein were cannulated with Silastic tubing. Anesthetic gases were removed and the rats were allowed to recover in a plastic restrainer for 60 min prior to administration of test compound and simultaneous measurement of arterial blood gasses and antinociceptive responses.

Following intravenous administration of test article, arterial blood was drawn into a syringe with heparin. The volume of blood taken was 0.15 ml. The syringe was capped and the blood was analyzed immediately (Ph/Gas Analyzer Synthesis 25 Model, Instrumentation Laboratory). Caution was taken to avoid exposing the arterial blood sample to atmospheric air.

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The antinociceptive assay was the standard tail-pinch test described previously (Wong et al., 1992). Briefly, the test was performed with the rat in a restrainer and pressure from an artery clamp was placed on the tail (one inch from the tip). The clamp remained in place until an escape response occurred (i.e. tail-flick or vocalization) or a maximum time of 20 seconds had elapsed. The escape response latency was recorded by means of a stopwatch.

Data were converted from the latency response time or unit into a maximal-percent-effect score as described below.

$$\text{MPE} = \frac{\text{response time} - \text{basal time}}{\text{maximum time} - \text{basal time}} \times 100$$

Sources of drugs. [^{3}H]DPDPE, [^{3}H]DAMGO, and [^{3}H]U69593 were purchased from Dupont-New England Nuclear, a division of Perkin-Elmer (Boston, MA). Purities were greater than 98%. CTOP and TIPP were purchased from Peninsula Laboratories, Inc. (Belmont, CA). Morphine, fentanyl, naloxone, U69593, and all other chemicals were reagent grade and purchased from Sigma Chemical Co. (St. Louis, MO). Novel compounds described in this study were synthesized at Ardent Research Laboratories and Burroughs Wellcome Co. (Research Triangle Park, NC) using standard protocols.

Calculations and Statistics. Pharmacologic data were analyzed by linear regression of the linear portion of the concentration-response curves to determine the EC_{50} or IC_{50} values using the computer program, Prism

(GraphPad Software Inc., 5755 Oberlin Drive, # 110, San Diego, CA 92121, USA).

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Results

Inhibition of [^{3}H]DPDPE, [^{3}H]DAMGO and [^{3}H]U69593 binding by piperazinyl methylbenzamidines. Previous studies have demonstrated that [^{3}H]DPDPE, [^{3}H]DAMGO, and [^{3}H]U69593 bind specifically and with high affinity to *delta*, *mu*, and *kappa* opioid receptors, respectively (Clark et al., 1988; Lahti et al., 1985; Yamamura et al., 1992). In rat brain membranes rich in *delta* and *mu* opioid receptors, the specific binding of [^{3}H]DPDPE to *delta* opioid receptors was 80-95% whereas the specific binding of [^{3}H]DAMGO to *mu* opioid receptors was 75-85% under the conditions used in this study. In membranes rich in *kappa* opioid receptors prepared from guinea pig cerebellum, the specific binding of [^{3}H]U69593 ranged from 75-95%. Monophasic Scatchard analysis and linear Hill plots with slopes of approximately 1.0 were consistent with these radioligands specifically binding to a single receptor. The structure of DPI-3290 is illustrated in Figure 1. The three chiral centers in this molecule create 8 theoretical diastereoisomers. As summarized in table 1, the RSR configuration (DPI-3290) yields a compound with the highest binding affinity for *mu*, *delta*, and *kappa* opioid receptors when compared with the remaining 7 possible diastereoisomers. The rank order for binding affinity at *mu* opiate receptors was RSR>RSS> RRS>RRR>SSS>SSR> SRS>SRR. The binding affinity of these 8 diastereoisomers at *delta* opioid receptors was distinct from that measured in *mu* opioid receptors yielding the following rank order of binding activity RSR>RRR>RRS>RSS>SRS>SRR >SSS>SSR.

