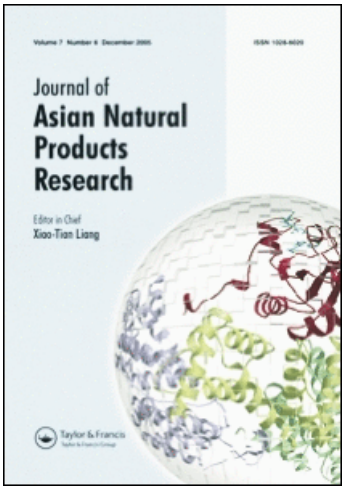


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# Effect of buagafuran on liver microsomal cytochrome P450 in rats

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## ORIGINAL ARTICLE

# Effect of buagafuran on liver microsomal cytochrome P450 in rats

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Buagafuran (BF), derived from α-agarofuran, is a promising anti-anxiety drug in phase I clinical trials. The present study was undertaken to examine the regulation of BF on liver cytochrome P450 (CYP) isoforms in rats. After being administered (4, 16, and 64 mg/kg) by gavage for 7 continuous days, the activities of CYP isoforms were measured by the qualification of six metabolites from CYP probe substrates using LC-MS/MS analysis. The mRNA and protein levels of CYPs were detected by reverse transcription polymerase chain reaction and Western blotting assay, respectively. Using phenacetin and chlorzoxazone as probe drugs, the activities of CYP1A2 and CYP2E1 were monitored *in vivo*. The result indicated that BF significantly increased the activity and protein levels of CYP1A2 and CYP2E1, while the mRNA levels were elevated to a certain extent. CYP2C6 and CYP2C11 were also slightly induced by BF, but no effect on liver CYP3A was detected in rats. Treatment of BF orally resulted in the decreasing of AUC, MRT and increasing of CL/F of phenacetin as well as production of acetaminophen in rats. The similar pharmacokinetic changes were also observed when using chlorzoxazone as a probe drug. Collectively, BF has inducing potential of liver CYP1A2 and CYP2E1 and may influence the corresponding pharmacokinetics of other drugs.

**Keywords:** buagafuran; cytochrome P450; liver; induction; CYP1A2; CYP2E1



## 1. Introduction

Anxiety disorders, with the increasing competition in modern life, have already become one of the most widespread psychiatric diseases. Although the therapeutic approaches are diverse, pharmacotherapy remains the first-line treatment [1], and the development of new anti-anxiety drugs with more efficiency and less side effects is a very active field.

Buagafuran (BF, 4-butyl-α-agarofuran, $C_{18}H_{30}O$, MW = 262.4; Figure 1, previously named as AF-5) is a synthetic derivative of agarofuran, which showed significant anti-anxiety activity in several animal models, with higher potency and lower toxicity compared with diazepam and buspirone [2]. The possible anti-anxiety mechanism of BF was related to the modulation of central monoamine neurotransmitters [3]. The preclinical pharmacokinetics studies indicated that the absorption of BF was extremely poor with an absolute bioavailability below 9.5%. The highest radioactivity of $^{3}$H-BF was found in the gastrointestinal tract, followed by the liver and kidney (unpublished results). Liver cytochrome P450

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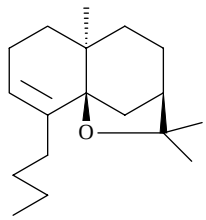


Figure 1. Chemical structure of BF.

(CYP) enzymes [4] and intestine P-glycoprotein [5] were involved in the biotransformation of BF and after multiple dosing the decrease in $AUC_{0-\infty}$ values and MTR was observed compared with single dosing, suggesting that there was an inductive effect of BF on the metabolism of itself in dogs [6]. However, there is still a lack of information about the influence of BF on CYP enzymes.

CYP is known to be a superfamily of mixed function oxidases that are responsible for the metabolism of most of the drugs, and numerous substrate drugs have been found to induce the activity and expression of CYP enzymes [7]. The induction of CYP enzymes may cause a decrease in plasma concentration of a co-administered drug that may compromise drug efficacy or enhance the formation of an active compound which results in an adverse event [8]. Therefore, the induction of CYP of an investigational new drug should be defined during the development duration.

Here, we report the results from both *ex vivo* and *in vivo* studies to evaluate the potential influence of BF on the major hepatic CYP enzymes in rats.

