



# Chemistry & Biology Interface

An official Journal of ISCB, Journal homepage; [www.cbijournal.com](http://www.cbijournal.com)

## Research Paper

### Pharmacokinetics and tissue uptake of arylpiperazine derived SARMs for benign prostatic hyperplasia management in rats

Jawahar Lal<sup>1,\*</sup>, Amit Sarswat<sup>2</sup>, Shailendra Kumar Pandey<sup>1</sup>, B. Heeralal<sup>3</sup>

<sup>1</sup>Pharmacokinetics & Metabolism Division, <sup>2</sup>Medicinal & Process Chemistry Division; CSIR-Central Drug Research Institute, Lucknow-226001, India; <sup>3</sup>NIPER, Pharmaceuticals, Raebareli-229010, India  
Received 2 March 2012; Accepted 14 March 2012

**Keywords:** Arylpiperazine, Benign prostatic hyperplasia, Hypothalamus, Pharmacokinetics, Prostate, SARM

**Abstract: Purpose.** This study was performed to study the pharmacokinetics and tissue uptake of three arylpiperazine derived SARMs, 1-(4-Nitro-2-trifluoromethyl-phenyl)-4-pyridin-2-yl-piperazine (**I**), 1-(4-Nitro-2-trifluoromethyl-phenyl)-4-(3-trifluoromethyl-phenyl)-piperazine (**II**) and 1-(4-Fluoro-phenyl)-4-(4-nitro-2-trifluoromethyl-phenyl)-piperazine (**III**) which have also shown better activity profile (reduced prostate weight) than the standard drug flutamide after 10 mg/kg oral administration in rats. **Methods.** Each compound was administered orally at a dose of 10 mg/kg in male Sprague Dawley rats and the concentration in biosamples were determined using HPLC-UV assay. **Results.** The compounds were quickly absorbed, distributed and eliminated from the serum with an MRT of ~7 h after dose. Their concentrations in prostate were higher than those in blood and hypothalamus. The clearance was smaller than the hepatic blood flow of the rat, suggesting an insignificant amount of extrahepatic elimination of the compounds. Moreover, the most promising compound (**I**) showed high stability in rat serum, SGF and SIF, and was not excreted through the urine and the fecal excretion was very low (~4%). **Conclusions.** These results indicated that the compounds have good absorption, negligible extrahepatic elimination and naturally target the prostate. Moreover, **I** which showed lower hypothalamus levels and better activity profile (reduced prostate weight) than **II** and **III** (13) indicating that **I** might be a promising candidate drug for the management of BPH.

## 1. Introduction

Benign prostatic hyperplasia (BPH) is the non-malignant enlargement of the prostate and one of the most common diseases in men older than 50 yr and is characterized by both obstructive and irritative components caused by the enlarged

prostate (1,2). The diagnostic evaluation and treatment choices for patients with moderate to severe symptoms of BPH are recommended by the American Urological Association (AUA) (3). However, the clinical application of the steroidal AR ligands has been limited by poor oral bioavailability, potential hepatotoxicity, lack of tissue selectivity, and occasionally, cross reaction with other steroid receptors (4,5). The discovery of nonsteroidal androgens not only provides an

Corresponding Author\*

Tel.: +91-522-2612411-18, Ext. 4406; Fax: +91-522-2623938/2623405; E-mail: [jlalcdri@rediffmail.com](mailto:jlalcdri@rediffmail.com)

opportunity to identify agents with superior therapeutic index and pharmacokinetic profiles to steroidal androgens but also implicates the possibility to obtain tissue-selective AR modulators (SARMs) (6-9). As a partial agonist in the prostate, SARMs may provide a completely new approach for androgen suppression in BPH treatment, with fewer side-effects.

Arylpiperazine derivatives have been reported as potent nonsteroidal AR antagonists (10,11) and some of them have exhibited better activity profile than bicalutamide both in vitro and in the testosterone propionate treated castrated rat model (12). Thus, aryl/heteroaryl/aralkyl/aroyl piperazine derivatives were synthesized as SARMs that can achieve AR blockade without causing increased testosterone levels. Of these, three compounds viz. 1-(4-Nitro-2-trifluoromethyl-phenyl)-4-pyridin-2-yl-piperazine (**I**), 1-(4-Nitro-2-trifluoromethyl-phenyl)-4-(3-trifluoromethyl-phenyl)-piperazine (**II**) and 1-(4-Fluoro-phenyl)-4-(4-nitro-2-trifluoromethyl-phenyl)-piperazine (**III**) (Figure 1), have shown better activity profile (reduced prostate weight by 47%, 43%, and 39%, respectively) than the standard drug flutamide (24% reduction) (13). We have previously reported that **I** at 10 mg/kg oral dose exhibited good absorption, negligible extrahepatic elimination, and rapid distribution to the target organ (prostate) but restricted entry through the blood-brain barrier. This communication reports the pharmacokinetics and their uptake by prostate (target tissues for main effects) and hypothalamus (target tissues for side effects) of **II** and **III** and excretion and stability studies of most promising compound **I** after single 10 mg/kg oral administration in order to develop a potential candidate drug for BPH management.

## 2. Materials and Methods

### 2.1. Materials

Compounds **I**, **II** and **III** were synthesized in our laboratories. The purities of these compounds were greater than 99%, as determined by high performance liquid chromatography (HPLC). HPLC grade *n*-hexane, analytical grade ammonium acetate and glacial acetic acid were obtained from E Merck Limited (Mumbai, India). Ultra pure water was from a Millipore MilliQ water purification system.