Isolated tissue studies. In studies measuring opioid receptor-mediated inhibition of tension development in mouse vas deferens, DPI-

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3209 is an extremely potent mixed opioid receptor agonist as illustrated in figure 2. The intrinsic activity of DPI-3290 at *delta* opioid receptors was assessed in MVD in the presence of the highly selective *mu* opioid receptor antagonist CTOP and the *kappa* opioid receptor antagonist nor-BNI. DPI-3290 produced *delta* opioid receptor-mediated, concentration-dependent inhibition of electrically stimulated smooth muscle contractions with a corresponding IC_{50} value of 1.0 $\pm$ 0.3 nM. Further studies in MVD indicated that DPI-3290 was also active at *mu* opioid receptors. The intrinsic activity of DPI-3290 at *mu* opioid receptors was determined in the presence of the highly selective *delta* opioid receptor antagonist TIPP and the selective *kappa* opioid receptor antagonist nor-BNI. Under these conditions, DPI-3290 again caused concentration-dependent inhibition of muscle contraction with a corresponding IC_{50} value of 6.2$\pm$2.0 nM. Although much less potent than at *mu* or *delta* opioid receptors, DPI-3290 elicited *kappa* opioid receptor-mediated inhibition of muscle contraction in MVD. The IC_{50} was shifted 30-fold to the right of *delta* opioid receptor activity and 7 fold rightward from the *mu* opioid receptor-mediate inhibition curves. When compared to fentanyl, morphine, or U69593, the activity of DPI-3290 was 600 to 24000 times more potent at *delta* opioid receptor-mediated changes and 2 to 1600 times more potent at *mu* opioid receptors in eliciting changes in tension development in the MVD.

The activity of DPI-3290 was further studied in GPI, a tissue previously shown to have predominant *mu* receptor density and activity, intermediate *kappa* opioid receptor presence, and little to no *delta* opioid receptor density or influence on muscle contraction. Increasing concentrations of DPI-3290 produced significant and concentration-related decreases in electrically stimulated GPI contractions as summarized in table

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2. The *mu* opioid receptor-mediate IC_{50} value in this tissue was 3.4 $\pm$ 1.6 nM, which is in good agreement with data obtained in the MVD. In addition, this agent also elicits significant *kappa* opioid receptor-mediated inhibition of ileal contractility with a corresponding IC_{50} value of 6.7 $\pm$ 1.6 nM, which is also in good agreement with the IC_{50} value determined in MVD. Furthermore, the activity of DPI- 3290 in GPI when compared to fentanyl, morphine, or U69593 are in agreement with the rank order of the activity of this compound on these opioid receptors when determined in MVD. Taken together, the rank order of activity for DPI-3290 to inhibit electrically stimulated tension development of MVD or GPI at opioid receptors is *delta>mu>>kappa*.

DPI-3290 mediated antinociception in rats. The *mu* opioid receptor-mediated nanomolar binding affinity and strong agonist intrinsic activities demonstrated in isolated muscles are characteristics DPI-3290 shares with narcotic analgesics. To determine the magnitude of analgesic activity associated with DPI-3290, antinociceptive experiments were carried out in conscious laboratory rats. As illustrated in figure 3, increasing doses of DPI-3290 produced significant and dose-related increases in MPE in rats. A bolus intravenous injection of DPI-3290 at doses $\geq$ 0.1mg/kg resulted in full antinociception recorded as 100% MPE. Antinociception resulting from DPI-3290 was rapid in onset and its duration of action exceeded 60 min following a 1mg/kg bolus injection. A 0.05 mg/kg intravenous dose of DPI-3290 caused a 50% antinociceptive response. This is in marked contrast to the 2.0 mg/kg intravenous dose of morphine that resulted in a 50% increase in MPE.

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Effects of naloxone or naltrindole on DPI-3290 analgesia in rats.

It is widely accepted that the analgesic actions of narcotics like morphine or fentanyl are mediated through *mu* opioid receptors. Therefore, *mu* opioid receptor antagonists like naloxone, effectively block this morphine-induced analgesia. When naloxone was administered s.c. to rats prior to a 1mg/kg i.m. administration of DPI-3290, the most striking effect was a dose-dependent and significant block of the DPI-3290-mediated antinociceptive response. This antagonist action of naloxone was measurable at 0.1 mg/kg and nearly complete at 0.5 mg/kg (fig. 4).

