In Vivo Pharmacological Characterization of Indiplon, a Novel Pyrazolopyrimidine Sedative-Hypnotic

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ABSTRACT

Indiplon (NBI 34060; N-methyl-N-[3-[3-(2-thienylcarbonyl)]pyrazolo[1,5-a]pyrimidin-7-yl]phenyl]acetamide), a novel pyrazolopyrimidine and high-affinity allosteric potentiator of GABA<sub>A</sub> receptor function, was profiled for its effects in rodents after oral administration. In mice, indiplon inhibited locomotor activity (ED<sub>50</sub> = 2.7 mg/kg p.o.) at doses lower than the nonbenzodiazepine hypnotics zolpidem (ED<sub>50</sub> = 6.1 mg/kg p.o.) and zaleplon (ED<sub>50</sub> = 24.6 mg/kg p.o.), a sedative effect that was reversed by the benzodiazepine site antagonist flumazenil. Indiplon inhibited retention in the mouse passive avoidance paradigm over a dose range and with a temporal profile that coincided with its sedative activity. Indiplon, zolpidem, and zaleplon were equally effective in inhibiting locomotor activity in the rat and produced dose-related deficits on the rotarod. In a rat vigilance paradigm, indiplon, zolpidem, and zaleplon produced performance deficits over a dose range consistent with their sedative effects, although indiplon alone showed no significant increase in response latency. Indiplon produced a small deficit in the delayed nonmatch to sample paradigm at a dose where sedative effects became apparent. Indiplon was active in the rat Vogel test of anxiety, but it showed only a sedative profile in the mouse open field test. The pharmacokinetic profile of indiplon in both rat and mouse was consistent with its pharmacodynamic properties and indicated a rapid T<sub>max</sub>, short t<sub>1/2</sub>, and excellent blood-brain barrier penetration. Therefore, indiplon has the in vivo profile of an efficacious sedative-hypnotic, in agreement with its in vitro receptor pharmacology as a high-affinity allosteric potentiator of GABA<sub>A</sub> receptor function, with selectivity for α1 subunit-containing GABA<sub>A</sub> receptors.

Benzodiazepine drugs have been used extensively for the treatment of insomnia, in addition to other central nervous system disorders. Although effective as hypnotics, benzodiazepines also produce undesirable side effects, such as memory impairment, have durations of action that can lead to drowsiness and functional impairment upon awakening (“next day hangover”), and are associated with dependence and abuse liability (Johnson and Chernik, 1982; Lister, 1985; Roehrs et al., 1994; Woods and Winger, 1995). Consequently, the development of drugs with sedative-hypnotic activity equivalent to the benzodiazepines, but with an improved side effect profile, would be of great benefit in the successful management of insomnia, which itself represents an enormous cost to society (Walsh and Engelhardt, 1999).

The benzodiazepine drugs act through potentiation of the effects of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), by binding to a specific site on the GABA<sub>A</sub> receptor to produce allosteric enhancement of anion flux through this ligand-gated chloride channel (McKernan and Whiting, 1996; Barnard et al., 1998; Möhler et al., 2002). GABA<sub>A</sub> receptors are hetero-oligomers formed primarily by the association of one or more α<sub>1–6</sub>, β<sub>1–3</sub>, and γ<sub>1–3</sub> subunits that can assemble in different combinations to form receptor subtypes that differ in their pharmacology and cellular and regional distribution (Barnard et al., 1998). The effects of benzodiazepine site ligands arise from their relative affinity and efficacy for GABA<sub>A</sub> receptor subtypes. These subtypes are formed by the assembly of different GABA<sub>A</sub> receptor subunits as hetero-oligomers containing, in particular, different α subunits (Pritchett et al., 1989; McKernan and Whiting, 1996; Barnard et al., 1998; Möhler et al., 2002). The diverse pharmacological properties of benzodiazepines, such as sedation, muscle relaxation, anxiolysis, anticonvulsant...
and memory impairment, have been attributed to interactions with these different receptor subtypes (Möhler et al., 2002), and α1 subunit-containing receptors have been associated with sedative-hypnotic activity (Rudolph et al., 1999).

The “nonbenzodiazepine” drugs represent a new generation of sedative-hypnotics (Miller, 2000) and are associated with greater selectivity for α1-containing GABA_A receptors than earlier benzodiazepines. Most notable of these are the imidazopyridine, zolpidem (Ambien), the cyclopyrrolone zopiclone (Imovane), and the pyrazolopyrimidine zaleplon (Sonata) (Fig. 1). The most extensively characterized member of this group is zolpidem. The advantages of these newer agents over benzodiazepines seem to result from their mode of interaction with the GABA_A receptor combined with a reduced duration of action. Thus, for example, zolpidem has a moderate affinity for α1-containing GABA_A receptors in radioligand binding experiments (10–100 nM; Arbilla et al., 1985; Smith et al., 2001; Sullivan et al., 2004), is fully efficacious (Smith et al., 2001; Sullivan et al., 2004), has selectivity for GABA_A receptors containing the α1 subunit (Damgen and Luddens, 1999; Smith et al., 2001), produces sedative activity in animals that is mediated through α1 subunit-containing GABA_A receptors (Crestani et al., 2000), and has a half-life in rodents and humans after oral administration of 2 to 3 h (Gaudreault et al., 1995; Greenblatt et al., 1998). Zopiclone displays a similar efficacy profile in animal tests (Perrault et al., 1990), but it has only marginal selectivity for GABA_A receptors containing the α1 subunit (Damgen and Luddens, 1999; Smith et al., 2001) and its half-life in humans of 3.5 to 6.5 h (Fernandez et al., 1995) has been associated with next day hangover effects, including impaired driving ability (Bocca et al., 1999; Vermeeren et al., 2002). Zaleplon has a half-life of 1 h in humans after oral administration (Greenblatt et al., 1998); however, this compound has a lower affinity than zolpidem at benzodiazepine binding sites and a duration of action. Thus, for example, zolpidem has a moderate selectivity for GABA_A receptors containing the α1 subunit-containing GABA_A receptors (Damgen and Luddens, 1999; Smith et al., 2001), produces sedative activity in animals that is mediated through α1 subunit-containing GABA_A receptors (Crestani et al., 2000), and has a half-life in rodents and humans after oral administration of 2 to 3 h (Gaudreault et al., 1995; Greenblatt et al., 1998). Zopiclone displays a similar efficacy profile in animal tests (Perrault et al., 1990), but it has only marginal selectivity for GABA_A receptors containing the α1 subunit (Damgen and Luddens, 1999; Smith et al., 2001) and its half-life in humans of 3.5 to 6.5 h (Fernandez et al., 1995) has been associated with next day hangover effects, including impaired driving ability (Bocca et al., 1999; Vermeeren et al., 2002). Zaleplon has a half-life of 1 h in humans after oral administration (Greenblatt et al., 1998); however, this compound has a lower affinity than zolpidem at benzodiazepine binding sites and a reduced selectivity for α1 subunit-containing GABA_A receptors (Damgen and Luddens, 1999). It is, therefore, of interest to make side-by-side comparisons of the in vivo pharmacology of the nonbenzodiazepine sedative-hypnotics in rodents to determine how their relative receptor pharmacology profiles and pharmacokinetic properties translate into in vivo efficacy.