## 2. Results and discussion

### *2.1 Effect of BF on the activities of rat hepatic CYP enzymes*

The activities of CYP enzymes were characterized by the metabolite formation of probe drugs using LC-MS/MS analysis. As shown in Figure 2, CYP1A2 and CYP2E1 activities were significantly enhanced to 1.56- to 3.07-fold and 1.40- to 1.79-fold by BF, respectively. In addition, CYP2C6 and CYP2C11 activities were also elevated to a certain extent,

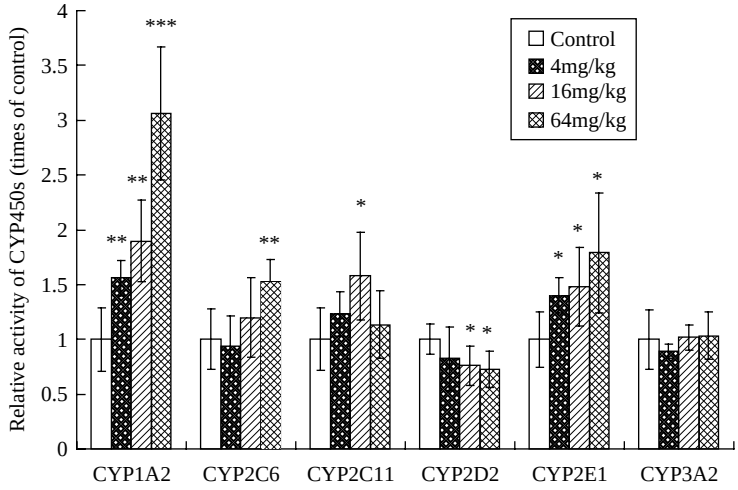


Figure 2. Effect of BF (4, 16, and 64 mg/kg × 7 days) on liver microsomal CYP1A2, CYP2C6, CYP2C11, CYP2D2, CYP2E1, and CYP3A2 activities in rats (means ± SD, $n = 5$). $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ vs. control group. The activities of CYP450s were represented by the metabolite formation of probe substrates. CYP1A2, CYP2C6, CYP2C11, CYP2D2, CYP2E1, and CYP3A2 activities in the control group were 1.06, 0.61, 2.16, 0.31, 0.72, and 0.11 nmol/min/mg protein, respectively.

whereas CYP2D2 activity was decreased by BF. No effect on CYP3A2 activity was observed. The induction of β-naphthoflavone and ethanol (as positive inducers) on CYP1A2 and CYP2E1 activities was similar as reported previously [9].

### 2.2 Effect of BF on CYP450s mRNA levels

Reverse transcription polymerase chain reaction (RT-PCR) assays were employed to characterize the specific CYP gene expression induced by BF in rats. The results showed that BF slightly increased the mRNA levels of *CYP1A2* and *CYP2E1* (Figure 3), followed by *CYP2C6* and *CYP2C11*, and no significant impact on *CYP2D2* and *CYP3A2* was observed in the rat liver (data not shown).

### 2.3 Effect of BF on CYP1A2 and CYP2E1 protein levels

The overexpression of liver CYP1A2 and CYP2E1 induced by BF was evaluated at protein levels by Western blotting. The results showed that BF elevated hepatic CYP1A2 and CYP2E1 protein levels to over 3.0- and 2.0-fold, respectively