Drug-free whole blood and tissues samples were collected from adult, healthy male Sprague Dawley rats provided by Laboratory Animal Services Division of the Institute and pooled to generate drug-free pool of the biosamples for use as control.

### 2.2. Animals

Male Sprague Dawley rats weighing  $250 \pm 25$  g were housed under a 12-h light/dark cycle in a room with controlled temperature and humidity. Food and water were provided *ad libitum*. All experiments, euthanasia and disposal of carcasses were carried out as per the guidelines of Local Ethics Committee for animal experimentation.

### 2.3. Pharmacokinetic and tissues uptake studies

In all the studies mentioned below the dose was administered after overnight fasting (12-16 h) in male Sprague Dawley rats (n=3 per time point). Suspension formulation of **I**, **II** and **III** was separately prepared by triturating the compounds with gum acacia and water in a pestle with mortar, and a single oral dose of 10 mg/kg was given to conscious rats by oral gavage. Rodent food and water were provided 2 h after the dose. The animals were sacrificed at various times up to 24 h after drug administration, and blood, prostate,

and hypothalamus were collected. Serum samples were also harvested. The hypothalamus of three rats were pooled and homogenized in order to ensure a measurable quantity. All samples were stored at -20°C until analysis.

#### **2.4. Excretion studies of compound I in rats**

The rats were given a single 10 mg/kg of **I** by the oral route and individually kept in modified Ballman cages for sample collection. Rodent food and water were provided 2 h after the dose. Urine and feces were collected at 0, 24, 48 and 72 h. The volume of urine was noted and the samples were centrifuged at 1000g for 10 min at 4°C to remove the debris and were stored at -20°C pending analysis. Feces samples were dried in vacuum desiccator, weighed, powdered and stored in desiccator. The concentration of **I** was determined by HPLC and cumulative amount of compound excreted was calculated in urine and feces samples.

#### **2.5. Stability studies of compound I in serum, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)**

Drug-free rat serum was preincubated in a water bath incubator at 37°C for 10 min and then spiked with **I** to obtain a concentration of 0.1, 1 and 10 µg/ml. Spiked serum was distributed into 24 glass extraction tubes having 500 µl each and incubated at 37°C and 50 oscillations/min. Three tubes (n=3 for each time point) were taken at 0, 0.5, 1.0, 1.5 and 2.0 h post incubation and the concentration was determined by HPLC. Pre-incubated SGF (without pepsin) and SIF (without pancreatin) were spiked individually with appropriate aliquot of formulation to obtain a concentration of 365 µg/ml and 100 µg/ml, respectively, and immediately subjected to incubation. These will be the resultant concentration of **I** in SGF and SIF for 60 kg human corresponding to 10

mg/kg rat's oral dose. Aliquots (100 µl) of the incubation mixture were sampled at 0, 5 min, 0.25, 0.5, 0.75, 1.0 and 2 h post incubation and the concentration of **I** was determined by HPLC. The initial concentration was considered as 100% and the remaining concentration versus time profiles was plotted to determine the degradation rate constants.

#### **2.6. Analysis of the compound in serum, tissues, urine and fecal samples**

##### **2.6.1. Serum and urine**

Aliquots of blank, spiked or test serum or urine samples (0.5 ml) taken in 10 ml test tube was added 2 × 3.5 ml n-hexane, vortex-mixed for 1 min and centrifuged at 1000g for 10 min. The supernatant was transferred to a 10 ml conical tube by snap freezing the aqueous layer in liquid nitrogen and evaporated to dryness in a Savant speed vac concentrator. The residue was reconstituted in 0.2 ml of mobile phase, centrifuged and the resulting clear solution was injected into the HPLC system. The external standard method was used for quantification. Test samples were analyzed along with calibration standards and QC samples prepared in the same biomatrix.

##### **2.6.2. Tissues**

For the analysis of the compounds in tissues samples, the sample preparation method was modified. The tissues were chopped into small pieces. Ten-volumes of physiological saline were added to the chopped tissues samples and were homogenized using tissue tearer. Tissue homogenate sample was spiked with the compound solution (25 µl/ml) to prepare calibration standards and QC samples. Aliquots of blank, spiked or test homogenate samples (0.5 ml) was extracted with n-hexane and assayed as described above.

##### **2.6.3. Analysis of feces samples**

A modified sample preparation method was used for the analysis of **I** in feces samples. Calibration standards of the compound were prepared by individually spiking powdered rat feces. For spiking, less than or equal to 2.5% of the spiking solution was used. Powdered 50 mg feces samples were transferred in a test tube and wetted with water (50  $\mu$ l) by gently tapping and then extracted with n-hexane and assayed as described above.

### 2.7. HPLC analysis

The concentration of the compounds was measured by HPLC as described previously (13) with modification. The HPLC system consisted of a pump (LC-10AT VP with FCV-10AL VP, Shimadzu), degasser (DGU-14A, Shimadzu) and auto-injector (SIL-HTc, fixed with a 100 $\mu$ l loop). Chromatographic separations was achieved on a Phenomenex C-18 column (5  $\mu$ m, 150 x 4.6 mm id) preceded with a guard column (5  $\mu$ m, 30 x 4.6 mm, id) packed with the same material under isocratic condition at a flow rate of 1 ml/min. Eluents were monitored at 260 nm with UV-Vis multiple wavelength detector (Shimadzu, Japan) and chromatograms were integrated using Class-VP (version 6.12 SP5) software. The mobile phase was prepared by mixing acetonitrile and 0.01 M ammonium acetate buffer (65:35, v/v) and mixed well, degassed for 15 min before use. The chromatography was performed at ambient temperature.

