# Population Pharmacokinetics, Brain Distribution, and Pharmacodynamics of 2nd Generation Dopamine Transporter Selective Benztropine Analogs Developed as Potential Substitute Therapeutics for Treatment of Cocaine Abuse

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**ABSTRACT:** A second generation of *N*-substituted 3α-[bis(4′-fluorophenyl)methoxy]-tropanes (GA 1–69, JHW 005 and JHW 013) binds with high affinity to the dopamine transporter (DAT) and are highly selective toward DAT compared to muscarinic receptor binding ($M_1$). The objective of this study was to characterize brain distribution, pharmacokinetics, and pharmacodynamics [extracellular brain dopamine (DA) levels] of three novel *N*-substituted benztropine (BZT) analogs in male Sprague–Dawley rats. The BZT analogs displayed a higher distribution ($V_d = 8.69$–$34.3$ *vs*. 0.9 L/kg) along with longer elimination ($t_{1/2}$: 4.1–5.4 *vs*. 0.5 h) than previously reported for cocaine. Brain-to-plasma partition coefficients were 1.3–2.5 *vs*. 2.1 for cocaine. The effect of the BZT analogs on extracellular brain (DA) levels ranged from minimal effects (GA 1–69) to several fold elevation (~850% of basal DA for JHW 013) at the highest dose evaluated. PK/PD analysis of exposure–response data resulted in lower $IC_{50}$ values for the BZT analogs compared to cocaine indicating their higher potency to inhibit DA reuptake (0.1–0.3 *vs*. 0.7 mg/L). These BZT analogs possess significantly different PK and PD profiles as compared to cocaine suggesting that further evaluation as cocaine abuse therapeutics is warranted.  J Pharm Sci 97:1993–2007, 2008

**Keywords:** pharmacokinetics; pharmacodynamics; dopamine; benztropine analogs; cocaine.

## INTRODUCTION

Cocaine abuse has resulted in an enormous health and social consequences and the epidemiological data further illustrate the growing need for an effective treatment strategy for drug addiction. Although cocaine interacts with several transporters/receptors in the brain, its reinforcing effects are primarily due to its interaction with the dopamine transporter (DAT) to block the

Abbreviations used: BZT, benztropine; GA1–69 (N-indoylethyl-3α-[bis(4′-fluorophenyl)methoxy]-tropane); JHW 005 (N-benzyl-3α-[bis(4′-fluorophenyl)methoxy]-tropane); JHW 013 (N-cyclopropyl methyl-3α-[bis(4′-fluorophenyl)methoxy]-tropane); GBR 12909, 1-[2-[bis(fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine

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reuptake of dopamine (DA) that results in an abnormally high concentration of DA in the synapse, which superstimulates DA receptors.[1,2] It has been hypothesized that the high abuse potential of cocaine is in part due to its rapid onset and short duration of action. Supportive evidence has come from positron emission tomography (PET) studies[3,4] and *ex-vivo* binding studies[5,6] that have shown that the rate at which cocaine enters the brain or binds to the DAT is the variable associated with the "high" rather than the presence of drug in brain.

One of the approaches to treat drug abuse is the use of substitute medications, such as methadone for opiate abuse, which can be used to minimize drug taking behavior. This strategy might be implemented for the treatment of cocaine abuse wherein the potential "substitute medication" would have high affinity for the DAT, but might inhibit DA reuptake at a slower rate than cocaine, that is, slower onset of effect and long duration of action.[7,8] A slower onset of action has been postulated as resulting in a reduced reinforcing effect, while a longer duration of action might attenuate the intense craving and thus reduce the urge for frequent cocaine administration. Hence, efforts to develop substitute medications have largely focused on DA uptake inhibitors since they share this neurochemical mechanism with cocaine. A wide variety of chemical structures have been used as templates in development of potential drugs, which include analogs of cocaine, methylphenidate, substituted piperazines and indanamines.[9,30] One compound from the substituted piperazine class GBR 12909 was considered as a potential substitute therapeutic agent but was terminated due to complications in phase I clinical trials.[9,30] A major concern however regarding the DA uptake inhibitors is their cocaine-like actions in animal models of addiction which may predict high abuse potential.[9,30]

Novel analogs of benztropine (BZT) have been developed as potential substitute therapeutic agents.[8,10,11] These analogs have high affinity for the DAT and are selective for DAT over other monoamine transporters.[12] The BZT analogs demonstrate reduced motor activity in rats as well as failure to substitute for cocaine in rats trained to discriminate cocaine from saline.[12,13] The 1st generation BZT analogs bind to DAT more selectively ($K_i$ for DAT $\lll K_i$ for serotonin transporter (SET), norepinephrine transporter (NET) and muscarinic $M_1$ receptor ($M_1$)) and with affinity higher than cocaine ($K_i$ for $DAT_{BZT\ analog} \lll K_i$ for $DAT_{cocaine}$).[13] In addition, they inhibit DA uptake with a higher potency in comparison to cocaine ($IC_{50BZT\ analog} \lll IC_{50cocaine}$). Preliminary studies with these analogs have provided intriguing results which show that the pharmacokinetics (PK) and pharmacodynamics (PD) of these analogs are distinct from cocaine, resulting in a significantly different behavioral profile from cocaine.[14,15,26] Structural modifications of the parent BZT molecule and resultant modifications of lipophilicity and other physical and pharmacological properties appear to influence blood–brain barrier (BBB) permeability and the PK/PD profile of these analogs. Results from previous studies have shown that (1) BZT analogs displayed a slower net transport across BBB *versus* cocaine, (2) BZT analogs' brain-to-plasma ratios were higher than cocaine, (3) BZT analogs' brain half-life was 12–25 fold longer than cocaine, (4) DA $T_{max}$ was 10 min for cocaine *versus* 120 min for BZT, and (5) DA profile after BZT dosing displayed a slow increase and slow elimination as compared to cocaine. These findings are consistent and may be supportive of development of one or more of these agents as a substitute medication as slow onset agonist drugs for the treatment for cocaine addiction.

The present study further examines the PK and PD of a second generation of *N*-substituted-BZT analogs that are more DAT selective than previously studied analogs (Fig. 1, Tab. 1). The present report focuses on analogs with heteroaromatic/cycloalkyl substitutions at the tropane nitrogen of 4′,4″-diF BZT. These substitutions at the tropane nitrogen have resulted in analogs that possess high affinity towards DAT along with lower affinity towards muscarinic receptors ($M_1$), SERT, and NET.[8] As such, systematic PK and PD evaluation of these analogs will provide evidence for their potential as substitute agents as well as identifying optimal structural requirements for efficacy.

## MATERIALS AND METHODS

### Materials

Sodium phosphate dibasic, triethylamine (TEA), ethylene diaminetetracetic acid (EDTA), monochloroacetic acid, ketamine, xylazine, and sodium hydroxide were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals and solvents were American Chemical Society (ACS) analytical

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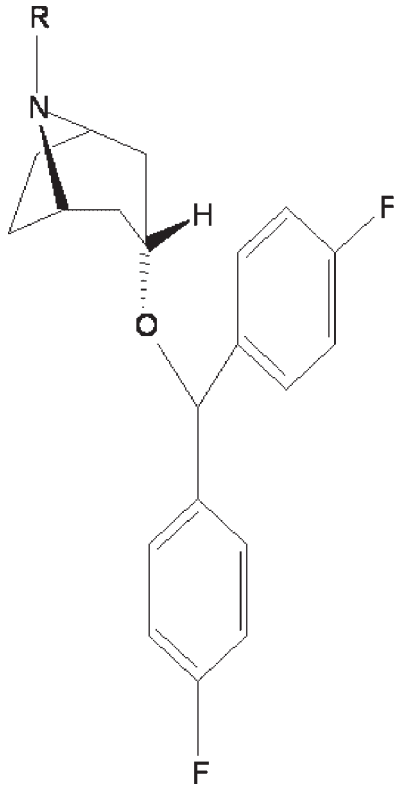


**Figure 1.** Chemical structure of *N*-substituted 3α-[bis(4′-fluorophenyl)methoxy]-tropane analogs; structural substituents presented in Table 1.

or High Pressure Liquid Chromatography (HPLC) grade. The BZT analogs were synthesized by Dr. Santosh Kulkarni and Ms. J. Cao (Medicinal Chemistry Section, National Institute on Drug Abuse, Intramural Research Program Baltimore, MD) as previously described.[11,25] Heparinized, sterile filtered rat plasma was obtained from Pelfreez (Houston, TX).