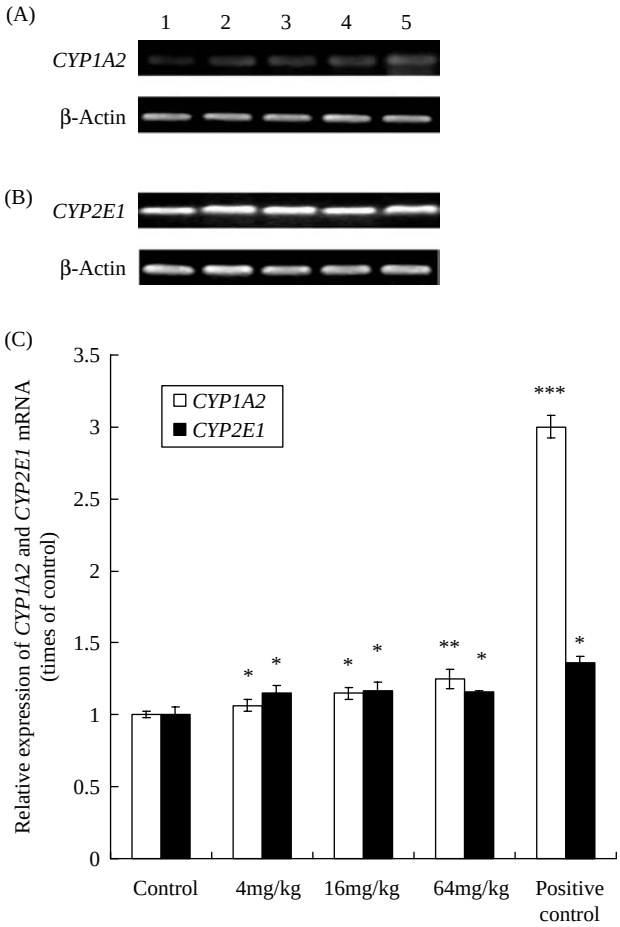


Figure 3. Effects of BF on liver *CYP1A2* and *CYP2E1* mRNA expression in rats. (A) and (B) Lanes 1, control; 2–4, BF 4, 16, and 64 mg/kg; 5, positive control (β-naphthoflavone and ethanol). (C) Ratio of PCR products relative to β-actin. Data were expressed as means ± SD ($n = 4$). $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ vs. control group.

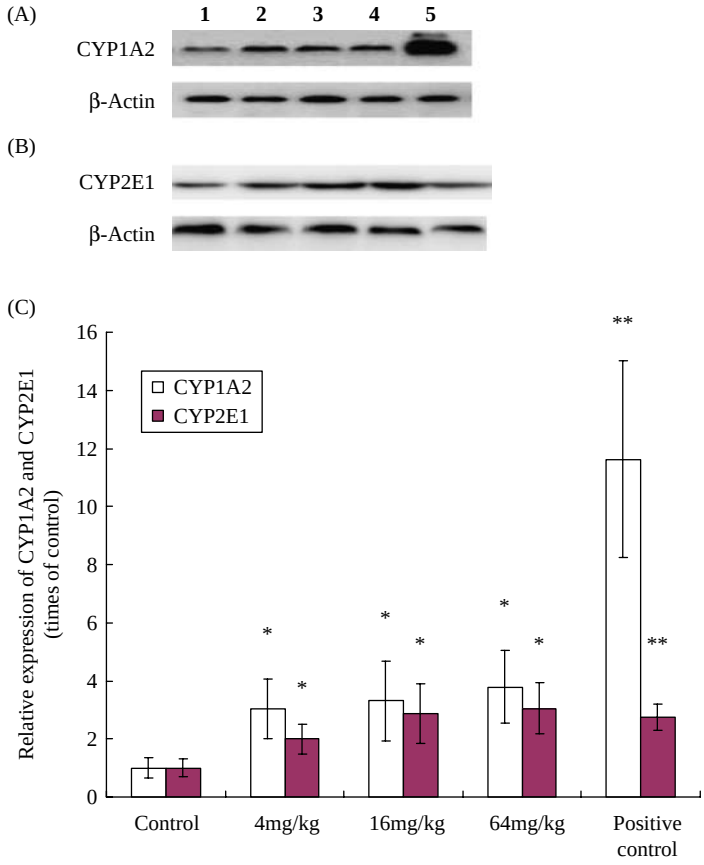


Figure 4. Effects of BF on liver CYP1A2 and CYP2E1 protein levels in rats. (A) and (B) Lanes 1, control; 2–4, BF 4, 16, and 64 mg/kg; 5, positive control (β-naphthoflavone and ethanol). (C) Ratio of CYP1A2 and CYP2E1 protein relative to β-actin. Data were expressed as means ± SD ($n = 4$). $*P < 0.05$, $**P < 0.01$ vs. control group.

(Figure 4). β-Naphthoflavone and ethanol, as the prototypical inducers, also enhanced the protein expression of CYP1A2 and CYP2E1 intensively.

### 2.4 *Effect of BF on pharmacokinetics of chlorzoxazone and phenacetin in rats* in vivo

The mean plasma concentration–time profiles of phenacetin/acetaminophen after oral administration of phenacetin (50 mg/kg) are shown in Figure 5, and the relevant pharmacokinetic parameters are listed in Table 1. After 7 daily doses of BF (16 mg/kg), the lower AUC (69%), shorter MTR (85%), and higher CL/F (147%) of phenacetin were observed. However, in BF-treated rats, the AUC and $C_{max}$ of acetaminophen were enhanced and the $T_{max}$ was shorter.

When chlorzoxazone was used as the probe drug, BF pretreatment caused the increasing clearance of chlorzoxazone and production of 6-hydroxychlorzoxazone. The relative plasma concentration–time profiles and pharmacokinetics parameters are summarized in Figure 6 and Table 2, respectively.