### 2.8. Pharmacokinetic calculations and statistics

The peak concentration ( $C_{max}$ ) and its time of occurrence ( $t_{max}$ ) are the observed values. The data sets were individually subjected to non-compartmental analysis using the WinNonlin program (version 1.5) to obtain pharmacokinetic parameters. The area under the serum concentration-

time curve from time zero to the time of final measurable sample ( $AUC_{0-t}$ ) was calculated using the linear trapezoidal method (14).

The results of tissue distribution of the compounds were expressed as ng/g tissue, calculated from  $C_t \text{ (ng/g tissue)} = C_s V_s / W$ , where  $C_t$  is the tissue concentration (ng/g),  $C_s$  is the supernatant concentration,  $V_s$  is the supernatant volume, and  $W$  is the weight of the tissue sample. Mean tissue concentration versus time curves were obtained, and pharmacokinetics parameters were determined, using the same equations described for serum.

Data are presented as the mean  $\pm$  SEM. Statistical analyses of the parameters were performed by one-way ANOVA analysis using Kaleidagraph software (Synergy Software, USA) and  $P < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Assay validation

The analysis method was proved to be specific and repeatable with reasonable detection sensitivity for plasma, tissue, urine and feces samples tested. With the HPLC conditions, the region was found to be free from endogenous impurities and the peak heights were linear with the concentrations. For serum and urine samples, the extraction recovery were always >95% of the QC samples, the lower limit of quantification was 10 ng/ml, and intra-day and inter-day variability were <15% (Table 1). For tissue and feces samples, the extraction recovery were always >90% with variations well within internationally recognized acceptance criteria for assay validations (15).

### 3.2. Pharmacokinetics and tissues uptake

The animals tolerated the treatment as no peculiarities in the animals' behaviour were observed. The time-dependent

concentrations of the compounds are illustrated in Figure 2. A non-compartmental model analysis was applied to calculate the pharmacokinetic parameters using WinNonlin (version 1.5) software and the calculated values are listed in Table 2. For comparison, the concentration-time profile and pharmacokinetic parameters of **I** is also shown. Compound **I** exhibited a double-peak profile and was detected up to 24 h. The first  $C_{\max}$  was observed at 0.5 h, and the second  $C_{\max}$  was at 2 h post dose. The  $AUC_{0-t}$ , mean residence time ( $MRT_{0-t}$ ) and clearance ( $Cl_{\text{oral}}$ ) were 712 ng.h/ml, 6.97 h and 0.01 L/h/kg, respectively. The elimination half-life ( $t_{1/2}$ ) and  $AUC_{0-\infty}$  could not be determined due to inability to define terminal elimination phase. However, the prostate levels achieved the  $C_{\max}$  which occurred after 1.5 and 6.0 h and the  $C_{\max}$  in hypothalamus occurred after 1.0 and 2.0 h post dose. Prostate-to-serum and hypothalamus-to-serum ratios for **I** were 7.94 and 2.81, respectively. The compounds **II** and **III** showed one peak serum concentration at 1 h and 2 h and the levels were detected up to 8 h and 24 h post dose, respectively. The serum  $C_{\max}$  of **I**, **II** and **III** showed significant variations ( $P < 0.05$ ). However, both the serum and prostate  $AUC_{0-t}$  of **I** and **II** showed insignificant variations ( $P > 0.05$ ). The  $MRT_{0-t}$  of **II** was lower than that of **I** and **III**. The serum clearance for **I** and **II** were similar but lower for **III**. However, the prostate and hypothalamus levels for **II** and **III** were higher than that of **I**. Although the serum-to-prostate ratio for **II** was lower than that of **I** but the prostate and hypothalamus levels for **II** and **III** were higher than that of **I** (Table 2).

### 3.3. Excretion of **I** in rat feces

In urine, no unchanged **I** was detected after 10 mg/kg oral dose. The unchanged **I** could be monitored up to 72 h post dose in feces. Cumulative amount of **I** excreted in feces versus collection time is shown in

Figure 3 and varied between 103.58 to 158.31  $\mu\text{g}$ .

### 3.4. Stability studies

Concentrations of **I** in serum, SGF and SIF were almost constant for up to 2 h of incubation (data not shown) indicating that the compound was stable in serum and at both acidic (SGF, pH 1.2) and slightly alkaline (SIF, pH 6.8) conditions.

### Discussion

The HPLC procedure developed in our laboratory was used to quantify the concentration of the compounds **I**, **II** and **III** in the biosamples (13) with minor modification. The procedure was validated before use and the recoveries of the analytes from the biosamples were  $>90\%$  with an LOQ of 10 ng/ml in rat serum, tissues, urine and feces with variations within the acceptable limit of variation (15). Compound **I** showed high stability in rat serum, SGF and SIF (16).

Following oral administration, **II** showed single peak in serum and was observed only up to 8 h whereas tissues exhibited double peak and were detected up to 24 h. Compound **III** exhibited single peak in serum and prostate but double peak in hypothalamus. However, **I** showed double peak in serum, prostate and hypothalamus. The double peak phenomenon has been widely observed with various classes of drugs (17-23). Possible mechanisms include variable absorption rates along the gastro-intestinal tract (24), storage and subsequent release from a post absorptive depot site (possibly liver parenchyma cells) (25). Although, additional studies are required to characterize the exact mechanism responsible for the double peak phenomenon, it may be the result of the poor aqueous solubility that might have resulted in the precipitation of the drug in the intestine where the slow dissolution of the compound in the

absorptive regions could have led to the discontinuous profile but not due to the enterohepatic recirculation as the second peak in the concentration-time profile was the larger than the first peaks (26). The systemic clearance values were similar and were smaller than the hepatic blood flow of the rat (2.9 L/h/kg; (27)), suggesting an insignificant amount of extrahepatic elimination of this compound (Tables 2). The  $C_{max}$  and  $AUC_{0-t}$  as a measure of bioavailability, MRT (a practical function similar to half-life) as a measure of time required to eliminate 63.2% of the administered dose are all variable.