## Animals

Adult male Sprague–Dawley rats (250–275 g) were used in this study and were purchased from Harlan Laboratories (Indianapolis, IN). The protocol was approved by Institutional Animal Care and Use Committee of the School of Pharmacy (SOP), University of Maryland. Rats were housed in the animal facility, SOP and were maintained at a temperature of $72 \pm 2^{\circ}$F. Their care followed the "Guide for the Care and Use of Laboratory Animals" according to NIH specifications. Animals were allowed food (Purina rodent chow) and water *ad libitum* and were closely observed with respect to appearance, appetite, and waste elimination to ensure that they were healthy. For the microdialysis studies, all necessary measures were taken before, during, and after surgical procedures to minimize pain and discomfort in the animals.

## Pharmacokinetics and Brain Distribution Studies

### *Benztropine Analogs PK Dosing and Sampling*

The BZT analogs (hydrochloride or oxalate salts) were dissolved in hydroxyl-propyl cyclodextrin solution (20%) and a dose of 5 or 10 mg/kg was administered *via* tail vein. In the case of the BZT analog GA 1–69, a lower dose range (2.5, 5.0 mg/kg) was evaluated. The dose range was based on a previous study evaluating the PK and brain distribution of *N*-substituted BZT analogs so that adequate comparisons of PK parameters could be made across the different analogs. The animals were fasted approximately 10 h prior to dosing when all food was removed and withheld. Animals were restrained only during dosing when they were placed in rodent restraining chambers. The total volume administered was 4.5 mL/kg. Sampling times used were based on previous studies performed with the BZT analogs. Cohorts of three animals were sacrificed by $CO_2$ asphyxiation predose and postdose at 5, 30, 60, 120, 240, 360, 480, and 600 min. Blood was collected by heart puncture using heparinized syringes, centrifuged for 10 min at 3000 rpm (Denville

**Table 1.** Structural Substitutions, Physiochemical Properties, DAT, $M_1$ Binding, and DA Uptake Inhibition Data on BZT Analogs (Newman and Kulkarni[8])

| Compounds | *R* | MW | clog*P*[a] | DAT[b] $K_i$ (nM) | DA Uptake[c] $IC_{50}$ (nM) | $M_1$ $K_i$ (nM) |
|---|---|---|---|---|---|---|
| GA 1–69 | Indoyl-ethyl | 553.48 | 5.84 | 44.6 | 1200 | 3280 |
| JHW 013 | Cyclopropyl methyl | 419.95 | 4.7 | 32.4 | 180 | 257 |
| JHW 005 | Benzyl | 535.5 | 5.7 | 82.2 | 290 | 1030 |

[a]Calculated log of partition coefficient.
[b]Affinity for the DAT by determination of displacement of [$^3$H] WIN 35428.
[c]Inhibition of [$^3$H] DA uptake.

Scientific 260D (Metuchen, NJ), and the plasma was stored at −70°C until HPLC analysis. Brain tissue was immediately excised, weighed, placed on ice, immediately frozen in liquid nitrogen and stored at −70°C until analysis.

### Benztropine Analog HPLC Analysis

A valid, specific HPLC method was used to quantify the BZT analogs in plasma and brain samples.[16] Plasma samples were analyzed using liquid–liquid extraction with hexane, followed by evaporation and reconstitution. Brain samples were homogenized with phosphate-buffered saline before extraction. Brain and plasma samples were reconstituted into 210 μL of mobile phase A (below). The chromatographic conditions consisted of a Supelcosil™ LC-ABZ Plus column ($C_{18}$, 250 × 4.6 mm, 5 μm; Supelco, Bellefonte, PA), UV detection ($\lambda_{max} = 220$ nm), gradient mobile phases [methanol/0.05 M $Na_2HPO_4$, pH = 3.0 (40/60 v/v) (A) and solvent Methanol/0.05 M $Na_2HPO_4$, pH = 3.0 (80/20 v/v) (B)] pumped over a 15 min gradient profile at 1 mL/min. Oxprenolol was used as an internal standard. Both plasma and brain HPLC chromatograms were without any interference and the calibration curves were linear in the range of 0.05–10 μg/mL.

### Population Pharmacokinetic Modeling

Data obtained after administration of the BZT analogs were analyzed in two stages. In the first stage a naïve-pooled analysis was performed followed by single-stage population modeling. In the first stage plasma concentration *versus* time data for a given BZT analog were pooled and analyzed using nonlinear least squares estimation method. Compartmental modeling was used to estimate various PK parameters using WinNonlin version 3.1 (Pharsight, Cary, NC). Both one- and two-compartment model were evaluated to determine the optimal structural model. Inequality in the variance of data along the concentration was accommodated using different weighing schemes (1, 1/observed concentration, 1/predicted concentration, 1/predicted concentration$^2$). Goodness of fit was based on visual inspection, final residual sum of squares, random distribution of residuals, Akaike's information criteria (AIC) and Schwartz criteria (SC).

Results from the naïve-pooled analysis were used as priors in the population PK modeling of data. A two-compartment model best described the disposition of the BZT analogs after single dose i.v. administration. Several simulation studies have shown that nonlinear mixed effects population modeling of destructively obtained data results in relatively accurate and unbiased estimates of theoretical PK parameters.[27] These studies also show that the estimates from nonlinear mixed effects modeling are more accurate than naïve pooling and noncompartmental analysis of destructively obtained data. In addition, this modeling approach allows us to statistically compare PK parameters across the analogs. Nonlinear mixed effect Population modeling was performed with NONMEM® version 5.0 (Globomax, Columbia, MD) using the first order (FO) approximation method. All the structural PK parameters were expressed as a function of fixed effects as well as random effects. The random effects consisted of two components, between subjects and within subject. Inter-individual variability (IIV) $\eta$ quantifies the unexplained random variability between individuals and was modeled using an exponential error model. Residual error ($\varepsilon$) or intra-subject variability quantifies the random error within subject due to assay error and/or model misspecification and was assumed to be identically and independently distributed with a mean 0 and variance $\sigma^2$. The residual error was modeled using additive or proportional models, "goodness of fit" was based on WSSR, %CV for parameters, AIC, SC, and plots of observed and model predicted concentration *versus* time, and time *versus* residual concentration. In order to avoid local minima on sum of squares surface, initial estimates were perturbed and the iterative process repeated to achieve convergence.

### Brain Distribution Analysis

Brain concentrations of the BZT analogs were determined at similar time points as those used to determine plasma concentrations. Partition coefficient ($R_i$), which is the ratio of amount of drug in brain to drug in plasma was determined to evaluate the extent of uptake in brain tissue compared to plasma.

$$R_i = \frac{\mathrm{AUC}(0-\mathrm{all})_{\mathrm{braintissue}}}{\mathrm{AUC}(0-\mathrm{all})_{\mathrm{plasma}}} \quad (1)$$

AUC in brain and plasma tissue were computed for a time frame where the drug levels were above the limit of quantitation (LOQ) using Bailer's method.[17] In addition, $C_{\mathrm{brain}}/C_{\mathrm{plasma}}$ ratios were determined at each sampling point.