Indiplon (NBI 34060; N-methyl-N-[3-[3-(2-thienylcarbonyl)-

![Chemical structures](image-url)

**Fig. 1.** Chemical structures of indiplon, desmethyl-indiplon, zaleplon, zolpidem, and triazolam.

pyrazolo[1,5-α]pyrimidin-7-yl]phenyl]acetamide; Fig. 1) is a novel pyrazolopyrimidine, which is a high-affinity allosteric potentiator of GABA_A receptor function, and possesses the characteristics of a compound with selectivity for α1 subunit-containing GABA_A receptors (Sullivan et al., 2004). Indiplon is currently being developed for the treatment of insomnia and has potential advantages over the currently marketed benzodiazepine and nonbenzodiazepine drugs. We now report the profile of activity of indiplon in rodent models of sedation, muscle relaxation, vigilance, amnestic liability, and anxiety and its pharmacokinetic profile in rodents. In most cases, we have made side-by-side comparisons between indiplon, zolpidem, zaleplon, and triazolam to directly compare the in vivo pharmacology of these drugs.

### Materials and Methods

**Materials**

Indiplon, N-desmethyl-indiplon, and zaleplon were synthesized by the medicinal chemistry department at Neurocrine Biosciences, Inc. Zolpidem and triazolam were purchased from Sigma/RBI (Natick, MA). Flumazenil was purchased from Toecis Cookson Inc. (Bristol, UK).

**Mouse Behavioral Studies**

**Subjects.** Adult male CD-1 mice (22–25 g) were housed in groups of 15 in the Neurocrine vivarium, where ambient temperature was 25–27°C, and a 12-h light/dark cycle was in effect (7:00 AM–7:00 PM). Mice were allowed food and water ad libitum. All studies were conducted during the light cycle.

**Drug Administration.** All compounds were given by oral gavage as suspensions in 45% 2-hydroxypropyl-β-cyclodextrin (HBC; Sigma/ RBI). Zolpidem, zaleplon, indiplon, and N-desmethyl-indiplon were given at doses ranging from 0.03 to 100 mg/kg, and triazolam was given at doses ranging from 0.0003 to 10 mg/kg. All drugs were given in a volume of 10 ml/kg.

**Locomotor Activity.** The sedative properties of zolpidem, zaleplon, indiplon, N-desmethyl-indiplon, and triazolam were compared using nonhabituated locomotor activity as an index of sedation. Thirty minutes after dosing, mice were placed into cages that were similar to their home cages but surrounded by an array of 16 photocell assemblies (Opto-Varimex-Mini Model B; Columbus Instruments, Columbus, OH). Total locomotor activity was defined as the number of beam breaks averaged over 10-min bins and was collected for a total of 10 to 60 min, depending on the requirements of the study. The dose-response function for each drug was characterized with a separate cohort of mice. In one study designed to assess the duration of effect for indiplon, mice were treated with 2 mg/kg p.o. and immediately placed into the locomotor activity test chambers. To study the effects of the benzodiazepine antagonist flumazenil on indiplon’s inhibition of locomotor activity, flumazenil was administered at 10, 30, or 45 mg/kg i.p. alone or concurrently with 1.5 mg/kg indiplon p.o., and locomotor activity was measured at 30 to 34 min postdose interval.

**Passive Avoidance Retention.** For the dose response studies with 24 h retention, male CD-1 mice were treated with indiplon (0.03–100 mg/kg), zaleplon (0.03–100 mg/kg), zolpidem (0.03–100 mg/kg), or triazolam (0.003–10 mg/kg) and 30 min later, placed into the brightly lit compartment of a passive avoidance apparatus. Latency to enter the dark compartment through a guillotine door was measured (typically 5–10 s). Mice that required more than 180 s to enter the compartment were not used in the study. Upon entry into the dark compartment, the guillotine door was closed, and footshock was administered (0.4 mA; 5 s). Mice were then taken out of the chamber and tested 24 h later. On the test day, mice were again placed into the lit portion of the apparatus, and the latency to enter
the dark compartment was measured. The test day latency was used as the measure of retention for the association between the dark compartment and footshock (Jarvik and Kopp, 1967).

For the studies to determine the importance of time interval length between drug administration and passive avoidance training in producing a retention deficit 24 h later, indiplon (2 mg/kg) was given by oral gavage either 30, 60, or 120 min before passive avoidance training. One group of animals also received indiplon immediately after training to assess their effects on memory consolidation (designated as the zero time point in Fig. 5).