The target organ is prostate, where it binds and exerts its activity, i.e., reduction of the prostate. All the three compounds are reaching the target organ but were also detected in hypothalamus (target tissues for side effects). The prostate-to-serum (P/S) ratios of **II** and **III** were higher than that of **I**. Although the hypothalamus-to-serum (H/S) of **I** and **III** were comparable but the hypothalamus levels of **III** were higher than that of **I**. Due to obvious reasons, a high hypothalamus levels is undesirable. **I**, which has shown higher reduction in prostate weight, exhibited the lowest hypothalamus levels than **II** and **III**. However, since some amount of **I** crosses the blood-brain barrier, its interference with the negative feedback mechanism resulted in increased serum testosterone levels during 0.5-6.0 h post dose (13). Similar rise in serum testosterone levels has been reported for bicalutamide (12). Moreover, the flip-flop serum testosterone levels correspond to the levels of **I** in serum, prostate, and hypothalamus. Nevertheless, its significantly higher levels of **I** in prostate than in brain (hypothalamus) kept the prostate weight suppressed (13).

With a view to develop the most active compound **I** as a candidate drug for BPH management, its stability and excretion in rats was also studied. It displayed high stability in rat serum and both acidic (SGF, pH 1.2) and slightly alkaline (SIF, pH 6.8) conditions. Unchanged **I** was not excreted through the urine and the fecal excretion was very low (~4%).

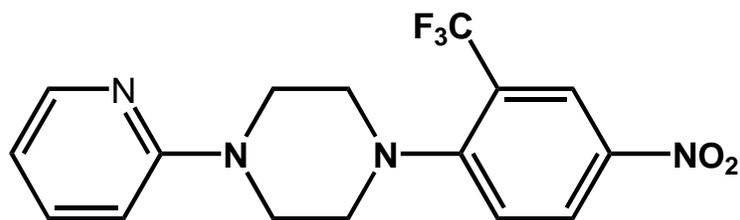
## Conclusion

Preclinical pharmacokinetics studies play a critical role in lead identification and optimization in the early drug discovery process. This study is the first preclinical pharmacokinetic evaluation of the three arylpiperazine derived SARMs (compounds **I**, **II** and **III**) for BPH management. These novel compounds were well absorbed, rapidly distributed to the target organ (prostate) and to the hypothalamus (the target organ for major side effect) and have the clearance smaller than the hepatic blood flow of the rat. Of these, **I**, having a high degree of efficacy and potency in animal models, high stability in rat serum, SGF and SIF, and acceptable pharmacokinetics in preclinical species, is a promising drug candidate SARM for clinical development for BPH management.

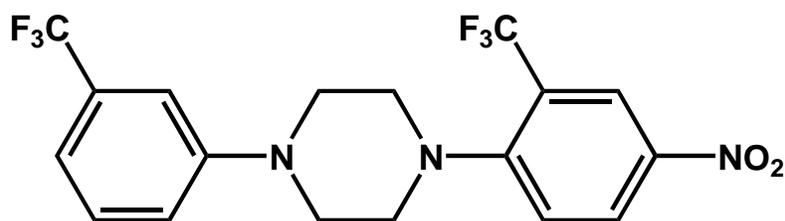
## Acknowledgments

The authors gratefully acknowledge the Director, CSIR-Central Drug Research Institute, Lucknow for providing facilities and infrastructure for the study. The financial assistance extended by the Council of Scientific and Industrial Research, India, to one of the authors (A.S.) is gratefully acknowledged. This paper is based on CDRI Communication no. 8216.

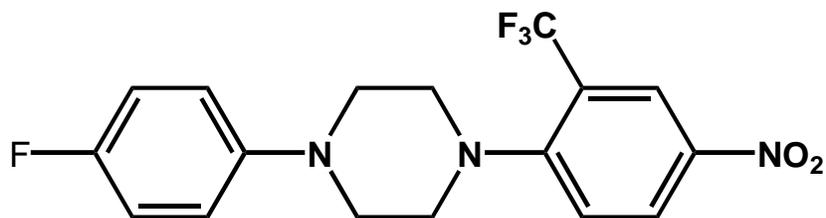
Figure 1. Chemical structure of compounds I, II and III