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## Pharmacodynamic Studies

### *In-Vivo Microdialysis Methods*

Rats were anesthetized for jugular vein cannulation and implantation of guide cannula by i.p. administration of ketamine/xylazine (80:12 mg/kg). After cannulation, each rat was placed in kopf stereotaxic apparatus, and a plastic intracerebral guide (CMA microdialysis, Acton, MA) was implanted. The guide cannula was stereotaxically implanted into the nucleus accumbens[29] using the following coordinates relative to bregma; +1.6 mm in anterioposterior plane, −1.7 mm in mediolateral plane and 6.0 mm below the dura in dorsoventral plane.[28] Guide cannulas were cemented in place using stainless steel screws and dental acrylic. After the surgery, animals were placed singly with *ad libitum* food and water with a minimum of 5 days to recover from surgery. A day prior to experiment, a microdialysis probe (CMA microdialysis) was lowered into the guide cannula and was continuously perfused with aCSF at a flow rate of 0.6 μL/min.[14,15] Disturbances in local cerebral blood flow may be altered during the first 2 h following the implantation of the microdialysis probe. As such, a 24-h recovery period is used to minimize DA depletion and high probe efficiency. On the day of the experiment, dialysate samples were collected every 20 min for an hour prior, providing baseline DA levels. After the 1-h period, rats were administered the BZT analogs, GA 1–69 (2.5, 5.0 mg/kg), JHW 005 (5.0, 10 mg/kg), and JHW 013 (5.0, 10 mg/kg) i.v *via* the jugular cannula. Dialysate samples were collected at 20, 40, 60, 120, 240, 360, 480, 600, and 1440 min for JHW 013. Samples were collected up to 120 and 1440 min for GA 1–69 and JHW 005, respectively. Samples were immediately injected into an HPLC-ECD system setup for detection of DA.

### *HPLC Analysis of Dialysate Samples*

Dialysate samples obtained after treatment with *N*-substituted BZT analogs were immediately analyzed for DA content by HPLC using electrochemical detection (ECD).[18] Standard solutions of DA ranged from 1 to 100 nM. The mobile phase consisted of 215 μM disodium EDTA, 145 mM sodium hydroxide, 150 mM monochloroacetic acid, 1.49 mM sodium octyl sulfate (SOS), 10 mM triethyl amine, 6% methanol, 6% acetonitrile at a final pH of 5.3. The HPLC system consists of syringe pump (ISCO, Inc., Lincoln, NE), electrochemical detector (Bioanalytical System, Inc., West Lafayette, IN) and a microbore unijet HPLC column (Bioanalytical System, Inc.; 1 mm ID × 150 mm). The optimal settings for the electrochemical detector were: applied potential = +650 mV, signal filter = 0.1 Hz, and range = 0.5 nA. The standard curve was found to be linear with an $r^2 > 0.99$ and the intra- and inter-day variability were ≤10%.

### *PK/PD Modeling*

The PK/PD analysis was carried out in two stages. The first stage consisted of PD model selection based on goodness of fit criteria. In the second stage, sequential PK/PD analysis was performed using the appropriate PD model where the PK parameters previously estimated were fixed and PD parameters estimated.

***Stage 1, Model Selection.*** A temporal delay was observed in the elucidation of effect for the BZT analogs (JHW 013 and JHW 005: $T_{max}$ for conc = 5 min, $T_{max}$ for effect = 20 min) however since the first PD sampling time for the *N*-substituted BZT analogs, JHW 005 and JHW 013, occurred at 20 min, the degree of the delay was not clear. Since the temporal delay does not appear to be significantly long, both types of models, direct and indirect were evaluated. The reason for evaluating indirect physiological response (IPR) model was based on the known mechanism of action of the BZT analogs.[8] The BZT analogs are known to bind to DAT and prevent the reuptake of DA thus resulting in elevation of brain extracellular DA levels. The rate of change of response was described as

$$\frac{dR}{dt} = K_{in} - K_{out}\left(1 - \frac{C_p}{IC_{50} + C_p}\right)R \qquad (2)$$

where R (response) is the brain DA level, $K_{in}$ is a zero-order input rate constant reflecting the release of DA in synapse, $K_{out}$ is a first-order response dissipation rate constant reflecting the uptake of DA from synapse, $C_p$ is the plasma concentration of the BZT analogs and $IC_{50}$ is the drug concentration producing 50% of maximum inhibition. The model assumes that DA levels are immediately and directly related to inhibition of the loss of DA as a result of blockade of the DAT.

To account for delay in response, the possibility of an "effect" compartment was also evaluated. This delay in response is assumed to be due to the temporal differences between systemic drug levels

and the BZT concentration achieved at the DAT. As such, in our case the synapse could be thought of as an effect compartment. The concentration-effect relationship was described by an $E_{max}$ model driven by concentration at effect compartment. The following equation describes the relationship:

$$E = E_0 + \frac{(E_{max-E_0}) \times C_e}{EC_{e50} + C_e} \quad (3)$$

where $E_0$ is the baseline DA level in the absence of drug, $E_{max}$ is the maximum effect produced, $C_e$ is the concentration in the effect compartment and $EC_{e50}$ is the effect compartment concentration required to produce 50% of $E_{max}$.

In order to select the appropriate PD model, the PK component of the model was fixed and DA profile from all the animals was pooled in and fitted to each model. Average PK parameters were used in the PK component of the model. Since the range of DA levels was small, only uniform weighting scheme was used to describe the error component. Model selection was based on standard goodness of fit criteria which include weighted sum of squares of residuals (WSSR), AIC, SC, residual plots, and plots of model-predicted concentration *versus* effect.

***Stage 2, PK/PD modeling.*** After an appropriate PD model was selected for the BZT analogs, the next stage consisted of performing sequential PK/PD modeling using the appropriate PD model. Since PK and PD studies were performed in different set of animals, the first step of PK/PD model consisted of simulating PK parameters to be used as a fixed component in the PK/PD model. Mean PK parameters and IIV was used to simulate full PK profiles using NONMEM v 5.0. Full plasma concentration time profile was simulated for six animals and the PK parameters were estimated for all six animals. Each set of PK parameters were randomly assigned to a PD data set. This process of simulations and random pairing was repeated for all the animals used for each BZT analog at both dose levels ($n = 5$–6 for 5 mg/kg, $n = 4$–6 for 10 mg/kg).

Simulated PK parameters and the DA profile for each animal were incorporated into appropriate PK/PD model. Sequential population PK/PD modeling was the performed using both (1) two stage approach and (2) single stage approach. In the standard two stage (STS) approach, individual PD parameters were estimated using WinNonlin® in the first step and population mean and variability estimated in the second step. Single stage population PK/PD was performed using NONMEM v 5.0. IIV was modeled using an exponential error model. In order to model residual variability both additive and proportional error models were evaluated.

### *Statistical Analysis*

Brain microdialysate levels at each time point were compared to baseline levels by a repeated measures ANOVA followed by Dunnett's post-hoc test. PK and PD parameters were compared by ANOVA followed by Dunnett's post-hoc analysis. Statistical significance was set at $p < 0.05$.