For the studies that assessed the impact of retention interval (24 or 72 h) on the indiplon-induced retention deficit, mice were given indiplon (3 mg/kg p.o.) 30 min before passive avoidance training. They were then tested either 24 or 72 h after training for retention of the association between context (dark compartment) and footshock.

Open Field Test. Thirty minutes after administration of indiplon (0.03–1 mg/kg p.o.) mice were placed into a clear Plexiglas arena (50 × 50 × 22 cm) surrounded by an array of photocell beams (Accuscan, Inc., Columbus, OH). A lamp directed on the center of the field provided a light level of 120 lux in the center of the arena. Testing was conducted during the light cycle in a room with constant white noise. Each animal was placed in the center of the arena to initiate the 10-min testing session. Horizontal activity and time spent in the center of the Plexiglas arena were recorded as photobeam breaks.

Data Analysis. Locomotor activity data were analyzed over time using a mixed design, repeated measures ANOVA, where the between groups variable was drug dose and the within-groups variable was time. Differences between dose groups at specified time points were analyzed using simple effects ANOVA followed by Fisher’s least significant difference test as the post hoc measure for group differences (provided that the simple effects ANOVA omnibus F-ratio was significant). ED_{50} estimates were calculated for each drug from the locomotor activity counts collected during the 30- to 40-min postdose interval, expressing these as percentage of inhibition versus vehicle control and plotting these data against the log_{10} drug dose. The resulting curve was analyzed, and ED_{50} values were derived, using the following equation: $y = maximum\ inhibition/(1 + 10^{x-ED_{50}})$, where $y$ is percentage of inhibition of locomotor activity and $x$ is the log_{10} drug dose (Prism; GraphPad Software, Inc., San Diego, CA). Passive avoidance data were analyzed by one-way ANOVA, with retention latencies as the dependent variable. The independent variable was dose, time of treatment relative to training, or retention test delay interval. Minimal effective doses (MEDs) were defined as the lowest dose to produce a statistically significant effect.

For the experiment where flumazenil was administered along with indiplon in the locomotor activity assay, the data were analyzed with a one-way ANOVA with post hoc analysis using Fisher’s protected least significant difference where appropriate.

Open-field data were analyzed using one-way ANOVA for two variables: horizontal activity and percent time in center, where Fisher’s least significant difference test was used as the post hoc test for group differences.

### Rat Behavioral Studies

**Subjects.** For all studies adult male Sprague-Dawley or Long-Evans rats (200–250 g) were housed in groups of two in the Neurocrine vivarium, where ambient temperature was 25–27°C, and a 12-h light/dark cycle was in effect (7:00 AM–7:00 PM). Rats were allowed food and water ad libitum, unless they were subjects in the vigilance and delayed nonmatch to sample studies, where they were food restricted so that they maintained 85% of their baseline body weight. Rats in the Vogel test were water deprived for 24 h before testing. All studies were conducted during the light cycle.

**Drug Administration.** All compounds were given by oral gavage as suspensions in 45% HBC (Sigma/RBI), unless they were used in studies where food was the reinforcer, such as the vigilance paradigm. For those studies, compounds were suspended in 1% Tween 180 and water, a vehicle that, in comparison with 45% HBC, occupies a smaller volume and therefore, is less likely to distend the stomach. Zolpidem, zaleplon, and indiplon were given at doses ranging from 1 to 30 mg/kg, and triazolam was given at doses ranging from 0.3 to 10 mg/kg. All drugs were given in a volume of 1 ml/kg.

**Locomotor Activity.** Male Sprague-Dawley rats were treated with either indiplon (1–10 mg/kg), zaleplon (1–10 mg/kg), zolpidem (3–30 mg/kg), or triazolam (0.04–10 mg/kg) or an equal volume of vehicle (45% HBC) by oral gavage. These dose ranges were based on the activity of the compounds in the mouse locomotor activity assay. Thirty minutes after dosing, rats were placed into clean home cages surrounded by an array of 16 photobeam assemblies (Opto-Varimex-Mini Model B; Columbus Instruments). Locomotor activity was defined as the number of beam breaks averaged over 10-min bins and was collected for a total of 30 min. The dose-response function for each drug was characterized with a separate cohort of rats. To compare the sedative effects of compounds under the conditions used in the vigilance assays, food-deprived, Long-Evans rats were used. Locomotor activity was measured as described above 30 min after a single oral dose of compound in 1% Tween 180 as the suspension vehicle by using the following doses: indiplon, 3 mg/kg; zaleplon 6 mg/kg; zolpidem, 25 mg/kg; and triazolam, 50 mg/kg.

**Rotarod Latency.** Male Sprague-Dawley rats were initially given three training trials on the rotating rod (6 revolutions/min; Economex accelerating rotarod for rats and mice; Columbus Instruments). Twenty-four hours later, they were given indiplon (1–10 mg/kg), zaleplon (1–10 mg/kg), zolpidem (3–30 mg/kg), or triazolam (0.3–10 mg/kg) by oral gavage. Thirty minutes after drug dosing they were again placed on the rotarod apparatus for three trials (60-s intertrial intervals). The average latency to fall off the rod was recorded for each rat. Dose ranges were based on the initial locomotor activity measurements in the mouse.