Compound I

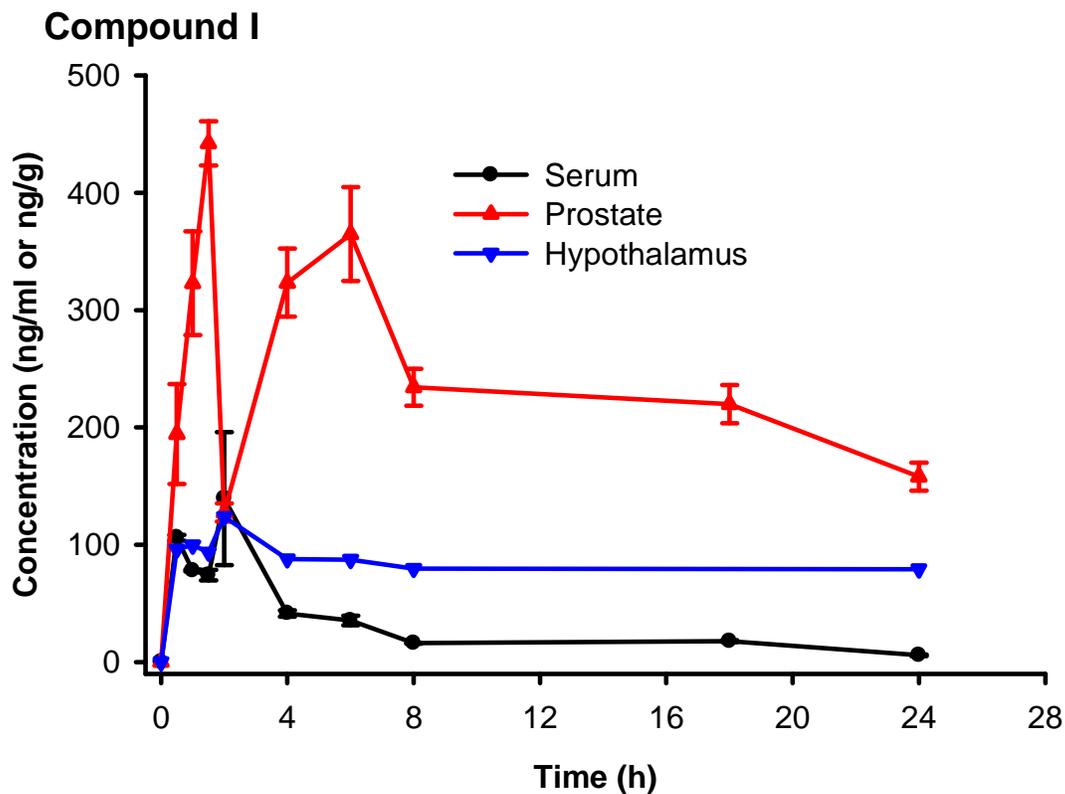


Compound II



Compound III

Figure 2. Concentration-time profile of compounds **I**, **II** and **III** in serum, prostate and hypothalamus after single 10 mg/kg oral dose in male Sprague Dawley rats. Bar represents SEM



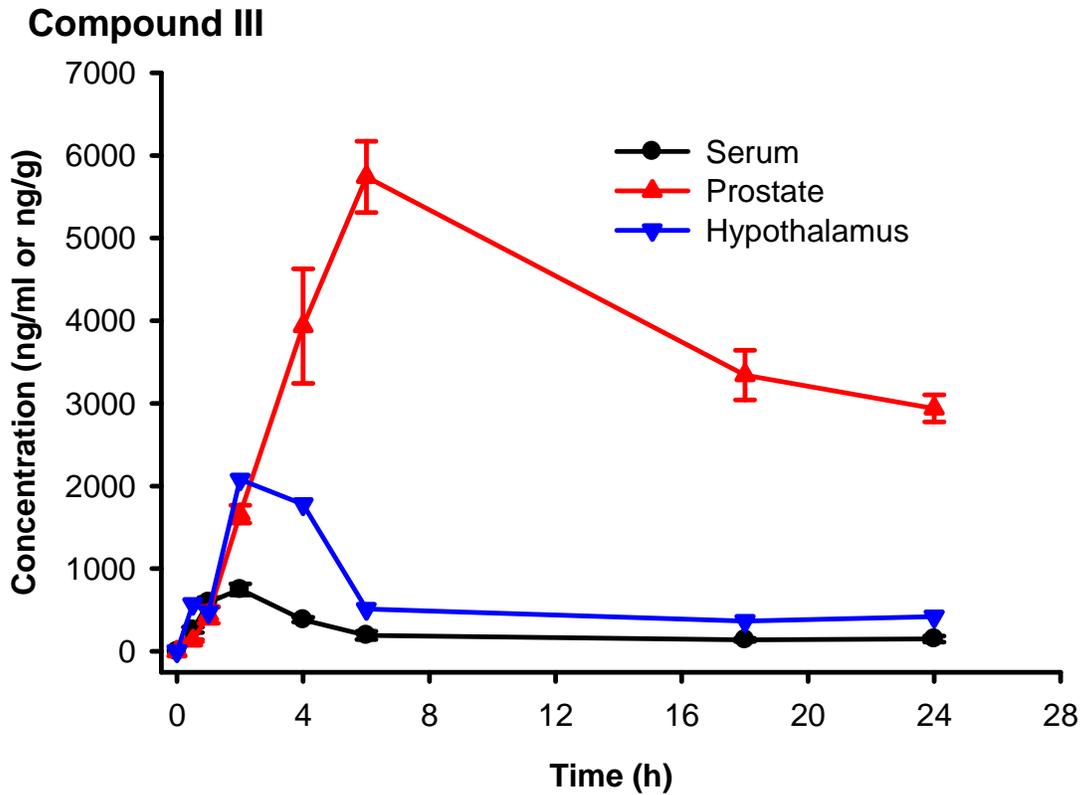
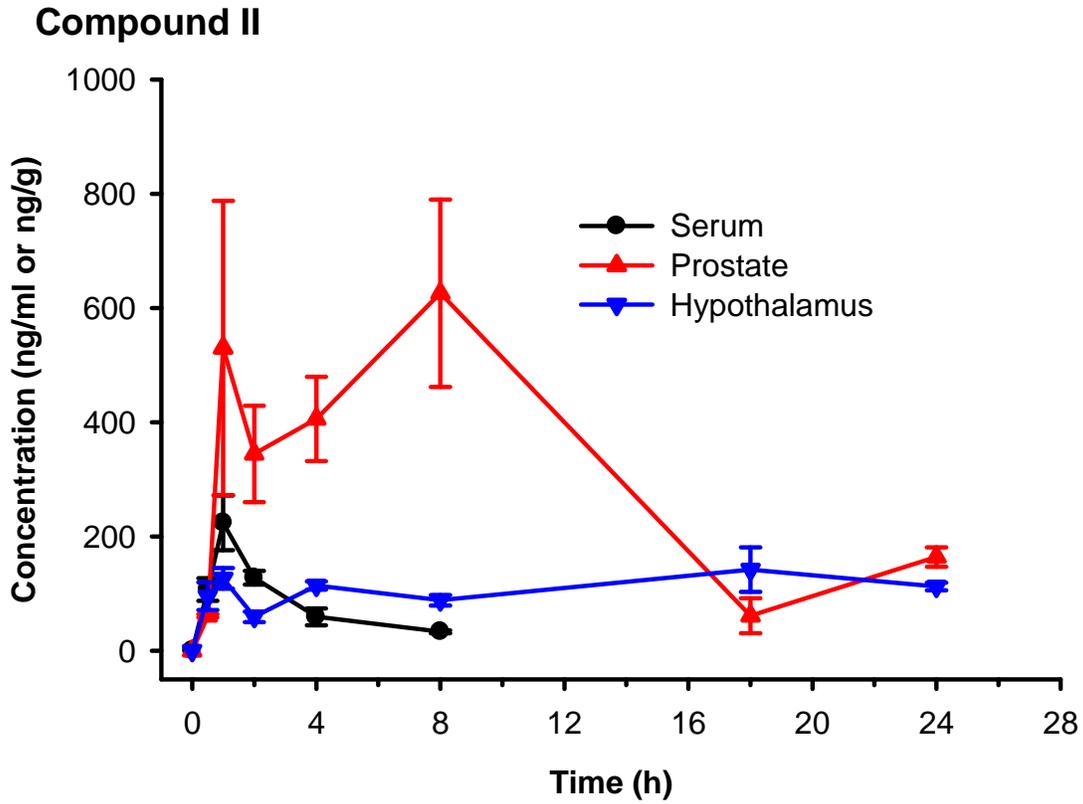