Unlike the nearly complete block of DPI-3290-mediated antinociception by naloxone, the *delta* opioid receptor antagonist, naltrindole only partially antagonized the actions of DPI-3290 on tail flick. In fact, the highest dose of naltrindole (2.0 mg/kg, sc) resulted in an approximate 60% block of the DPI-3290-mediated antinociceptive effects.

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Discussion

We have discovered that of the 8 diastereoisomers resulting from the three chiral centers of 4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl-N-(3-fluorophenyl)-N-methylbenzamide, the RSR configuration (DPI-3290) confers upon the molecule the highest affinity for δ, μ, and κ opioid receptors. Furthermore, consistent with these results from radioligand binding studies, we have demonstrated that DPI-3290 has greater efficacy than morphine or fentanyl in functional studies conducted in isolated smooth muscle, and for morphine in antinociception in whole animals. The differences in the binding affinities between these 8 diastereoisomers may result from physiochemical differences, and we are investigating this possibility.

In vas deferens isolated from mice or ileal strips removed from guinea pigs, the concentration-related inhibition of electrically induced muscle contraction elicited by DPI-3290 was mediated by opioid receptors. DPI-3290 had the greatest binding affinity and in vitro efficacy for the *delta* opioid receptor, followed by its radioligand binding and pharmacologic activity at *mu* opioid receptors, with the least activity at *kappa* opioid receptors. The intrinsic activity of this compound was demonstrated in isolated smooth muscle utilizing CTOP, TIPP and nor-BNI to differentiate the activity of DPI-3290 at δ, μ, and κ opioid receptors. CTOP is a high affinity opioid receptor antagonist with a 2000-fold selectivity for the *mu* subclass of opioid receptors (Pelton et al 1986, Hawkins et al 1989). TIPP is currently the most selective *delta* opioid receptor antagonist with selectivity for *delta* receptors in the range of 10,000-fold when compared at other opioid receptors (Schiller et al 1993) and nor-BNI is a selective kappa

receptor antagonist. Thus, these studies clearly indicate that DPI-3290 is a potent mixed opioid receptor agonist with its greatest activity at the *delta* opioid receptor. Since neither CTOP, TIPP, nor nor-BNI are able to differentiate between the reported opioid receptor subtypes within the three subclasses of these receptors, it is not clear if DPI-3290 acts at a particular receptor subtype to elicit its activity.

When administered i.v. to rats, the predominant effect of DPI-3290 was a marked increase in the tail pinch latency thus consistent with the compound's strong antinociceptive action. This antinociception was dose-dependent, persistent and partially blunted by the selective *delta* opioid receptor antagonist, naltrindole whereas naloxone almost completely blocked the antinociceptive actions of DPI-3290. In this regard, it is likely that the antinociceptive actions of DPI-3290 result from its combined action at both *delta* and *mu* opioid receptors. Studies reported previously by O'Neill et al. (1997) describe the complex interaction that exists between *delta* and *mu* opioid receptors and that *delta* opioid receptors alone are linked to antinociceptive activity in mice (Wild et al., 1993). Therefore, the actions of DPI-3290 would be unlike traditional narcotic analgesics such as morphine and fentanyl that elicit their analgesic action primarily by selectively activating *mu* opioid receptors.

In the clinical setting, the most serious and life threatening side effect of narcotic analgesics is respiratory depression (Inturrisi, 1990). The effects on respiratory function are believed to result from the *mu* opioid receptor-mediated inhibition of neuronal transmission in the rostrodorsal surface of the pons, the nucleus tractus solitarius and nucleus ambiguous (Spanagel et al., 1994; Hassen et al., 1984). One recently proposed hypothesis that could suggest a marked difference between DPI-3290 and other narcotic analgesics

on respiratory depression is the likelihood that *delta* opioid receptors modulate or counteract the respiratory depression induced by *mu* opioid receptors (Su *et al*., 1998; O'Neill et al., 1997). Alfentanil-induced respiratory depression was inhibited in a dose-dependent manner with the non-peptide *delta* opioid receptor agonist +BW373U86. These investigators reported similar effects on alfentanil-induced respiratory depression after icv administration of DPDPE and other highly selective *delta* opioid agonists. Taken together, this interaction between *delta* and *mu* opioid receptors could form the basis for a marked difference between the effects of DPI-3290 and traditional opioid analgesics like morphine or fentanyl on respiratory function.