**Vigilance.** Food-restricted male Long-Evans rats were trained in Coulbourn Instruments (Allentown, PA) operant boxes fitted with MED Associates (St. Albans, VE) five-choice nosepoke panel and
software. The methods used were based upon the Robbins version of Leonard’s five-choice serial reaction time task for humans (Cole and Robbins, 1987). The nosepoke panel consisted of five openings on the wall opposite to the food hopper. Trials were conducted in the dark, where illumination of the pellet feeder started a trial. When the feeder was illuminated, the rat was required to nosepoke in the pellet feeder and then turn to the opposite wall and wait until one of the five openings was illuminated. The rat was then required to nosepoke in the illuminated opening to receive the food reward in the pellet feeder. Trials were conducted in the dark, where illumination of the pellet feeder started a trial. When the feeder was illuminated, the rat was required to nosepoke in the pellet feeder and then turn to the opposite wall and wait until one of the five openings was illuminated. The rat was then required to nosepoke in the illuminated opening to receive the food reward in the pellet feeder. Illumination of the openings was conducted in a random sequence, with a duration of 3 s. If the rat gave a nosepoke in the wrong opening, or did not make an immediate nosepoke response, a 7-s dark time-out period was engaged. If the response was correct, a 3-s intertrial interval was engaged, ending with the illumination of the pellet feeder. Rats were trained to a criterion of less than 15 omission errors (per 100 attempts; an omission error is a failure to nosepoke in response to illumination) and a nosepoke latency of less than 1.75 s before testing. On the test day, rats were given indiplon (1–30 mg/kg), zaleplon (0.3–10 mg/kg), or zolpidem (0.3–30 mg/kg) by oral gavage in 1% Tween 180, 30 min before the onset of testing. Average latency to nosepoke, number of correct trials/total trials, and omission errors were recorded (Cole and Robbins, 1987). In a second study, indiplon (3 mg/kg p.o.) was administered 10 h before the onset of vigilance testing. The same animals were tested in the locomotor activity assay 30 min after treatment (9.5 h before vigilance testing) to ascertain that indiplon at the selected dose reduced locomotor activity by approximately 50% compared with vehicle controls. Because the vigilance task required food as a motivation, and the 45% HBC vehicle we had used for the previously discussed studies could have caused stomach distension, we used a 1% Tween 180/water vehicle for this and any other tasks that were appetitively motivated. We tested the sedative effects of indiplon, zaleplon, zolpidem, and triazolam in this vehicle in food restricted rats compared with a group that had free access to food and found that the vehicle reduced the effects of zaleplon and zolpidem and that food restriction reduced the effect of triazolam, but not the nonbenzodiazepine hypnotics. We then titrated the doses of all four hypnotics in the Tween vehicle and food-restricted rats to produce a 50% reduction in locomotor activity compared with vehicle-treated animals. The following data express the average number of photobeams broken by each group during the first 30 min of observations, and for each drug the corresponding percentage of inhibition: vehicle, 2412 ± 192; 3 mg/kg indiplon, 1333 ± 240 (45% inhibition); 6 mg/kg zaleplon, 1371 ± 142 (43% inhibition); 25 mg/kg zolpidem, 1216 ± 192 (52% inhibition); and 50 mg/kg triazolam, 1287 ± 96 (47% inhibition). Because the sedative effects of triazolam were so severely altered by food restriction, a comparison with triazolam was not made in the vigilance studies.

Delayed Nonmatch to Sample. Food-restricted male Long-Evans rats were trained in Coulbourn Instruments operant cham-
bers. Two retractable levers extended on either side of the food hopper/nosepoke apparatus. The hopper/nosepoke apparatus contained photocells that monitored pellet delivery and receipt. Rats were initially trained on a fixed ratio 1 schedule where the animal would initiate a trial with a nosepoke in the hopper, which produced extension of one lever. Lever extension varied from left to right throughout the entire session during this initial training period, where the animal learned to initiate a trial with a nosepoke and to receive a reward by pressing the extended lever. During the second phase of training, trial initiation again produced only one lever; this now served as the “study” phase of the trial. After variable delays of 0.5, 1, or 2 s, both levers would extend, and the animal would be required to choose the lever alternate to that presented during the study phase. Rats continued in this phase of training until they reached an 80% criterion with 100 obtained reinforcements. Training then proceeded with variable delays of 1, 2, 4, 8, 12, 16, and 20 s until rats achieved a 70% accuracy criterion regardless of delay. Delays were presented in a random order, using a list without replicate scheme. Rats were then assigned to treatment groups based upon their accuracy at the 20-s delay. Indiplon was administered by oral gavage 30 min before testing at a dose low enough to allow at least 70% of vehicle responding (2 mg/kg). During testing, rats were presented with variable delays of 1, 4, 16, and 32 s. Each test session continued for 30 min, with number of trials initiated and number of accurate choices recorded as dependent variables. Both of these variables were averaged over trials for each delay. In a second study, indiplon (2 mg/kg p.o.) was administered 10 h before the onset of delayed nonmatch to sample testing.

**Vogel Test.** Male Sprague-Dawley rats were water deprived for 24 h before testing in operant chambers (Coulbourn Instruments). After 200 free (our unpunished data) licks, rats received a 100-ms, 0.4-mA shock every 10 licks. The test session commenced after the first shock was received and lasted for 5 min. Indiplon was administered by oral gavage in 45% HBC vehicle. Both the number of shocks taken and the total number of licks over the test session were recorded as indices of conflict behavior.

**Data Analysis.** Locomotor activity data were analyzed over time using a mixed design, repeated measures ANOVA, where the between groups variable was drug dose and the within-groups variable was time. Differences between dose groups at specified time points were analyzed using simple effects ANOVA followed by Fisher’s least significant difference test as the post hoc measure for group differences (provided that the simple effects ANOVA omnibus F ratio was significant). Curves were fitted and ED$_{50}$ estimates were calculated for each drug from the locomotor activity counts during the 30- to 40-min postdose interval, expressing these as 10-min locomotor activity averages as percentage inhibition of vehicle control and plotting these data against the log$_{10}$ drug dose, as described above for the mouse data. Rotarod data were analyzed by one-way ANOVA, with latency to fall as the dependent variable. The independent variable was dose. Curves were fitted and ED$_{50}$ values estimated as described above. Vigilance, DNMTS, and Vogel data were analyzed...
using one-way ANOVA with dose as the independent variable, and Fisher’s least significant difference as the post hoc test for group differences. The total reinforcement, choice accuracy, omission error, and response latency variables for the vigilance test were averaged for the 1-, 2-, and 3-s stimulus durations before analysis, because there were no group differences when these stimulus durations were assessed separately.