Figure 3. Cumulative amount of compound I excreted in feces after 10 mg/kg oral dose in male Sprague Dawley rats (n=3). Bar represents SEM

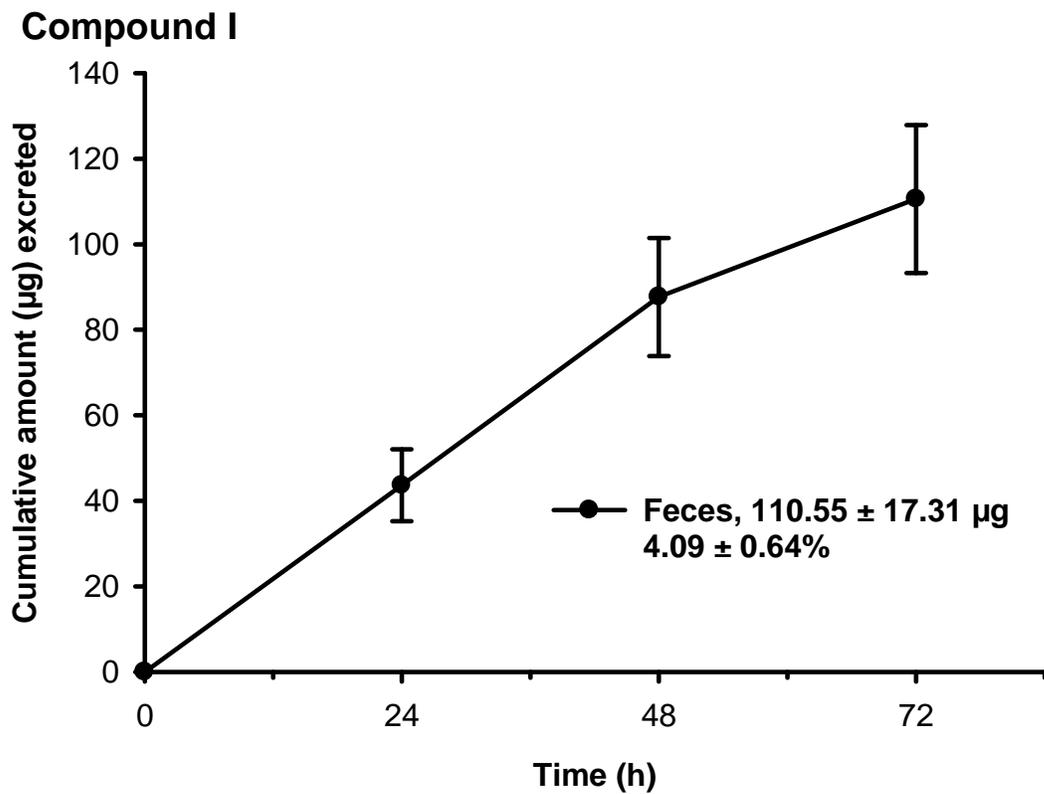


Table 1. Intra- and inter-assay accuracy (%bias) and precision (%RSD) of compounds I, II and III in rat serum