In summary, the extensive in vitro and in vivo pharmacologic and radioligand binding data outlined here are consistent with the strong antinociceptive actions of DPI-3290. The mechanism(s) of action responsible for this activity is centered at opioid receptors since opioid receptor antagonists block this effect. Since opioids are the principal agents used for the treatment of moderate-to-severe pain there are several interesting implications to the data reported here. The establishment that mixed opioid receptor agonist activity can be contained within a single chemical entity with varying degrees antinociception and respiratory depression opens the possibility for further advances in the treatment of severe pain. Currently, studies are underway to assess the dependence, tolerance, and gastrointestinal effects of DPI-3290. Needless to say, this potential breakthrough in the management of pain could be the first in a series of pharmacophores with mixed opioid receptor agonist activity that produce strong, safe and effective analgesia.

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TABLE 1

K_i values for DPI-3290 and its diastereoisomers.
The results summarized represent the mean ± S.E.M from 3-4 separate experiments.

	Configuration (α, 2, 5)	**[^{3}H]DPDPE (nM)**	**[^{3}H]DAMGO (nM)**	**[^{3}H]U69593 (nM)**
DPI-3290	RSR	0.18 ± 0.02	0.46 ± 0.05	0.62 ± 0.09
DPI-5201	SSR	150.3 ± 0.10	>1000	
DPI-673	SRS	384.7 ± 55.2	64.4 ± 5.6	306.8 ± 63.1
DPI-5402	RRS	12.7 ± 1.3	7.3 ± 0.89	13.8 ± 2.1
DPI-5336	RRR	41.8 ± 2.2	6.4 ± 0.84	414.2 ± 65.8
DPI-5337	SRR	429.9 ± 40.0	320.2 ± 59.7	>1000
DPI-5701	SSS	119.3 ± 10.7	405.5 ± 64.4	231.4 ± 28.6
DPI-5702	RSS	3.8 ± 0.7	11.6 ± 8.7	19.1 ± 0.9

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TABLE 2

IC_{50} values for opioids effects on the contractility of electrically stimulated mouse vas deferens.

The results summarized represent the mean ± S.E.M from 6-8 independent tissue.

	Delta (nM)	**Mu (nM)**	**Kappa (nM)**
DPI-3290	1.0 ± 0.3	6.2 ± 2.0	25 ± 3.3
BW373U86	0.49 ± 0.03	134 ± 9	201 ± 7
Fentanyl	492 ± 15	14 ± 3	868 ± 19
Morphine	19,700 ± 170	1,090 ± 30	37,750 ± 140
U69593	5,200 ± 1,200	10,000 ± 2,000	24 ± 2.2

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TABLE 3

IC_{50} values for opioids effects on the contractility of electrically stimulated guinea pig ileum.

The results summarized represent the mean ± S.E.M from 6-8 independent tissue.

	Mu (nM)	**Kappa (nM)**
DPI-3290	3.4 ± 1.6	6.7 ± 1.6
BW373U86	150 ± 7	325 ± 9
Fentanyl	4.5 ± 1.4	146 ± 9
Morphine	51 ± 4	1,370 ± 24
U69593	500 ± 15	4.2 ± 1.1

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Figure 1. The chemical structure of DPI-3290 (+) 3-((α-R)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl-N-(3-fluorophenyl)-N-methylbenzamide}.

Figure 2. Decreases in contractile force with cumulative addition of DPI-3290 measured in mouse vas deferens alone ( ) or following pretreatment with 1 μM CTOP + 15nM nor-BNI (■), 3μM TIPP + 15 nM nor-BNI (□), 1μM CTOP + 3μM TIPP (▲), or 3μM TIPP + 1μM CTOP + 15nM nor-BNI. Values represent the mean ± S.E.M. of eight experiments.

Figure 3. Effects of DPI-3290 or morphine on MPE in conscious laboratory rats. DPI-3290 or morphine were intravenously injected via the jugular vein prior to measurement of tail-pinch response latency. MPE values illustrated were measured at the peak of the response. Values represent the mean ± S.E.M. of six experiments.