**Pharmacokinetic Studies.** Indiplon was administered by oral gavage to CD-1 mice (4 mg/kg; n = 2) or CD rats (5 mg/kg; n = 4) in a vehicle of 0.5% hydroxypropylmethylcellulose (HPMC) suspension solution. Terminal blood and brain samples were taken at predetermined times for composite sampling. All plasma and brain samples were flash frozen in liquid nitrogen immediately after harvest and stored at −21°C until analysis. The bioanalytical method applied for the measurement of indiplon in plasma along with added internal standard consisted of precipitation with 200 μl of acetonitrile from 50 μl of plasma, centrifugation and recovery of the supernatant, drying down under vacuum, and then reconstitution in 30:70 acetonitrile/water before introduction into a high-performance liquid chromatography-fluorescence detector system for analysis. The analytical method applied for the measurement of indiplon in brain tissue consisted of dividing the brain sagittally into two halves, homogenizing one-half of the brain tissue with a Tissue Tearor, extraction of indiplon along with added internal standard with 2 ml of 70:30 acetonitrile/water from the homogenate, centrifugation and recovery of the supernatant, drying down under vacuum, and then reconstitution in 30:70 acetonitrile/water before introduction into an LC-MS/MS system for analysis. The lower limits of quantification for the analytical methods were 20 ng/ml and 5 ng/g for plasma and brain samples, respectively. All pharmacokinetic parameters were calculated from a noncompartmental model using WinNonlin program version 1.2.

**Results**

**Pharmacokinetics in the Rat and Mouse.** After oral dosing in the mouse, peak plasma levels of indiplon (T_{max}) occurred at 30 min with a t_{1/2} of 1 h (Fig. 2A). The levels of indiplon were higher in the brain and paralleled the time course in the plasma, peaking at 30 min, with a brain/plasma ratio of 1.7, indicating excellent blood-brain barrier penetration. A similar plasma profile was observed after oral dosing of indiplon in the rat (Fig. 2B): T_{max} was 30 min and the estimated t_{1/2} was 1 h. On the basis of these data, behavioral tests were conducted 30 min after oral administration of drug, unless otherwise indicated.

**Locomotor Activity in the Mouse.** Indiplon produced a dose-dependent reduction in nonhabituated locomotor activity (Fig. 6,590) = 15.5; p < 0.0001) over the 60-min observation period, with a maximal effect during the first 10 min of observation (F(8,117) = 23.1; p < 0.0001) (Fig. 3A). Similar effects were observed for zaleplon (F(8, 126) = 9.7; p < 0.0001), zolpidem (F(8,106) = 13.0; p < 0.0001) and triazolam (F(10,95) = 22.2; p < 0.0001). Maximal drug effects were observed early in the observation period because locomotor activity decreased over time for all animals as a function of gradual habituation to the test environment (F(5,590) = 65.7; p < 0.0001).

Dose-response curves derived from these data (Fig. 3B) indicated that indiplon was more effective than zaleplon and zolpidem, with ED_{50} values being indiplon, 2.7 mg/kg; zaleplon, 6.1 mg/kg; and zolpidem, 24.6 mg/kg. Triazolam was the most potent of the drugs tested with an ED_{50} value of 0.04 mg/kg. Indiplon, zolpidem, and triazolam achieved a similar maximum level of inhibition (approximately 90% versus vehicle). The maximum inhibition by zaleplon seemed lower at approximately 70%, although doses higher than 100 mg/kg would be needed to clearly define the maximal effect of this drug. We also tested desmethyl-indiplon (Fig. 1), the major metabolite of indiplon, which was inactive up to a dose of 100 mg/kg p.o. [F(8,125) = 0.6; p < 0.771] (Fig. 3B).

The duration of the sedative activity of indiplon was evaluated in mice at a dose that approximated its ED_{50} value in the locomotor activity assay. At 2 mg/kg p.o., indiplon produced inhibition of locomotor activity that persisted for 2 h [F(1,132) = 33.8; p < 0.0001; F(2 h)1(22) = 5.7; p < 0.02] (Fig. 3C), consistent with the pharmacokinetic profile of the drug in mice (vide supra).

To confirm that the effects of indiplon on locomotor activity were due to an interaction with the benzodiazepine site on the GABA<sub>A</sub> receptor, the effects of the benzodiazepine site antagonist flumazenil on the inhibition of locomotor activity produced by indiplon were examined. An approximate ED_{50} dose of indiplon (1.5 mg/kg p.o.) was administered to mice, along with three doses of flumazenil and locomotor activity measured for a 4-min period starting 30 min after the indiplon dose. This timing was chosen to take into account the rapid clearance of flumazenil from mouse plasma and brain (d’Argy et al., 1987; Potier et al., 1988). Flumazenil produced a dose-dependent reversal of the inhibition of locomotor activity produced by indiplon [F(8,91) = 5.4; p < 0.001] (Fig. 3D). Flumazenil alone had no effect on locomotor activity at 10, 30, or 45 mg/kg i.p. (data not shown).