Concentration (ng/ml)	Compound I			Compound II			Compound III		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Theoretical	25 ng/ml	100 ng/ml	1000 ng/ml	25 ng/ml	100 ng/ml	1000 ng/ml	25 ng/ml	100 ng/ml	1000 ng/ml
Observed (Mean $\pm$ SD)	23.83 $\pm$ 1.71	103.81 $\pm$ 5.82	995.57 $\pm$ 29.75	25.4 $\pm$ 3.4	103 $\pm$ 12.37	973.9 $\pm$ 85.8	22.77 $\pm$ 3.80	96.73 $\pm$ 11.92	974.73 $\pm$ 86.87
%Bias <sub>intra-assay</sub>	-2.61	3.89	-1.16	3.1	0.12	- 3.25	- 10.83	-10.86	-7.88
%Bias <sub>inter-assay</sub>	-3.32	2.32	-1.88	-8.29	-0.05	-2.16	-8.58	-1.96	-1.71
RSD <sub>intra-assay</sub> (%)	6.98	4.47	1.91	2.78	1.36	1.54	6.63	6.01	1.26
RSD <sub>inter-assay</sub> (%)	7.68	7.76	4.97	13.54	13.64	8.81	8.56	12.05	8.91

Table 2. Pharmacokinetic parameters of compounds I, II and III after single oral administration of 10 mg/kg in male Sprague Dawley rats<sup>a</sup>

Parameters		Compounds I			Compounds II			Compounds III		
		Serum	Prostate	Hypothalamus	Serum	Prostate	Hypothalamus	Serum	Prostate	Hypothalamus
C <sub>max</sub> (ng/ml or ng/g)	1	106.01 ± 2.47	442.19 ± 18.82	99.47	239.89 ± 48.05*	530.15 ± 257.49	113.88	749.16 ± 67.38*	5740.2 ± 431.00*	2077.43
	2	139.31 ± 2.47	364.73 ± 39.94	123.82	-	625.95 ± 163.96	141.99	-	-	123.82
t <sub>max</sub> (h)	1	0.5	1.5	1.0	1.0	1.0	4.0	2.0	8.0	1.0
	2	2.0	6.0	2.0	-	8.0	18.0	-	-	2.0
AUC <sub>0-t</sub> (ng h/ml or ng h/g)		712	5653	1999	657	7525	2665	5546*	89830*	15475
T/S ratio		1.00	7.94	2.81	1.00	11.45	4.06	1.00	16.20	2.79
MRT (h)		6.97	10.98	11.57	2.76*	8.23	13.59	8.59	11.48	8.55
CL <sub>oral</sub> (L/h/kg)		0.010	0.001	0.001	0.010	0.001	0.004	0.0012	0.0001	0.0004

<sup>a</sup>Each value represents the average of three rats dosed orally at 10 mg/kg. Serum and prostate concentrations are mean ± SEM from three rats. Hypothalamus concentrations were the mean of replicates from the pooled tissue of three rats. \*Significant difference ( $P < 0.05$ )

## References

- [1] S.J. Berry, D.S. Coffey, P.C. Walsh, L.L. Ewing. The development of human benign prostatic hyperplasia with age. *J. Urol.*, **1984**, 132, 474-479.
- [2] P.C. Walsh. Benign prostatic hyperplasia: Benign prostatic hyperplasia, In: P.C. Walsh, A.B. Retin, T.A. Stamey, Vaughan, E.D. (eds). *Campbell's Urology*. WB Saunders, Philadelphia, **1992**, pp 1007.
- [3] American Urological Association Guideline on Management of Benign Prostatic Hyperplasia. Chapter 1: Guideline on the Management of Benign Prostatic Hyperplasia (BPH), 2010. Available at: [http://www.auanet.org/content/guidelines-and-quality-care/clinical-guidelines/main-reports/bph-management/chap\\_1\\_GuidelineManagementof\(BPH\).pdf](http://www.auanet.org/content/guidelines-and-quality-care/clinical-guidelines/main-reports/bph-management/chap_1_GuidelineManagementof(BPH).pdf)
- [4] S. Basaria, A.S. Dobs, Risks versus benefits of testosterone therapy in elderly men. *Drugs Aging*, **1999**, 15, 131-142.
- [5] E. Nieschlag. Testosterone treatment comes of age: new options for hypogonadal men. *Clin. Endocrinol.*, **2006**, 65, 275-281.
- [6] A. Schmidt, D.B. Kimmel, C. Bai, A. Scafonas, S. Rutledge, R.L. Vogel, S. McElwee-Witmer, F. Chen, P.V. Nantermet, V. Kasparcova, C.T. Leu, H.Z. Zhang, M.E. Duggan, M.A. Gentile, P. Hodor, B. Pennypacker, P. Masarachia, E.E. Opas, S.A. Adamski, T.E. Cusick, J. Wang, H.J. Mitchell, Y. Kim, T. Prueksaritanont, J.J. Perkins, R.S. Meissner, G.D. Hartman, L.P. Freedman, S. Harada, W.J. Ray, Discovery of the selective androgen receptor modulator MK-0773 using a rational development strategy based on differential transcriptional requirements for androgenic anabolism versus reproductive physiology. *J. Biol. Chem.*, **1999**, 285, 17054-7064.
- [7] A. Negro-Vilar, Selective androgen receptor modulators (SARMs): a novel approach to androgen therapy for the new millennium. *J. Clin. Endocrinol. Metab.*, **1999**, 84, 3459-3462.
- [8] S. Segal, R. Narayanan, J.T. Dalton, Therapeutic potential of the SARMs: revisiting the androgen receptor for drug discovery. *Expert Opin. Investig. Drugs*, **2006**, 15: 377-387.
- [9] W. Gao, J.T. Dalton, Expanding the therapeutic use of androgens via selective androgen receptor modulators (SARMs). *Drug Discov. Today*, **2007**, 12, 241-248.