Figure 4. Decreases in DPI-3290-mediated maximal percent effect in laboratory rats following pretreatment with naloxone administered subcutaneously (□, 0.1mg/kg; ◇, 0.2mg/kg; ○, 0.5mg/kg). Values represented are the mean ± S.E.M. of six experiments.

Figure 5. Decreases in DPI-3290-mediated maximal percent effect in laboratory rats following pretreatment with naltrindole administered subcutaneously (■, 0.2mg/kg; ▲, 0.5mg/kg; ○, 2.0mg/kg). Values represented are the mean ± S.E.M. of six experiments.

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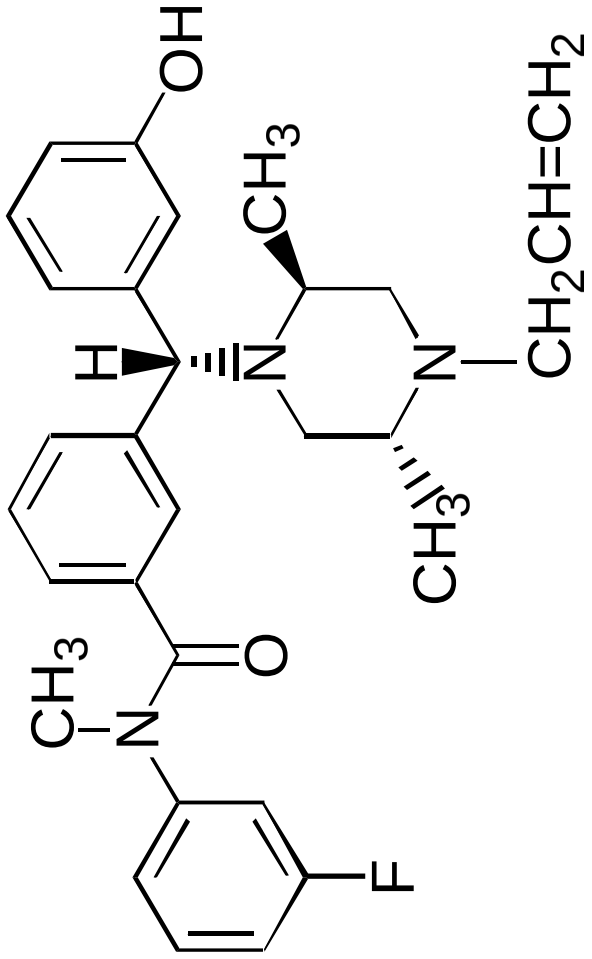

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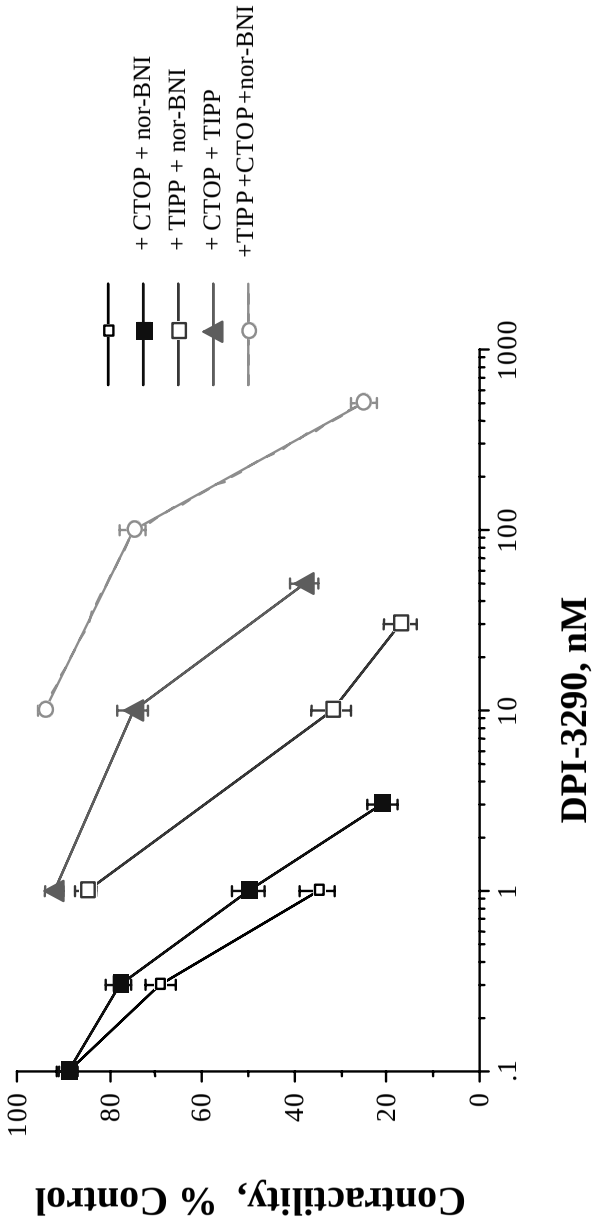