**Passive Avoidance in the Mouse.** Indiplon significantly reduced retention latency [F(6,84) = 12.8; p < 0.0001] (Fig. 4A) with an MED of 1 mg/kg (p < 0.0001). Retention latency was also reduced by zaleplon [F(10,91) = 9.8; p < 0.0001], MED = 3 mg/kg (p < 0.001), zolpidem [F(10,103) = 17.4; p < 0.0001], MED = 30 mg/kg (p < 0.0001), and triazolam [F(10,101) = 18.3; p < 0.0001], MED = 0.1 mg/kg (p < 0.0001) (Fig. 4, B–D). A further experiment examined the importance of the time interval between treatment and training with a 2-mg/kg dose of indiplon (Fig. 5). No significant retention deficit occurred when indiplon was administered 120 or 60 min before the training session, but a significant effect was seen with 30 min pretreatment (p < 0.03), consistent with the results in Fig. 4A. Treatment with indiplon immediately after the passive avoidance training gave no
significant deficit 24 h later (Fig. 5; zero time point). To examine the effect of the degree of difficulty on passive avoidance retention, an experiment was carried out where the time period between training and test periods was lengthened to 72 h. Vehicle-treated mice showed a nonsignificant trend toward reduced retention 72 h after training relative to the 24 h paradigm ($p < 0.17$). Mice treated with 3 mg/kg indiplon 30 min before training showed a significant retention deficit at 24 ($p < 0.0001$) and 72 ($p < 0.01$) hours after training. Increasing the time between training and testing from 24 to 72 h did not enhance the retention deficit ($p = 0.58$ at 24 versus 72 h) in indiplon-treated mice (data not shown).

**Locomotor Activity in the Rat.** Locomotor activity in the rat was recorded from 30 to 60 min after oral administration of the test compounds. Indiplon significantly reduced locomotor activity compared with vehicle in a dose-dependent manner ($F(3,54) = 13.9; p < 0.0001$) (Fig. 6, A and B). Dose-dependent inhibition was also observed with zaleplon ($F(3,56) = 66.0; p < 0.0001$), zolpidem ($F(3,56) = 22.1; p < 0.0001$), and triazolam ($F(4,70) = 36.5; p < 0.0001$) (Fig. 6B). 

ED$_{50}$ values (milligrams per kilogram) derived from the dose-response relationships were indiplon, 2.5; zaleplon, 0.8; zolpidem, 2.4; and triazolam, 1.4.

**Rotarod Performance in the Rat.** Indiplon significantly reduced the latency to fall from the rotarod when compared to vehicle in a dose-dependent manner ($F(3,36) = 12.9; p < 0.0001$) (Fig. 7A). Dose-dependent reductions in latency were also observed for zaleplon ($F(3,28) = 15.2; p < 0.0001$) (Fig. 7B), zolpidem ($F(3,28) = 32.4; p < 0.0001$) (Fig. 7C), and triazolam ($F(3,28) = 21.4; p < 0.0001$) (Fig. 7D). ED$_{50}$ values (milligrams per kilogram) derived from the dose-response relationships were indiplon, 5.9; zaleplon, 5.4; zolpidem 14.8; and triazolam, 4.8.

**Vigilance Measures in the Rat.** The results of the vigilance studies are shown in Fig. 8, A–D. Indiplon significantly reduced the total number of trials initiated ($F(4,47) = 5.4; p < 0.001$), increased the number of omission errors ($F(4,47) = 10.8; p < 0.0001$) and reduced the proportion of correct trials in relation to the total number of trials initiated ($F(4,47) = 6.5; p < 0.0003$), with an MED for all of these measures of 3 mg/kg ($p < 0.03$). Indiplon had no significant effect on the average latency to respond to a cue light stim-
ulus (nosepoke latency). Zaleplon significantly reduced the total number of trials initiated \(F(4,44) = 8.7; p < 0.0001\], increased the number of omission errors \(F(4,44) = 5.9; p < 0.0006\), reduced the proportion of correct trials in relation to the total number of trials initiated \(F(4,44) = 6.5; p < 0.003\], and increased the average latency to nosepoke \(F(4,44) = 4.8; p < 0.003\). The MED for zaleplon in all measures except latency to respond was 3 mg/kg (\(p < 0.04\)). Zolpidem significantly reduced the total number of trials initiated \(F(5,32) = 10.2; p < 0.0001\], increased the number of omission errors \(F(5,32) = 21.8; p < 0.0001\], reduced the proportion of correct trials in relation to the total number of trials initiated \(F(5,32) = 10.2; p < 0.0001\], and increased the average latency to nosepoke \(F(5,32) = 9.7; p < 0.0001\). The MED for zolpidem in all measures was 10 mg/kg (\(p < 0.02\)). When the above-mentioned parameters were measured in the vigilance paradigm 10 h after indiplon administration (3 mg/kg p.o., a dose that produced a significant inhibition of locomotor activity in the same animals from 30 to 60 min after dosing \(F(1,17) = 10.9; p < 0.004\)), no significant difference from vehicle was observed in any parameter (data not shown), indicating no residual sedative effects 10 h after treatment.

### Delayed Nonmatch to Sample Test in the Rat

As shown in Fig. 9A, indiplon produced deficits in responding at the 4- and 32-s delay intervals \(F(1,72) = 13.3; p < 0.001\); post hoc \(p < 0.02\), but this was accompanied by a generalized reduction in responding, as measured by the number of reinforcements obtained at each delay interval \(F(1,72) = 13.8; p < 0.001\). Reduced responding was statistically significant even at the 1-s delay interval \(p < 0.006\), even though accuracy was maintained (Fig. 9B). When the task was repeated 10 h after dosing, no deficits in response accuracy \(F(1,21) = 5.5; p < 0.051\); post hoc \(p < 0.14\) or number of reinforcements obtained \(F(1,21) = 1.4; p < 0.27\) were observed in the indiplon group versus vehicle (Fig. 9, C and D).

### Discussion

The studies described here characterize indiplon as an effective sedative-hypnotic in rodents, with the pharmacological profile expected for an allosteric potentiator of GABA\_A receptor activity that has preference for \(\alpha_1\) subunit-containing GABA\_A receptors.