### 2.5 *Discussion*

CYP enzymes, located in the hepatic endoplasmic reticulum, were believed

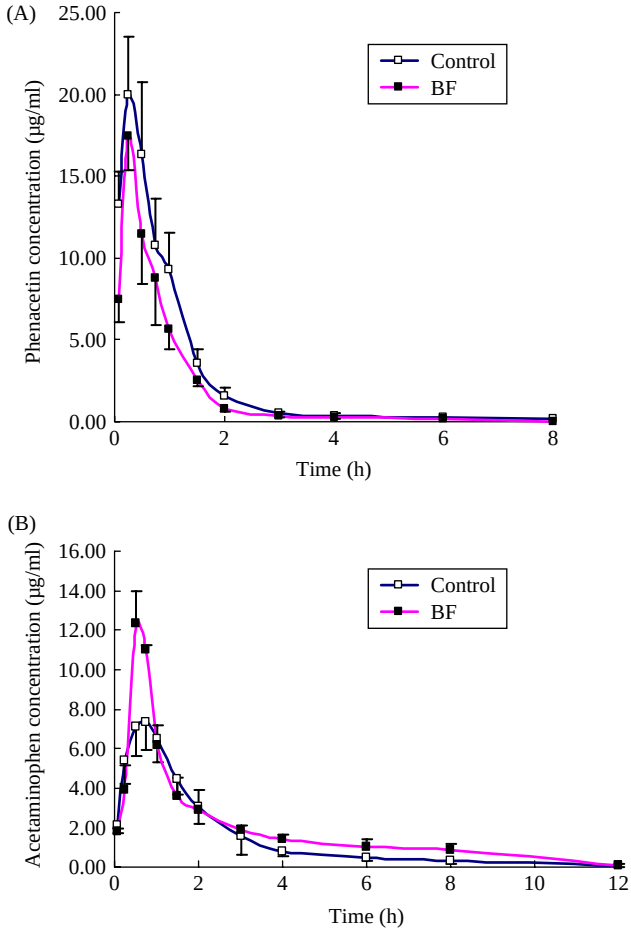


Figure 5. Mean plasma concentration–time profiles of (A) phenacetin (50 mg/kg, p.o.) and (B) acetaminophen in untreated and BF (16 mg/kg × 7 days) pretreated rats ($n = 5$). Error bars represent SD.

to cause hepatic elimination primarily. Previous studies have demonstrated that most of the CYP forms were subjected to the induction by xenobiotics [10]. The subsequent result usually appeared to decrease the exposure of the affected compound, leading to the therapeutic failure (e.g. rifampicin and oral contraceptive steroids) or toxicological implications by higher levels of undesired metabolites [11].

Generally, the anxiety patients may possess complex co-morbid conditions (including depression, alcohol or drug abuse, etc.) and receive a variety of medications besides anxiolytic. Therefore, the unpredictable induction of CYP by BF should be demonstrated to provide beneficial suggestions for both disposition studies and clinical safety. In the present study, we found that BF could induce hepatic CYP1A2 and CYP2E1, and had no intensive impact on CYP2C6, CYP2C11, CYP2D2, and CYP3A2 in rats.

CYP1A2, mainly expressed in the liver, catalyzes the biotransformation of several commonly used clinical drugs such as theophylline, caffeine, imipramine, paracetamol, and propranolol [12]. As compared to some other CYP forms, CYP2E1 has

Table 1. Pharmacokinetic parameters of phenacetin/acetaminophen in untreated and BF (16 mg/kg) pretreated rats.

| Parameter | AUC (μg/ml h) | $t_{1/2}$ (h) | $T_{max}$ (h) | MRT (h) | CL/F (l/h/kg) | $C_{max}$ (μg/ml) |
|---|---|---|---|---|---|---|
| *Phenacetin* | | | | | | |
| Control | 21.1 ± 4.25 | 2.35 ± 0.2 | 0.25 | 1.22 ± 0.10 | 2.34 ± 0.4 | 19.95 ± 3.60 |
| BF | 14.6 ± 2.67* | 2.03 ± 0.56 | 0.25 | 1.04 ± 0.15* | 3.45 ± 0.49** | 17.46 ± 2.12 |
| *Acetaminophen* | | | | | | |
| Control | 16.4 ± 2.22 | 2.25 ± 0.644 | 0.75 | 2.15 ± 0.33 | 3.01 ± 0.365 | 7.50 ± 1.34 |
| BF | 21.8 ± 2.65** | 2.23 ± 0.847 | 0.5 | 2.84 ± 0.44** | 2.06 ± 0.695** | 12.7 ± 1.34** |