## RESULTS

### Pharmacokinetic and Brain Distribution

### *Benztropine Analog Pharmacokinetics*

Figure 2 presents the mean plasma concentration *versus* time profile for the BZT analogs after single dose i.v. administration in Sprague–Dawley rats. The BZT analogs appear to follow a biphasic disposition with a two-compartment model providing the optimal fit. Figure 2 also shows the WinNonlin® predicted and observed concentration *versus* time fits for the BZT analogs. The mean parameter estimates from the naïve-pooled analysis (WinNonlin®) were used as initial estimates to perform single-stage mixed effects population modeling to obtain individual parameter estimates using NONMEM®. The Inter-animal variability component of random effects was best described by an exponential error model while the residual error component was best described by proportional error model. PK parameters for the BZT analogs are presented in Table 2. The BZT analogs displayed a wide range with respect to $V_{dss}$ (steady state volume of distribution), 8.6–34.3 L/kg. Amongst the BZT analogs, JHW 005 had the highest $V_{dss}$ which was significantly higher than the rest of the analogs. The rank order for $V_{dss}$ amongst the analogs was GA 1–69 < JHW 013 < JHW 005. Amongst the BZT analogs, JHW 005 had the longest distribution half-life ($t_{1/2}$-$\alpha$ = 0.211 h) while GA 1–69 had the shortest (0.128 h). Elimination half-life of the BZT analogs ($t_{1/2}$-$\beta$) was within the range of 3.4–5.4 h with JHW 005 having the longest $t_{1/2}$-$\beta$ (5.4 h). Elimination half-life ($t_{1/2}$-$\beta$) for JHW 005 was significantly higher than JHW 013. Clearance from central compartment (CL) was highest for JHW

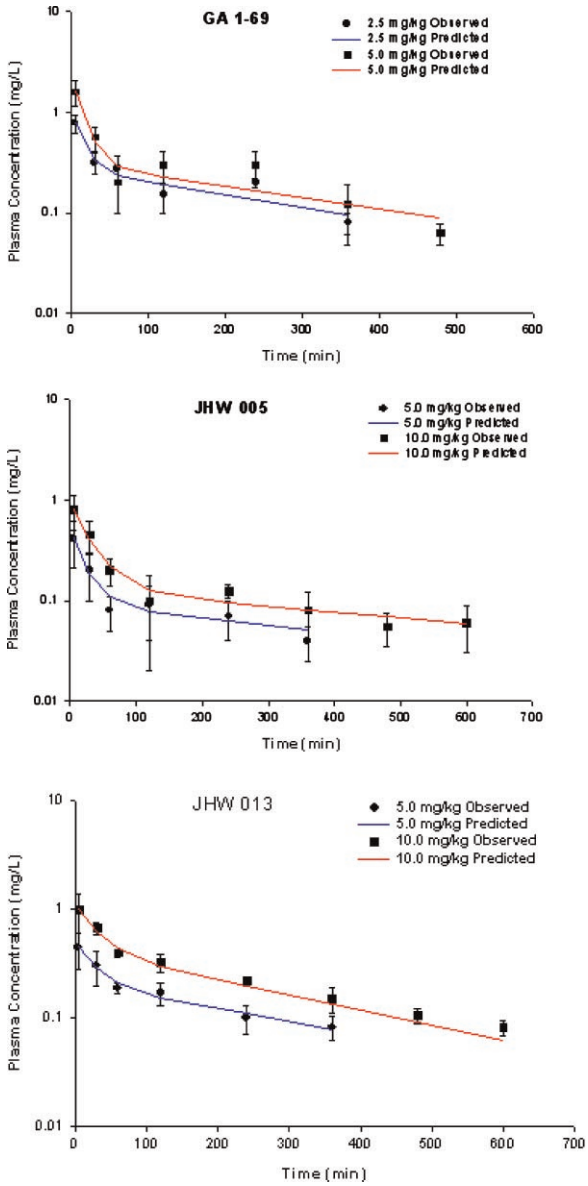


**Figure 2.** Observed and predicted plasma concentration *versus* time profiles of GA 1–69 (2.5, 5.0 mg/kg i.v.), JHW 005 (5.0, 10.0 mg/kg i.v.), and JHW 013 (5.0, 10.0 mg/kg i.v.) in male Sprague–Dawley rats using WinNonlin. Data displayed as mean ± SD ($n = 3$/time point/dose).

005 (5.41 L/h/kg), while GA 1–69 had the lowest clearance amongst the series (1.56 L/min/kg).

### *Brain Distribution of the BZT Analogs*

Figure 3 presents the brain concentration *versus* time profiles for the BZT analogs after naïve-pooled averaging of data. Brain uptake ratios ($R_i$) and $t_{1/2\text{-}\beta}$ values are presented in Table 2. Rapid distribution to brain compartment was observed with peak brain levels for all BZT analogs seen at first sampling time after i.v. administration (5 min). Rapid entry in the brain compartment suggests that there is not delay in the entry in brain tissue. Amongst the BZT analogs, GA 1–69 ($R_i = 1.32$) and JHW 013 ($R_i = 1.50$) displayed similar extent of distribution in brain as compared to plasma. Brain half-lives for all the BZT analogs were several fold higher than that of plasma half-lives. Figure 4 represents $C_{brain}/C_{plasma}$ ratio at each sampling point for the BZT analogs evaluated. In general, the ratio for both JHW 005 and JHW 013 remains the same for the entire sampling period indicating equilibrium between plasma and brain compartment. In the case of GA 1–69 there is a gradual increase the $C_{brain}/C_{plasma}$ ratio observed at the later end of sampling period (~360 min after dosing).

## Pharmacodynamic Study

### *Effect on Extracellular DA Levels after i.v. Administration of BZT Analogs*

Administration of the low dose of GA 1–69 (2.5 mg/kg) had minimal effect on extracellular DA levels with only the first sampling point at 20 min after dose being significantly above the baseline DA level (Fig. 5). Increasing the dose to 5.0 mg/kg had no further effect on extracellular DA levels. At 40 min after dose, the DA levels were similar to baseline. Maximum elevation ($R_{max}$) seen with both the dose levels was ~145% of basal DA. Higher doses were not evaluated as potentially toxic effects were observed in some animals at higher doses.

Administration of JHW 005 had a dose-dependent graded effect on extracellular DA levels (Fig. 5). Both dose group (5 and 10 mg/kg) resulted in a significant increase in extracellular DA levels above baseline. Increase in DA levels was rapid with $T_{max}$ being the first sampling point at 20 min for both the dose levels. Maximum extent of DA rise was ~160% with the low dose (5.0 mg/kg) and ~270% with the 10 mg/kg. DA levels remained significantly elevated above baseline for the high dose group for almost 2 h after dosing. Elevated DA levels gradually returned back to baseline after 2 h postdose.

Similar to JHW 005, administration of JHW 013 resulted in dose-dependent graded increases in DA levels (Fig. 5). The increase at both the dose levels was, however, much greater than JHW 005.

**Table 2.** Pharmacokinetic Parameters and Inter-Animal Variability (% CV) for BZT Analogs after Single Dose i.v. Administration to Male Sprague–Dawley Rats

| Parameter | GA 1–69 | JHW 005 | JHW 013 |
|---|---|---|---|
| $V_{dss}$ (L/kg) | 8.69 (45.1%) | 34.3 (33.1%) | 20.1 (5.01%) |
| Clearance (L/h/kg) | 1.5 (27.6) | 5.4 (11.3) | 3.6 (6.1) |
| $t_{1/2}$-$\alpha$ (h) | 0.128 (22.8%) | 0.211 (29.7%) | 0.16 (28.5%) |
| $t_{1/2}$-$\beta$ (h) | 4.46 (58.0%) | 5.45 (36.1%) | 4.1 (6.45%) |
| AUCbr/AUCpl | 1.32 | 2.52 | 1.51 |
| Brain—$t_{1/2}$-$\beta$ (h) | 7.08 | 3.7 | 7.16 |

Dose: GA 1–69 (2.5, 5.0 mg/kg), JHW 005 and JHW 013 (5, 10 mg/kg).