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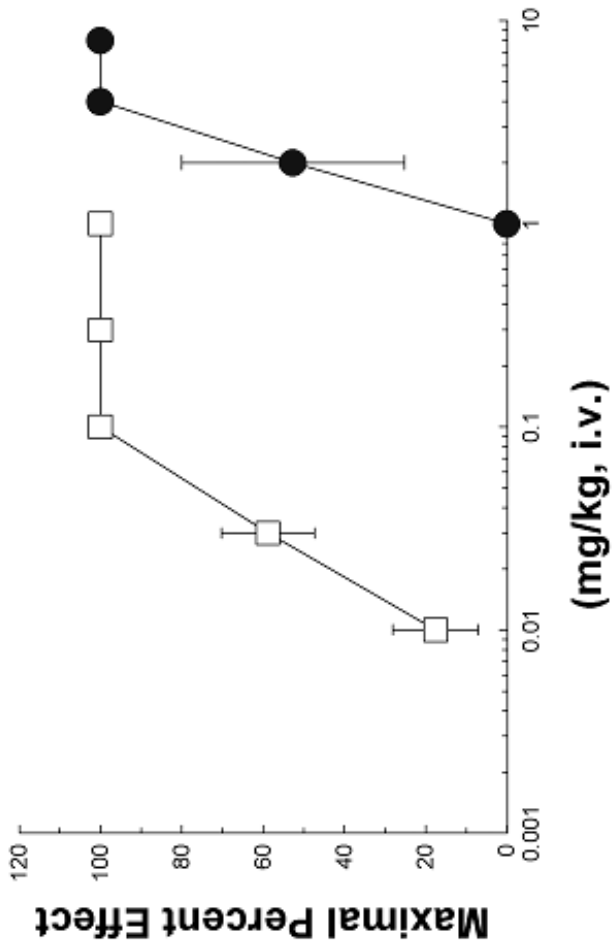

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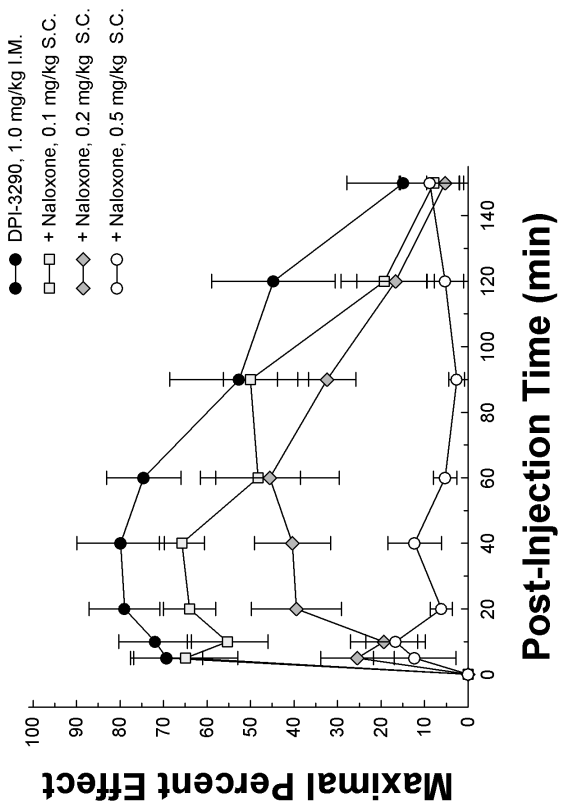

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