Notes: Phenacetin (50 mg/kg) was given orally to rats. Plasma was collected at 0.083–24 h after administration. *$P < 0.05$ and **$P < 0.01$ vs. control group (paired-samples *t*-test, $n = 5$). AUC, area under curve; $t_{1/2}$, plasma half-life; $T_{max}$, the time of the peak concentration; MRT, mean residence time; CL/F, apparent clearance; $C_{max}$, peak concentration.

only a minor role in the metabolism of clinical therapeutic agents including diethyl ether, halothane, paracetamol, chlorzoxazone, and methoxyflurane [13]. The induction of CYP1A2 and CYP2E1, for example by smoking and alcoholism, may cause the weakening of the therapeutic effect (e.g. the enhancive requirements for theophylline among asthmatics and haloperidol among psychiatric patients) and the potentiating toxicities (leading to elevation of dichloromethane converted to carbon monoxide) [14].

The present paper showed that BF elevated the activities of CYP1A2 and CYP2E1 in a dose-dependent manner in rats. Nevertheless, by comparison of the induction level with other classical inducers (β-naphthoflavone and ethanol), BF seemed to be less potent in this regard. To explore the upregulation mechanism at transcriptional and/or post-transcriptional levels, we further investigated the changes at the mRNA and protein level of CYP enzymes following BF treatment. It was found that the elevation of BF on protein levels of two isoforms was much more significant than that on mRNA levels, which suggested that the elevation of CYP1A2 and CYP2E1 activities may originate from enhanced protein expression by post-transcription regulation.

To evaluate the biological responses caused by the induction of BF on CYP1A2 and CYP2E1, we determined the activity of CYP1A2 and CYP2E1 *in vivo* using phenacetin and chlorzoxazone as the chemical probes. After BF pretreatment, the decrease in parent drugs and increase in their major metabolites were observed in the plasma, which reflected the enhancement of hepatic elimination through CYP1A2 and CYP2E1 inductions. The impact on pharmacokinetic parameters of the probe drug was consistent with the increasing of activities and proteins of CYP1A2 and CYP2E1, which suggested that when BF is prescribed concomitant with other drugs, the above induction

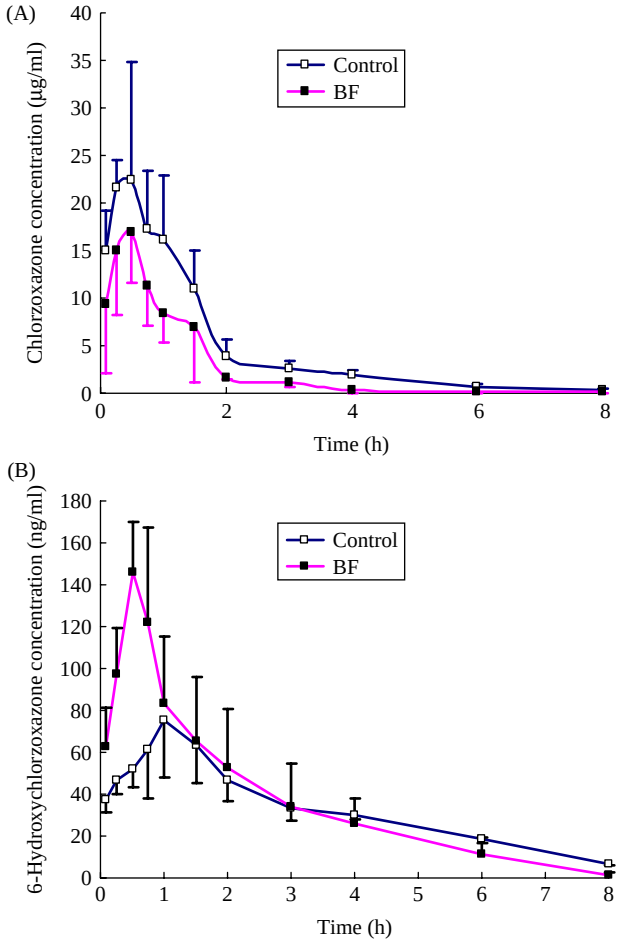


Figure 6. Mean plasma concentration–time profiles of (A) chlorzoxazone and (B) 6-hydroxychlorzoxazone after p.o. administration of chlorzoxazone (50 mg/kg) to untreated and BF (16 mg/kg × 7 days) pretreated rats ($n = 5$). Error bars represent SD.

deserves attention. Previous study showed that the AUC of chlorzoxazone and phenacetin can be decreased more than 90 and 40% by 3-methylcholanthren and alcohol treatment [15,16], which indicated that the influence of BF on CYP1A2 and CYP2E1 was much weaker than for classical inducers.