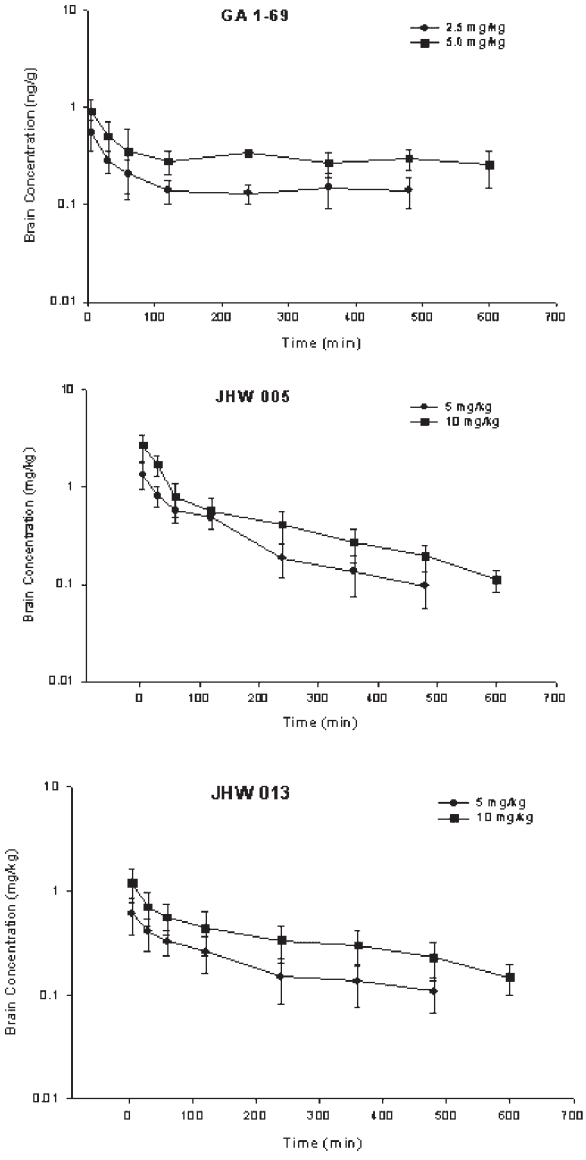


**Figure 3.** Naïve-pooled averaging based representative brain concentration *versus* time profiles of *N*-substituted BZT analogs. Data displayed as mean ± SD ($n = 3$/time point/dose).

Increase in DA levels was rapid with $R_{max}$ seen at 20 min after dose. Maximum increase in DA levels at lower dose (5 mg/kg) was ~400% of basal DA. Decline in DA levels was slow, with levels remaining significantly elevated above baseline for almost 10 h after dose. At the higher dose level, the maximum extent of increase was ~850% of basal DA. Elevated DA levels declined rather rapidly during the first 2 h and then stabilized at ~400% for almost 8 h.

### *PK/PD Analysis*

Since the delay in production of effect (elevation of DA) was not significantly long (Fig. 6), both direct (Sigmoidal $E_{max}$) and indirect PD models (Link and IPR) were evaluated for JHW 005 and JHW 013. The *N*-substituted BZT analog, GA 1–69 produced negligible effects on extracellular brain DA levels at both doses (2.5, 5.0 mg/kg i.v. administration), so no PK/PD modeling was performed with this analog. In the first stage of PK/PD modeling DA profiles from all the animals were pooled together and modeled using direct (Sigmoidal $E_{max}$), effect compartment and indirect physiologic response models to select an appropriate PD model. Mean PK parameters of the BZT analogs were used to drive the PK component of the model. Based on model suitability metric values (AIC, SC, and WSSR) and good estimation of PD parameters, the IPR model incorporating the inhibition of loss of response was selected as the appropriate PD model for both JHW 005 and JHW 013. Simulated PK parameter and the DA profile for each animal were incorporated into the appropriate PK/PD model to obtain individual PD parameters. In the STS method, individual PD parameters were estimated using WinNonlin® in the first stage and mean and variability associated with these PD parameters in the second stage. In the single stage approach, population mean

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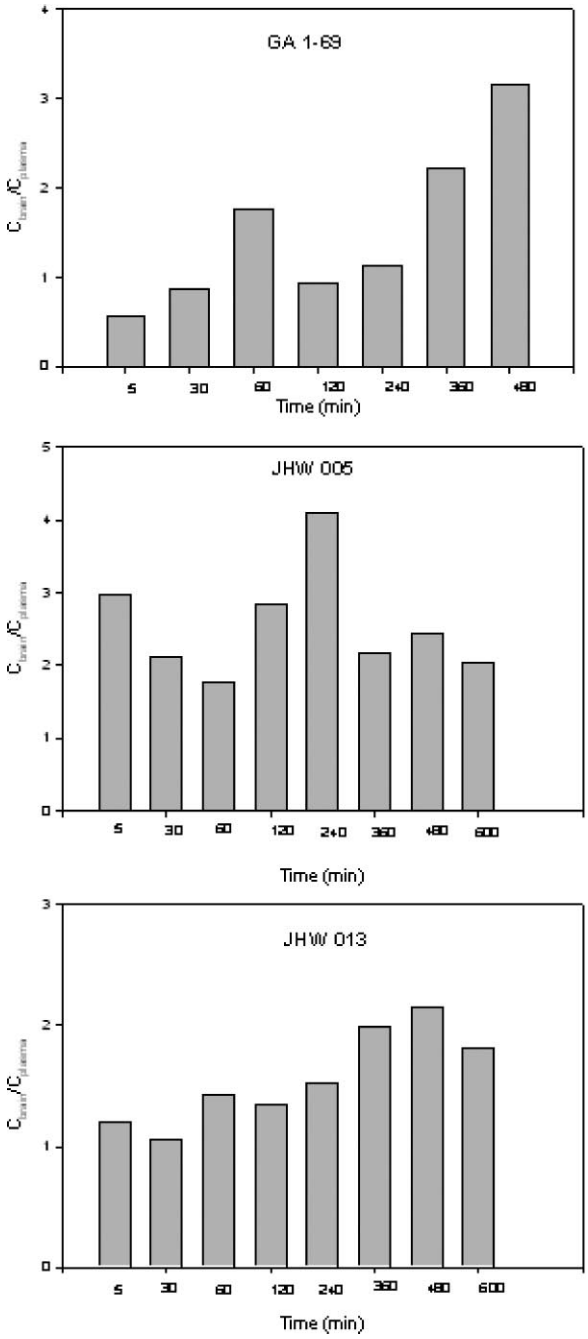


**Figure 4.** Brain/plasma ratios for BZT analogs GA 1–69, JHW 005, and JHW 013 at various time points after single dose i.v. administration. Dose: 10 mg/kg for JHW 005 and JHW 013, 5.0 mg/kg for GA 1–69.