The in vivo pharmacology of indiplon is entirely consistent with in vitro studies that show this compound to be a high-affinity allosteric potentiator of GABA\_A receptors. Indiplon has low nanomolar affinity for \(^{3}H\)Ro 15-1788 and \(^{3}H\)Ro 15-4513 binding sites in rat brain membranes, and the binding of \(^{3}H\)indiplon itself shows a pharmacology and regional distribution of binding sites in rat brain consistent with that for \(\alpha_1\) subunit-containing GABA\_A receptors (Sullivan et al., 2004). Indiplon is also a fully efficacious potentiator of GABA\_A receptor-mediated responses in neurons (Sullivan et al., 2004). The predominantly sedative activity of indiplon in rats and mice, and reversal of this by the benzodiazepine site...
antagonist flumazenil is in good correspondence with this in vitro receptor profile, as is the fact that indiplon is active in the passive avoidance and Vogel tests, and in blocking pentylentetrazole-induced convulsions (our unpublished observations), activities consistent with an allosteric potentiator of GABA<sub>A</sub> receptors.

The sedative activity of indiplon was apparent in multiple assays in both rats and mice. Inhibition of locomotor activity in both species, reduced ability to maintain balance on the rotarod, reduced accuracy, increased omission errors, and general response suppression in the vigilance assays along with inhibition of activity in the open field test all suggested a consistent sedative profile. Indeed, the activity of indiplon in the passive avoidance paradigm in mice, and the delayed nonmatch to sample paradigm in rats, both widely used as measures of learning and memory, may have resulted from the sedative activity of the compound, rather than a direct influence on the neuronal substrates of learning and memory. This follows from the observations that the doses of indiplon that were active in these paradigms closely matched the doses that produce sedation in mice and rats (Table 1). In the passive avoidance paradigm, the temporal profile of indiplon’s action also matched that of its sedative effects. Furthermore, indiplon did not alter passive avoidance retention if it was given immediately after training, suggesting that it did not have effects on memory consolidation (Cherkin, 1969; McGaugh, 1972). In the delayed nonmatch to sample paradigm, short-term (4–32-s delay intervals) memory deficits were observed at a dose of indiplon that clearly produced a reduction in overall responding, indicated by a decrease in

![Fig. 8. Vigilance analysis in the rat using the five-choice paradigm. Testing began 30 min after oral gavage. A, total reinforcements for indiplon (5–18 rats/group), zaleplon (5–15 rats/group), and zolpidem (3–11 rats/group). *, p < 0.04 versus vehicle. B, omission errors for indiplon (5–18 rats/group), zaleplon (5–15 rats/group), and zolpidem (3–11 rats/group). *, p < 0.03 versus vehicle. C, choice accuracy as measured by percentage of correct responses for indiplon (5–18 rats/group), zaleplon (5–15 rats/group), and zolpidem (3–11 rats/group). *, p < 0.02 versus vehicle. D, latency to respond for indiplon (5–18 rats/group), zaleplon (5–15 rats/group), and zolpidem (3–11 rats/group). *, p < 0.0005 versus vehicle.](https://jpet.aspetjournals.org/content/127/4/555/F8)
total reinforcements obtained. Furthermore, there was no evidence of retrograde amnestic effects as indicated by a lack of effect in the delayed nonmatch to sample paradigm 10 h after dosing. Interestingly, there were also no residual sedative effects 10 h after dosing, as measured by the five-choice serial reaction time test of vigilance. The above-mentioned findings suggest that the indiplon-induced memory deficits were a function of generalized response suppression. Together, the delayed nonmatch to sample and passive avoidance data do not provide strong evidence that indiplon has specific effects on short-term memory processes or on memory consolidation, although this must be more fully evaluated in future studies.

The activity of indiplon in the Vogel test is consistent with that observed for both benzodiazepine and nonbenzodiazepine ligands in this, and other, conflict paradigms (Gardner and Piper, 1982; Martin et al., 1993; Nazar et al., 1997; Griebel et al., 1998) and may indicate that the compound has anxiolytic activity. However, in the mouse open field test, where the anxiolytic effects of benzodiazepine ligands are also apparent (Crawley, 1985), indiplon showed no increase in center time, but a decrease in this parameter and in total horizontal activity. This suggests that the potential anxiolytic effects of indiplon were overshadowed in this test by the compound’s sedative effects, and the dose range over which this occurred is consistent with the inhibition of locomotor activity in the mouse observed previously. Interestingly, the dose range over which indiplon is active in the Vogel test also coincides with the sedative dose range in the rat. One explanation for these observations could be that both the anxiolytic and sedative activities of the compound occur over the same dose range and that the sedative effects of the compound can be overridden in a conflict paradigm, where animals have a considerable drive to respond, whereas in the more “natural” setting of the open field test, sedation dominates. Similar observations have been made previously in conflict versus ethologically derived tests comparing different benzodiazepine site ligands. Thus, “nonbenzodiazepine”

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Fig. 9. Delayed nonmatch to sample task in the rat. A and B, effect of indiplon (2 mg/kg p.o.) 30 min after oral gavage (8–18 rats/group) on percentage of correct choices (A) and number of reinforcements obtained for each delay (B). C and D, effect of indiplon (2 mg/kg p.o.) 10 h after oral gavage (4–5 rats/group) on percentage of correct choices (C) and number of reinforcements obtained for each delay (D). *, p < 0.006 versus vehicle.
agonists with selectivity toward GABA\(_A\) receptors containing \(\alpha1\) subunits, including zolpidem and zaleplon, have a different profile to benzodiazepines such as diazepam and clorazepate (Griebel et al., 1996a,b,c, 1998; Nazar et al., 1997). Consistently, zolpidem and zaleplon show “anxiolytic” effects in conflict procedures, and anxiolytic activity is weak or absent in measures such as the open field test, light-dark box, and elevated plus maze. In addition, the effects of zolpidem and zaleplon in anxiety models overlays their sedative activity. Therefore, the profile of indiplon with similar dose ranges for activity in assays for sedation, learning and memory and anxiolytic activity (Table 1) is consistent with that for allosteric potentiators of GABA\(_A\) receptor function that have preference for \(\alpha1\) subunit-containing GABA\(_A\) receptors.