CYP3A was considered the most abundantly expressed subfamily and was involved in the breakdown of at least 120 diverse categories of medications. The induction of CYP3A plays a major role in enzyme induction-based drug interactions [17]. Moreover, human CYP2C9, CYP2C19, and CYP2D6 were involved in a variety of drug metabolisms (especially a number of psychotropic drugs, the non-steroidal anti-inflammatory drugs, the hypoglycemic agents, the proton pump inhibitors, and the anti-arrhythmics) [18]. We measured the activities of liver microsomal CYP3A2, CYP2C6, CYP2C11, and CYP2D2 in BF-treated rats. No significant change on CYP3A2 activity, mild induction on CYP2C6, CYP2C11, and suppression of CYP2D2 by BF were found in this study. The low effect of BF on CYP3A, CYP2C6, and CYP2C11 may not influence the hepatic elimination

Table 2. Pharmacokinetic parameters of chlorzoxazone/6-hydroxychlorzoxazone in untreated and BF (16 mg/kg) pretreated rats.

| Parameter | AUC | $t_{1/2}$ (h) | $T_{max}$ (h) | MRT (h) | CL/F (l/h/kg) | $C_{max}$ |
|---|---|---|---|---|---|---|
| *Chlorzoxazone* | (μg/ml h) | | | | | (μg/ml) |
| Control | 35.2 ± 5.43 | 1.94 ± 0.479 | 0.5 | 1.81 ± 0.184 | 1.48 ± 0.371 | 27.6 ± 7.95 |
| BF | 22.8 ± 4.23* | 1.50 ± 0.324 | 0.5 | 1.29 ± 0.368* | 2.42 ± 0.430* | 16.9 ± 4.33 |
| *6-Hydroxychlorzoxazone* | (ng/ml h) | | | | | (ng/ml) |
| Control | 266.9 ± 31.0 | 2.2 ± 0.169 | 1 | 2.80 ± 0.162 | 172.8 ± 20 | 80.9 ± 26.2 |
| BF | 344.2 ± 61.9* | 1.6 ± 0.37* | 0.5 | 2.0 ± 0.25*** | 284.3 ± 64** | 148.4 ± 27.7** |

Notes: Chlorzoxazone (50 mg/kg) was given orally to rats. Plasma was collected at 0.083–24 h after administration. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ vs. control group (paired-samples *t*-test, $n = 5$). For the description of terms, see Table 1.

of co-administration drugs metabolized by the above isozymes.

In summary, the present study clearly identified BF as a co-inducer of CYP1A2 and CYP2E1 on activity, mRNA and protein expression to a certain extent, leading to the decrease in plasma concentrations of the probe drug due to the induction, which suggested that BF has a potential influence on the metabolism of combination drugs. Certainly, because of the species differences of CYP subfamilies between human and rats, further investigation using human origin should be considered to validate the conclusion.

## 3. Materials and methods

### 3.1 Chemicals and reagents

BF (purity >99.8%) was synthesized and provided by the Laboratory of Chemical Synthesis (Chinese Academy of Medical Sciences). Phenacetin, *S*-mephenytoin, dextromethorphan, chlorzoxazone, midazolam, acetaminophen, 4′-hydroxymephenytoin, dextrorphan, 4′-hydroxydiclofenac, 6-hydroxychlorzoxazone, 1′-hydroxymidazolam, and β-naphthoflavone were purchased from Sigma-Aldrich (St Louis, MO, USA). Diclofenac was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Redzol® reagent and oligonucleotide primers were obtained from SBS Genetech (Beijing, China). RT-PCR kits were purchased from Takara Biotechnology Co. Ltd (Otsu, Shiga, Japan). Rabbit anti-rat β-actin antibody was the product of Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Rabbit anti-rat CYP2E1 and mouse anti-rat CYP1A2 antibodies were obtained from Abcam (Cambridge, MA, USA). All other reagents were of analytical grade or HPLC grade.