- [10] I. Kinoyama, N. Taniguchi, T. Yoden, H. Koutoku, T. Furutani, M. Kudoh, M. Okada, Synthesis and pharmacological evaluation of novel arylpiperazine derivatives as nonsteroidal androgen receptor antagonists. *Chem. Pharm. Bull.*, **2004**, 52, 1330-1333.
- [11] I. Kinoyama, N. Taniguchi, E. Kawaminami, E. Nozawa, H. Koutoku, T. Furutani, M. Kudoh, M. Okada, N-Arylpiperazine-1-carboxamide Derivatives: A novel series of orally active nonsteroidal androgen receptor antagonists. *Chem. Pharm. Bull.*, **2005**, 53, 402-409.
- [12] I. Kinoyama, N. Taniguchi, A. Toyoshima, E. Nozawa, T. Kamikubo, M. Imamura, A. Matsuhisa, K. Samizu, E. Kawanimani, T. Niimi, N. Hamada, H. Koutoku, T. Furutani, M. Kudoh, M. Okada, M. Ohta, S. Tsukamoto, (+)-(2R,5S)-4-[4-Cyano-3-(trifluoromethyl)-phenyl]-2,5-dimethyl-N-[6-(trifluoromethyl) pyridin-3-yl] piperazine-1-carboxamide (YM580) as an orally potent and peripherally selective nonsteroidal androgen receptor antagonist. *J. Med. Chem.*, **2006**, 49, 716-726.
- [13] A. Sarswat, R. Kumar, L. Kumar, N. Lal, S. Sharma, Y.S. Prabhakar, S.K. Pandey, J. Lal, V. Verma, A. Jain, J.P. Maikhuri, D. Dalela, G. Gupta, V.L. Sharma, Arylpiperazines for management of benign prostatic hyperplasia: Design, synthesis, quantitative structure-activity relationships, and pharmacokinetic studies. *J. Med. Chem.*, **2011**, 54, 302-311.
- [14] M. Gibaldi, D. Perrier, *Pharmacokinetics*. Marcel Dekker, Inc., New York, 1982.
- [15] US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine. Guidance for Industry: Bioanalytical Method Validation, May 2001. Available at: <http://www.fda.gov/cder/guidance/index.htm>
- [16] L. Di, E.H., Kerns, Y. Hong, H. Chen, Development and application of high throughput plasma stability assay for drug discovery. *Int. J. Pharmaceut.*, **2005**, 297, 110-119.
- [17] R.F. Bergstrom, D.R. Kay, T.M. Harkcom, J.G. Wagner, Penicillamine kinetics in normal subjects. *Clin. Pharmacol. Ther.*, **1981**, 30, 404-413.
- [18] D.C. Garg, D.J. Weidler, F.N. Eshelman, Ranitidine bioavailability and kinetics in normal male subject. *Clin. Pharmacokinet.*, **1983**, 33, 445-452.
- [19] M.M. Hammarlund, L.K. Paalzow, B. Odland, Pharmacokinetics of furosemide in man after intravenous and oral administration. Application of moment analysis. *Eur. J. Clin. Pharmacol.*, **1987**, 26, 197-207.
- [20] R.L. Oberle, G.L. Amidon, The influence of variable gastric emptying and intestinal transit rates on the plasma level curve of cimetidine: an explanation for the double peak phenomenon. *J. Pharmacol. Biopharm.*, **1987**, 15, 529-544.
- [21] Y. Plusquellec, G. Campistron, S. Staveris, J. Barre, L. Jung, J.P. Tillement, G. Houin, A double peak phenomenon in the pharmacokinetics of veralipride after oral administration: a double-site model for drug absorption. *J. Pharmacokin. Biopharm.*, **1987**, 15, 225-239.
- [22] M. Piquette-Miller, F. Jamali, Pharmacokinetics and multiple peaking of acebutolol enantiomers in rats. *Biopharm. Drug Dispos.*, **1997**, 18, 543-556.
- [23] Y. Wang, A. Roy, L. Sun, C.E. Lau, A double peak phenomenon in the pharmacokinetics of alprazolam after oral administration. *Drug Metab. Dispos.*, **1999**, 27, 855-859.
- [24] B.P. Imbimbo, S. Daniotti, A. Vidi, D. Foschi, F. Saporiti, L. Ferrante, Discontinuous oral absorption of cimetoprium bromide, a new antispasmodic drug. *J. Pharm. Sci.*, **1986**, 75, 680-684.
- [25] J.B. Houston, D.G. Upshall, J.W. Bridges, Further studies using carbamate esters as model compounds to investigate the role of lipophilicity in the gastrointestinal absorption of foreign compounds. *J. Pharmacol. Exp. Ther.*, **1975**, 195, 67-92.
- [26] K.R. Godfrey, P.A. Arundel, Z. Dong, R. Bryant, Modelling the double peak phenomenon in pharmacokinetics. *Comput. Methods Programs Biomed.*, **2011**, 104, 62-69.
- [27] B. Davies, T. Morris, Physiological parameters in laboratory animals and humans. *Pharm. Res.*, **1993**, 10, 1093-1095.