Our understanding of the roles played by GABA\(_A\) receptor subtypes in benzodiazepine ligand pharmacology has recently been confirmed and extended in an elegant series of experiments where the affinity of the benzodiazepine site has been manipulated by point mutations in the different \(\alpha\) subunits with a subsequent “knock-in” approach to create mutant mice that have lost benzodiazepine sensitivity in GABA\(_A\) receptor subtypes containing specific \(\alpha\) subunits (Möller et al., 2002). This work has shown that certain pharmacological effects of benzodiazepine ligands are a result of an interaction with particular \(\alpha\) subunits, for example sedation with \(\alpha1\), anxiolysis with \(\alpha2\), and memory effects with both \(\alpha1\) and \(\alpha5\) (Rudolph et al., 1999; Low et al., 2000; McKernan et al., 2000; Crestani et al., 2002). As an efficacious allosteric potentiator with preference for \(\alpha1\) subunit-containing GABA\(_A\) receptors, indiplon’s in vivo profile is entirely consistent with this work because it produces a predominantly sedative profile in animals.

A major aim of the present study was to make a side-by-side comparison between the in vivo sedative effects of indiplon with particular \(\alpha\) subunits, for example sedation with \(\alpha1\), anxiolysis with \(\alpha2\), and memory effects with both \(\alpha1\) and \(\alpha5\) (Rudolph et al., 1999; Low et al., 2000; McKernan et al., 2000; Crestani et al., 2002). As an efficacious allosteric potentiator with preference for \(\alpha1\) subunit-containing GABA\(_A\) receptors, indiplon’s in vivo profile is entirely consistent with this work because it produces a predominantly sedative profile in animals.

### TABLE 1
Comparison of the affinities and effective doses of indiplon, zaleplon, zolpidem, and triazolam in rodent assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Indiplon</th>
<th>Zolpidem</th>
<th>Zaleplon</th>
<th>Triazolam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flumazenil binding(^a)</td>
<td>1.8</td>
<td>11.2</td>
<td>68.5</td>
<td>0.55</td>
</tr>
<tr>
<td>Rat cortex membranes (K(_i), nM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potentiation of GABA currents(^a)</td>
<td>11.6</td>
<td>152</td>
<td>630</td>
<td>26.5</td>
</tr>
<tr>
<td>Rat cultured neurons (EC(_{50}), nM)</td>
<td>2.7</td>
<td>24.6</td>
<td>6.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Inhibition of locomotor activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse (ED(_{50}), mg/kg p.o.)</td>
<td>1</td>
<td>30</td>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td>Passive avoidance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse (MED, mg/kg p.o.)</td>
<td>2.5</td>
<td>2.4</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Inhibition of locomotor activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat (ED(_{50}), mg/kg p.o.)</td>
<td>5.9</td>
<td>14.8</td>
<td>5.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Rotarod impairment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat (ED(_{50}), mg/kg p.o.)</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td>ND</td>
</tr>
<tr>
<td>Vigilance impairment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat (MED, mg/kg p.o.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(ND\), not determined.

\(^a\) In vitro data from Sullivan et al. (2004).
lon, zaleplon, zolpidem, and triazolam, and this has yielded some interesting results. Table 1 compares the relative activities of these compounds across the in vivo measures taken and also includes a summary of in vitro receptor affinity (Sullivan et al., 2004). First, when assessed over all of the assays, indipren emerges as the most effective sedative versus zolpidem and zaleplon, consistent with its higher affinity in the receptor binding assays. When the rank order is compared, the relationship between receptor affinity and efficacy in the mouse and rat locomotor activity, rat rotord and mouse passive avoidance assays holds true between triazolam, indipren and zolpidem, with zaleplon being an outlier. Consequently, zaleplon seems to be more effective in vivo than would be predicted from its receptor affinity. Interestingly, dose-response curves for zaleplon seemed to be atypical compared with the other benzodiazepine site ligands. In the mouse locomotor activity assay, inhibition by zaleplon seemed to reach a lower maximum inhibition with a more shallow dose-response curve (Fig. 3B); similarly, the dose-response function for zaleplon in the passive avoidance assay also seemed to be shallow compared with the other drugs tested. These differences are not explained by the allosteric potentiation by this compound of the GABA<sub>A</sub> receptor, because all of these ligands seem to be fully efficacious (Sullivan et al., 2004). The reportedly high oral bioavailability of zaleplon in the rat at 80% (Beer et al., 1997) (cf. oral bioavailability of zolpidem in the rat is 27%; Garrigou-Gadenne et al., 1989) may contribute to the effectiveness of zaleplon in vivo, although its blood-brain barrier penetration is similar to zolpidem (brain/plasma ratio of approx. 0.5; Garrigou-Gadenne et al., 1989; Gaudreault et al., 1995) and inferior to that for indipren (brain/plasma ratio = 1.7; vide supra). One possibility could be the existence of an active metabolite for zaleplon, although the major metabolites are apparently weak or inactive displacers of benzodiazepine binding (Vanover et al., 1994). Another possibility may be an additional pharmacological activity of the compound itself, or a metabolite.

In conclusion, the in vivo pharmacological profile of indipren in rats and mice shows this compound to be an effective sedative-hypnotic, consistent with its in vitro profile as a high-affinity, allosteric potentiator of GABA<sub>A</sub> responses, with preference for α<sub>1</sub> subunit-containing GABA<sub>A</sub> receptors. The combination of high affinity, rapid onset and short duration of action, as determined in both pharmacodynamic and pharmacokinetic assays, should make this compound an attractive candidate as an improved treatment for insomnia, a concept which is currently being tested in multiple clinical studies.

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