### 3.2 Animals and treatments

Male Sprague–Dawley rats weighing 220–240 g were obtained from Beijing

Table 3. CYP probe substrates, reactions, and metabolites.

| CYP | Substrate | Reaction | Metabolite |
|---|---|---|---|
| 1A2 | Phenacetin | *O*-Deethylation | Acetaminophen |
| 2C6 | Diclofenac | 4′-Hydroxylation | 4′-Hydroxydiclofenac |
| 2C11 | *S*-Mephenytoin | 4′-Hydroxylation | 4′-Hydroxymephenytoin |
| 2D2 | Dextromethorphan | *O*-Demethylation | Dextrorphan |
| 2E1 | Chlorzoxazone | 1′-Hydroxylation | 6-Hydroxychlorzoxazone |
| 3A2 | Midazolam | 6-Hydroxylation | 1′-Hydroxymidazolam |

Vital River Experimental Animal Co. Ltd, Beijing, China. The rats were housed in cages, and fed *ad libitum* at 22°C with a 12 h light/dark cycle. All experimental procedures were in compliance with the guidelines of China for animal care, which was conformed to the internationally accepted principles in the care and use of experimental animals.

Rats were given BF at 4, 16, and 64 mg/kg per day (suspended in 0.5% sodium carboxymethyl cellulose) by gavage for 7 consecutive days. Other rats received vehicle, β-naphthoflavone (80 mg/kg per day for 3 days, intraperitoneally) and 20% ethanol (5 mg/kg per day for 4 days orally) as positive controls, respectively. The animals were sacrificed at 24 h after the last administration. The liver tissues were removed rapidly for microsome preparation, and were frozen in liquid nitrogen for subsequent total RNA isolation.

### 3.3 Preparation of liver microsomes

Liver microsomes were prepared by differential centrifugation as described previously [19]. The pellet was resuspended in a homogenization medium and stored at −80°C until use. Protein concentrations of liver microsomes were determined using the Bradford method [20].

### 3.4 Measurement of CYP activities with probe substrates

Six probe substrates (phenacetin for CYP1A2, diclofenac for CYP2C6, *S*-mephenytoin for CYP2C11, dextromethorphan for CYP2D2, chlorzoxazone for CYP2E1, and midazolam for CYP3A2) were chosen to determine the activities of CYP isoforms in rat liver microsomes. The selective CYP substrates and reactions used in the method are listed in Table 3.

The incubation mixture (final volume = 0.5 ml) contained 50 mM Tris (pH = 7.4), 4 mM $MgCl_2$, and 1 mg/ml microsomal protein. The reaction was initiated by the addition of NADPH-generating system (1 mM NADP+, 1 IU glucose-6-phosphate dehydrogenase, 1.11 mM glucose-6-phosphate) and terminated by adding an equal volume of ice-chilled acetonitrile. All analyses of metabolites were performed with validated LC-MS/MS methods as previously described [21–23].

### 3.5 RT-PCR analysis

Total RNA from liver tissue was isolated with Redzol® reagent according to the manufacturer's instructions. The quality of RNA solutions was determined using an ultraviolet light spectrophotometer. A 500 ng of total RNA was used for cDNA synthesis and 10 μl of each reverse transcription product was added to 40 μl of the reaction mixture containing 10 μl of 5 × PCR buffer, 0.25 μl of 5 U/μl *Ex Taq*® DNA polymerase, 1 μl of 100 μM corresponding primers, and 27.75 μl of $ddH_2O$ for PCR amplification. The endogenous reference gene, *β-actin*, was used to measure the constitutive level of the gene and to regulate the variations in RNA recoveries from each specimen.

Table 4. Primer sequences (5′ → 3′) used in this study.

| Gene | Primer sequence | Cycle no. | Annealing temperature (°C) | Product length (bp) |
|---|---|---|---|---|
| *CYP1A2* | Sense: GTCACCTACGGGAATGCTGTG<br>Anti-sense: GTTGACAATCTTCTCCTGAGG | 26 | 58 | 236 |
| *CYP2E1* | Sense: CTCCTCGTCATATCCATCTG<br>Anti-sense: GCAGCCAATCAGAAATGTGG | 24 | 58 | 473 |
| *β-Actin* | Sense: CCACAGCTGAGAGGGAAATCG<br>Anti-sense: AGAGGTCTTTACGGATGTCAACG | 30 | 55 | 277 |

The *CYP* primer sequences are listed in Table 4.

PCR was initiated at 94°C for 2 min followed by 24–30 cycles at 94°C for 30 s, 55–59°C for 30 s, and 72°C for 30 s, and was ended with a 10 min final extension at 72°C after the last cycle. The number of cycles and annealing temperature for each primer pair were optimized, as shown in Table 4. The PCR products were separated by electrophoresis on a 1.5% agarose gel at 100 V for 45 min and the 100 bp DNA ladder was used as the molecular marker. The bands were visualized with ethidium bromide and analyzed by BandScan.