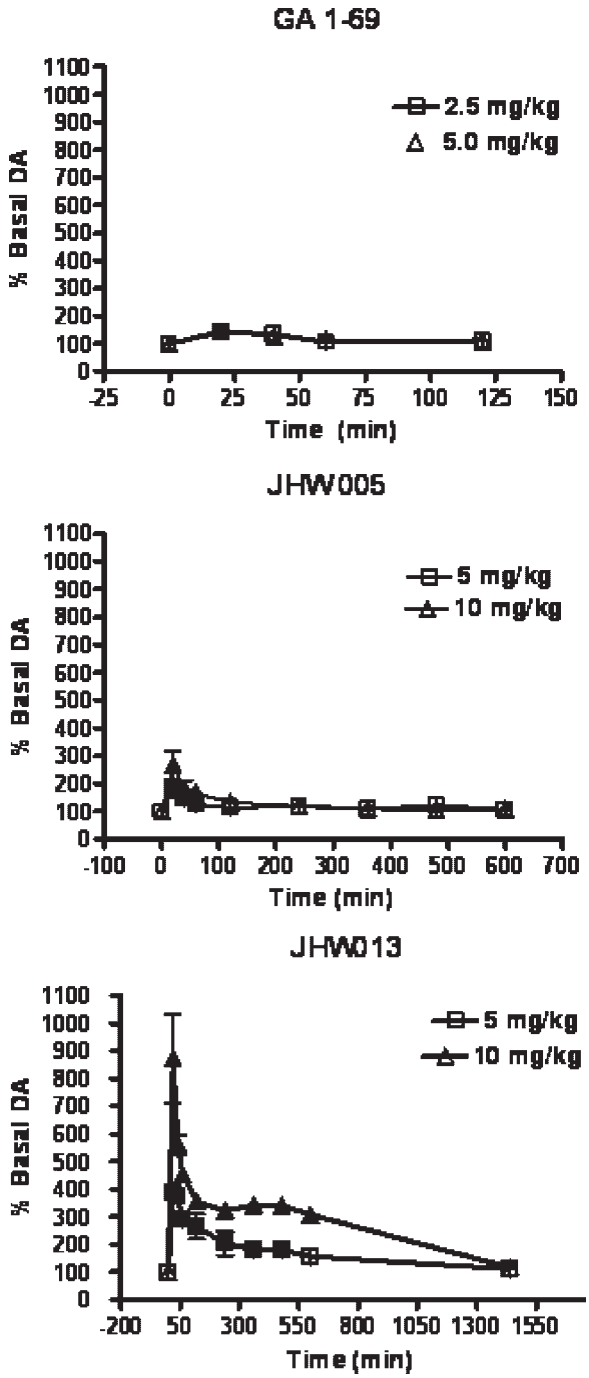


**Figure 5.** % Basal DA levels after single dose i.v. administration of BZT analogs to Sprague–Dawley rats (A) GA 1–69, (B) JHW 005, (C) JHW 013. (GA 1–69: $n = 6$/dose, JHW 005: $n = 5$/dose, JHW 013: $n = 5$/dose.) Filled symbols significant above baseline ($p < 0.05$).

and individual PD estimates were estimated simultaneously. The structural component of the model was (1) PK component: two compartment model with FO elimination as found appropriate in population PK analysis of these analogs (2) PD component: indirect response model II with inhibition of loss. To model IIV on the PK parameters, exponential error model was preferred over the additive model since the PD parameters are assumed to be log-normally distributed

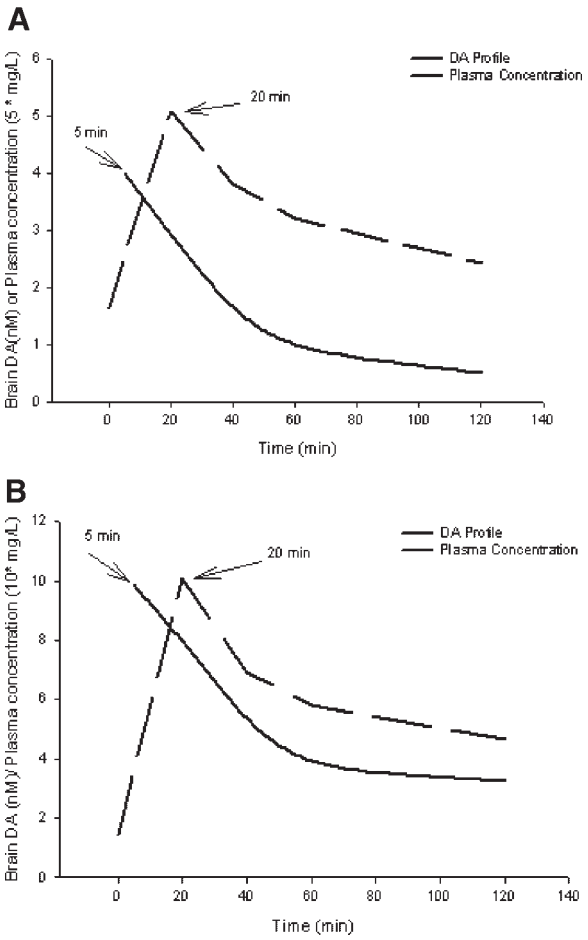


**Figure 6.** Plasma concentration and effect (DA profile) *versus* time profile for BZT analogs after single dose i.v. administration (10 mg/kg) (A) JHW 005, (B) JHW 013 to Sprague–Dawley rats.

and also the approach helps to constrain the parameter values to positive numbers.[27] Plot of absolute residual *versus* time displayed uniformity in residuals and hence the additive error model was used to model residual error.

The indirect response model with inhibition of loss of response appears to adequately describe the response-time profile for the BZT analogs based on goodness of fit plots and acceptable precision of estimated PD parameters. There appears to be a good correlation between observed and individual predicted DA levels for the *N*-substituted BZT analogs JHW 005 (Fig. 7) and JHW 013 (Fig. 8). The residuals *versus* time plot for both the analogs JHW 005 and JHW 013 using both the population PK/PD approaches show a random scatter with no obvious pattern suggesting adequacy of the structural model.

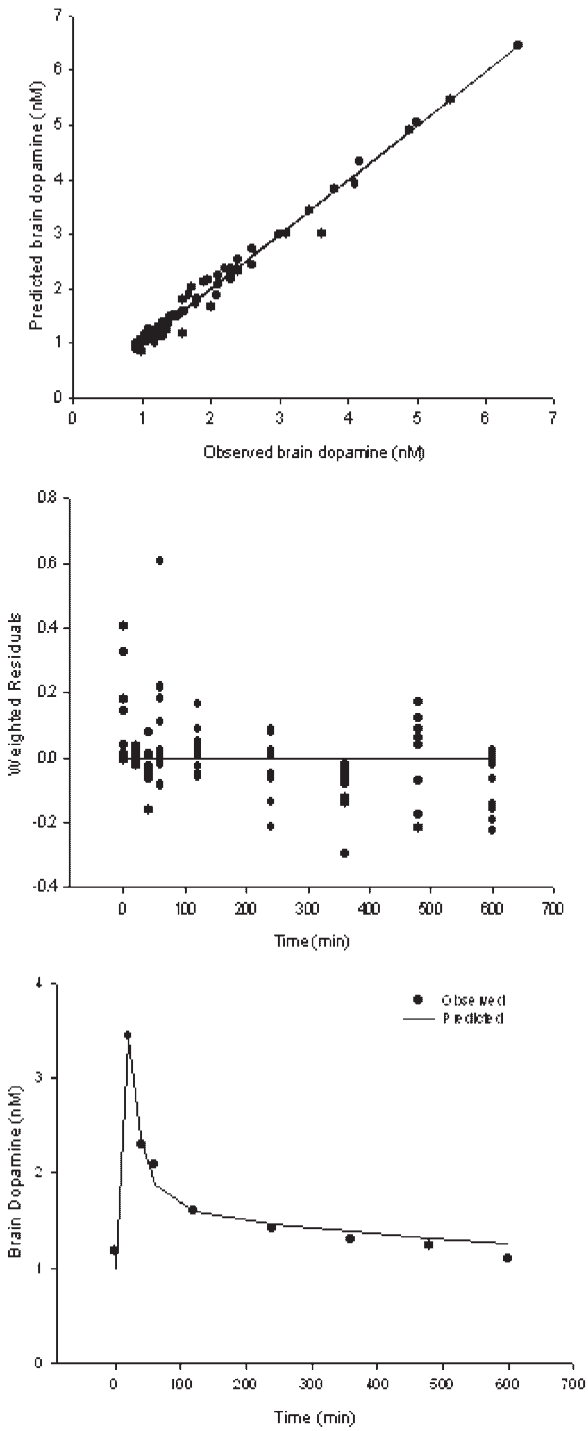


**Figure 7.** Goodness of fit plots based on individual estimated parameters (indirect response model II) for JHW 005 (A) observed *versus* model-predicted dopamine ($n = 10$) (B) weighted residuals *versus* time (C) model-predicted and observed dopamine levels *versus* time representative fit for 10 mg/kg dose animal.

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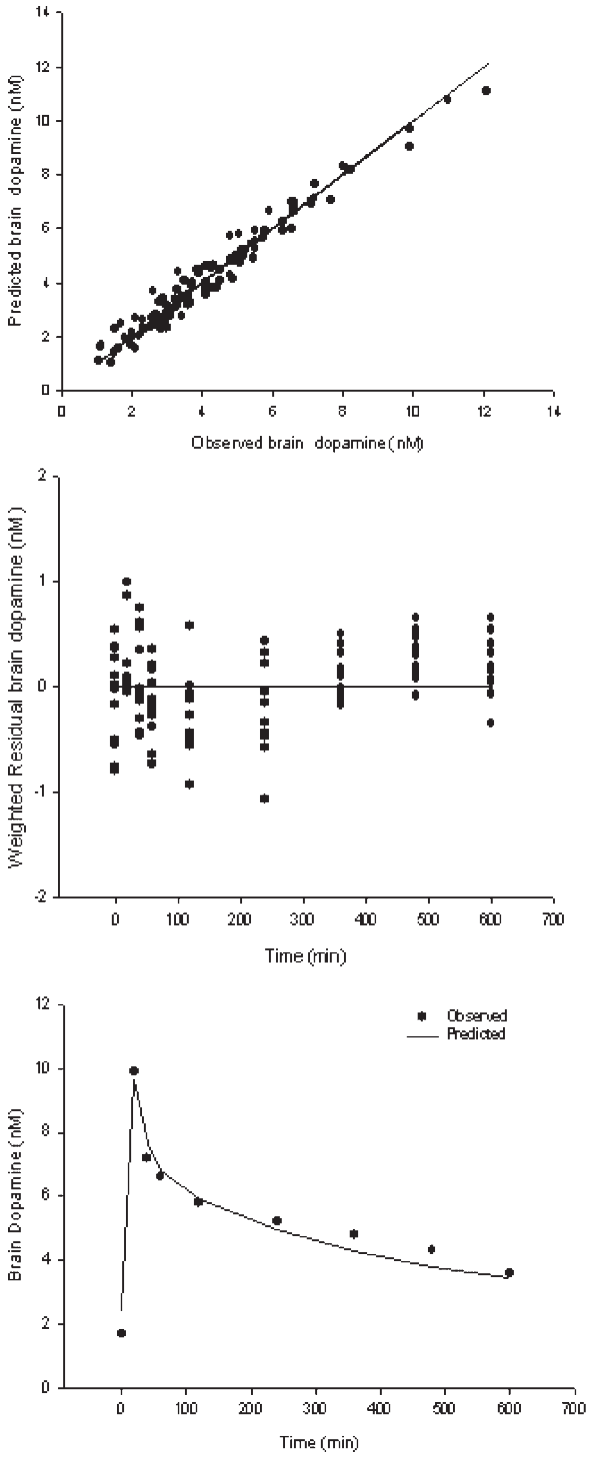


**Figure 8.** Goodness of fit plots based on individual estimated parameters (indirect response model II) for JHW 013 (A) observed *versus* predicted dopamine ($n = 10$) (B) weighted residuals *versus* time (C) model-predicted and observed dopamine levels *versus* time representative fit for 10 mg/kg dose animal.

PD parameters for the BZT analogs using both POPPK/PD approach are summarized in Table 3. Similar population PD parameters were estimated for JHW 005 using the population PK/PD approach. The zero-order rate constant for production of response ($K_{in}$) (release of DA in synapse) was estimated around 0.3 nM/min (0.39, 0.32) using both approach. Population parameter estimate of the rate constant for production of response ($K_{in}$) was around 0.3 nM/min (0.35, 0.29) and the $IC_{50}$ around 0.3 mg/L (0.34, 0.24). The IIV across the animals for all the PD parameters was in the range of 41–58%.

For JHW 013, the PD parameter estimates for both $K_{in}$ (1.08 *vs*. 0.63) and $K_{out}$ (0.66 *vs*. 0.41) were higher with STS approach as compared to single stage approach. The inhibition potency parameter $IC_{50}$ however was similar at around 0.1 mg/L with both population PK/PD approaches.

## DISCUSSION

The BZT analogs are potent DA uptake inhibitors developed as potential substitute therapeutic agents for treatment of cocaine abuse.[8] Based on their high binding affinity to DAT it might be expected that they would behave similarly to cocaine, however, these analogs possess a significantly different behavioral profile in animal models compared to cocaine.[12,19,20] Previously performed PK[14,26] and PD studies[27] with the BZT analogs have shown that the BZT analogs display (1) slower onset of effect, (2) longer disposition profile (3) longer duration of effect. In light of "*Rate theory*" these results suggest that the BZT analogs possess some of the desirable properties of a substitute therapeutic agent.[21] Structural substitutions at the tropane nitrogen have resulted in DAT-selective analogs, especially reducing the affinity for muscarinic receptors.[8] In the present report we have evaluated PK, brain distribution and PD of three, more DAT selective *N*-substituted BZT analogs. The previous report focused on the BZT analogs that possessed straight chain alkyl substitutions at the tropane nitrogen (*N*-H, methyl, allyl, and *n*-butyl)[14] while in the present report we have evaluated the BZT analogs with either aromatic (benzyl-JHW 005, indoyl-ethyl—GA 1–69) or conformationally constrained cycloalkyl (cyclopropyl methyl—JHW 013) substitution.

PET studies have shown that the rate at which cocaine enters the brain and binds the DAT is the

**Table 3.** Mean and Inter-Individual Variability (IIV) for Both Individually Estimated (WinNonlin®) and Single-Stage (NONMEM®) Population Pharmacodynamic Parameters Based on Indirect Response Model (Model II) with a Two Compartment PK Model as Fixed Component

| BZT Analog | $K_{in}$ | $K_{out}$ | $IC_{50}$ |
|---|---|---|---|
| JHW 013 | | | |
| Two-stage POPPK/PD | 1.08 (56.6) | 0.66 (43.0) | 0.13 (42.6) |
| Single-stage POPPK/PD | 0.63 (9.3) | 0.41[a] | 0.10[a] |
| JHW 005 | | | |
| Two-stage POPPK/PD | 0.39 (49.0) | 0.35 (55.1) | 0.34 (41.8) |
| Single-stage POPPK/PD | 0.32 (54.7) | 0.29 (57.7) | 0.24 (42.3) |

Units: $K_{in}$ = nM/min, $K_{out}$ = 1/min, $IC_{50}$ = mg/L.
[a] IIV < 1%.

variable associated with the "high reinforcing" effects rather than the presence of drug itself in brain.[3] Based on this observation a substitute therapeutic might be designed to enter the brain slowly resulting in a slower onset of effects. In the present report, however, all the BZT analogs entered the brain rapidly with peak levels observed within 5 min of dosing. These results are similar to previously reported AHN and chloro series of BZT analogs.[14,26] However, results from the *ex-vivo* transporter binding study have shown that several BZT analogs displayed a slower apparent binding rate at the DAT compared to cocaine.[6] Therefore, although the BZT analogs enter the brain rapidly, binding to DAT and inhibiting DA reuptake is at a slower rate as compared to cocaine. This slow association and long duration of action has been postulated to be related to the lack of cocaine-like behavior observed for these BZT analogues in rodent models of cocaine abuse.[6]

In general the BZT analogs displayed preferential distribution in the brain compartment in comparison to plasma, ($R_i > 1$) which is a favorable attribute for a CNS active drug. The overall brain distribution was similar to that reported for cocaine ($R_i \sim 2.0$).[14] The BZT analogs have shown high permeability *in vitro* in cell culture studies and high brain to plasma partition coefficients ($R_i > 1$) *in vivo*.[13–15] The blood volume in the brain tissue ranges from 2.7% in the white matter to 5.2% in the gray matter with an average of around 3.7% in the whole rat brain.[31,32] Therefore for a whole rat brain, which averages around 1.5 g, one would expect to have remaining blood volume of 55 μL in the vasculature. Having observed brain to plasma partition coefficients of ≥1 for the BZT analogs, it is highly unlikely that the amount of drug in 55 μL after being diluted in the whole brain (around 27-fold dilution), will have any significant contribution to the high concentrations observed in the brain homogenate. Therefore, approaches such as brain perfusion to remove blood in the vasculature were deemed unnecessary for the BZT analogs, even though it may be necessary for other drugs with low permeability.

Even though there are structural similarities between JHW 013 and the previously studied AHN 2-005 (cyclopropyl group of JHW 013 can be considered as conformationally rigid congener or bioisostere of the allyl group in AHN 2-005), brain uptake of AHN 2-005 is more than twofold higher than JHW 013.[14] Hence, conformational rigidity of JHW 013 appears to hinder the movement across the BBB lowering its overall brain uptake in comparison to the allyl substituted AHN 2005.

Elimination half-lives of the BZT analogs were in the range of 3.4–5.4 h and were much higher than those previously reported for cocaine (0.5 h).[14] Even though the clearance for JHW 013 and JHW 005 was similar or higher compared to cocaine, extensive distribution of the BZT analogs (high $V_{dss}$) prolongs their overall residence time in plasma. It should be reiterated that previous studies have suggested that the "high" associated with cocaine ingestion is due to rate at which it enters the brain and/or the rate at which it binds to the DAT.[3–6] An agent that binds to the DAT with a higher affinity coupled with a longer retention time than cocaine may reduce repeated administration of cocaine. Hence, the "*Rate theory*" suggests that the slower elimination of these analogs might prolong the duration of action resulting in reduced frequency of craving of the abused drug.[21] Sustained effect on locomotor activity has been reported with two BZT analogs, AHN 1055

and 4Cl-BZT, which also have long elimination half-lives.[12,22,26] Administration of methylphenidate, a DA uptake inhibitor, has been reported to produce euphoric effects in subjects with a history of cocaine abuse; however this drug is not reported to be abused.[4] One of the reasons proposed for its lack of abuse potential is its long half-life in plasma and brain resulting in persistent blockade of DAT.

Both the *N*-substituted BZT analogs JHW 005 and JHW 013 produced a dose-dependent graded effect on extracellular brain DA levels. GA 1–69 however had minimal effects on DA levels at both doses tested (2.5, 5.0 mg/kg). A critical variable that is associated with the reinforcing effects of drugs of abuse in general is the rate at which they produce the change in DA levels (ΔDA/time).[4] DA levels are reported to increase by 630% of baseline within 15 min after acute i.v. cocaine administration (3 mg/kg).[24] The BZT analog, JHW 005 produced a maximum elevation of 170% and 260% above baseline after 5 and 10 mg/kg of dose. At comparable doses of cocaine, the extent of DA rise is much lower. This lower rate of DA increase might not completely eliminate reinforcing effects; however the effects might be significantly blunted as compared to cocaine.

Based on *in-vitro* binding data and the microdialysis data presented here, it appears that GA 1–69 behaves more like a "DA sparing blocker" due to its high affinity towards DAT and at the same time very low potency to inhibit DA reuptake (DA uptake $IC_{50}$; GA 1–69:1200 *vs*. 120 nM cocaine)[25] resulting in minimal effect on basal DA levels. Since the substitute therapeutic agent might be concurrently administered during the period of cocaine abuse, GA 1–69 could preferentially bind to DAT due to its higher binding affinity effectively blocking cocaine from binding to DAT and at the same time having no reinforcing effects of its own due to its minimal effect on DA reuptake.

Amongst the *N*-substituted BZT analogs evaluated, the *N*-cyclopropyl methyl substituted JHW 013 produced the highest elevation in DA levels at both dose levels (5, 10 mg/kg). A combination of several factors appears be responsible for the maximum elevation in DA levels observed with JHW 013 as compared to other *N*-substituted BZT analogs, including: high DAT affinity; $K_i$: 32.4 nM, and relatively high potency to inhibit DA uptake, $IC_{50} = 180$ nM). Although Δ DA/time is considerably high during the rising phase at both dose levels; however it shows a much slower decline. We might expect a behavioral profile for JHW 013 which is similar to another DA uptake blocker methylphenidate, which shows rapid binding to DAT that lasts for a long period of time.[23] Methylphenidate is reported to produce some degree of euphoria in subjects with a history of cocaine abuse but there are no reports of methylphenidate addiction, which may be mainly due to its long duration of action. If indeed JHW 013 does produce a similar behavioral profile, this would be a positive feature for its potential use as substitute therapeutic agent.

In the present study, we have performed mechanism based PK/PD modeling of the BZT analogs to characterize the exposure–response relationship between the BZT analogs and microdialysate brain DA levels with indirect response model (IPR) adequately describing the exposure–response relationship. Amongst the *N*-substituted analogs, JHW 013 ($IC_{50}$: 0.10 mg/L) was found to be more potent than JHW 005 ($IC_{50}$: 0.24 mg/L) which is in agreement with the *in-vitro* binding and DA uptake inhibition data.[25] Previously reported *N*-substituted BZT analogs had a much slower rise in DA levels with $T_{max}$ of 60–120 min compared to the present series of BZT analogs suggesting that structural substitutions have a profound impact on their functional profile. Studies on DAT binding kinetics have shown that cocaine, which acts by a similar mechanism as the BZT analogs and shows no delay in effecting increases in basal DA levels has a higher apparent rate of association at DAT as compared to the BZT analogs evaluated herein, which have shown considerable delay in response.[6] Based on this *in-vitro* data, we speculate that the BZT analogs (JHW 005, JHW 013) which do not show a considerable delay in response might have a higher apparent rate of association at DAT in comparison to previously studied BZT analogs.

In conclusion, we have characterized PK and PD of second generation of more DAT-selective BZT analogs. The PK profiles of these newer analogs are significantly different from cocaine, characterized by a higher distribution and a longer elimination half-life. The lower extent of DA elevation along with longer duration of elevation compared to cocaine might result in lower reinforcing effects for these BZT analogs. Having some degree of reinforcing effect might be beneficial for substitute therapeutic agent as it might increase compliance in addiction treatment programs. However, without a medication to treat cocaine abuse and addiction, the absolute nature

of a successful candidate remains elusive. The BZT analogs evaluated in this study are highly selective for DAT, thus lowering the chances of side effects due to action at other receptor/transporters. Thus, selectivity and favorable PK and PD profile of the DAT inhibitors suggests that further evaluation as cocaine abuse therapeutics is warranted.

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