### 3.6 *Western blotting analysis*

The protein levels of CYP1A2, CYP2E1, and β-actin were determined by Western blotting. Rat liver microsomal proteins were separated on 12% (w/v) SDS-polyacrylamide gel and then transferred to Poly-Screen PVDF membranes at 80 V for 40 min in transfer buffer (20 mM Tris–base, 154 mM glycine, and 20% methanol). The membranes were blocked with 5% non-fat milk powder in buffer (200 mM NaCl, 0.05% Tween 20, and 50 mM Tris–HCl, pH 7.4) at room temperature for 2 h, incubated with primary antibodies at 4°C for 14 h, washed and incubated with secondary antibodies at room temperature for 1 h. The blots with HRP-conjugated antibodies were developed by the addition of ECL reagents (Applygen Technologies Inc., Beijing, China). The densities of bands were semi-quantified by gelpro32 software program.

### 3.7 *Pharmacokinetic study of phenacetin and chlorzoxazone* in vivo

Phenacetin or chlorzoxazone (suspended in 0.5% sodium carboxymethyl cellulose) at a dose of 50 mg/kg was administered to rats intragastrically. Blood samples (approximately 250 μl) were collected from retrobulbar plexus at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after oral dosing. Plasma was separated by centrifugation and stored at −20°C for later analysis.

The plasma sample diluted five times with blank plasma was precipitated with a double volume of acetonitrile and was centrifuged at 14,000 rpm twice. The supernatant was collected for LC-MS/MS analysis according to reported methods [24,25].

Pharmacokinetic analysis was performed using non-compartmental and compartmental methods via the proprietary Drug and Statistics computer software package (version 2.0; Anhui Provincial Center for Drug Clinical Evaluation, China).

### 3.8 *Statistical analysis*

All data were expressed as means ± SD. Statistical analysis was performed using analysis of variance and the Student–Newman–Keuls *post hoc* test. Differences between groups were considered significant at $P < 0.05$.

## References

[1] J.R. Davidson, *J. Clin. Psychiatry* **70** (Suppl 2), 25 (2009).

[2] Q. Liu, D.H. Wang, C. Li, D. Lu, W.J. Wang, and J.Y. Guo, *Chinese J. Med. Chem.* **13**, 125 (2003).
[3] Y. Zhang, W.J. Wang, and J.J. Zhang, *Eur. J. Pharmacol.* **504**, 39 (2004).
[4] N. Li, J.L. Zhang, and T.H. Zhou, *Acta Pharm. Sin.* **3**, 528 (2001).
[5] E. Li and Y. Li, *Acta Pharm. Sin.* **43**, 361 (2008).
[6] J.P. Hu, H. Chen, and Y. Li, *J. Pharm. Biomed. Anal.* **47**, 383 (2008).
[7] F.J. Gonzalez and H.V. Gelboin, *Prog. Clin. Biol. Res.* **372**, 11 (1991).
[8] J.S. Cropp and H.I. Bussey, *Pharmacotherapy* **17**, 917 (1997).
[9] Y. Deng, H. Bi, L.Z. Zhao, F. He, Y.Q. Liu, J.J. Yu, Z.M. Ou, L. Ding, X. Chen, Z.Y. Huang, M. Huang, and S.F. Zhou, *Xenobiotica* **38**, 465 (2008).
[10] F.P. Guengerich, *FASEB J.* **6**, 745 (1992).
[11] B.K. Park, N.R. Kitteringham, and M. Pirmohamed, *Br. J. Clin. Pharmacol.* **41**, 477 (1996).
[12] M. Eichelbaum and A.S. Gross, *Pharmacol. Ther.* **46**, 377 (1990).
[13] J.G. Gillum, D.S. Israel, and R.E. Polk, *Clin. Pharmacokinet.* **25**, 450 (1993).
[14] S.N. Kim, J.Y. Seo, D.W. Jung, M.Y. Lee, Y.S. Jung, and Y.C. Kim, *Drug Metab. Dispos.* **35**, 1754 (2007).
[15] C. Girre, D. Lucas, and E. Hispard, *Biochem. Pharmacol.* **47**, 1503 (1994).
[16] J.M. Pierre, J.J. Richard, and N. Jan, *J. Pharmacol. Exp. Ther.* **225**, 153 (1982).
[17] L. Bertilsson, Y.Q. Lou, and Y.L. Du, *Clin. Pharmacol.* **51**, 388 (1992).
[18] P.K. Honig, D.C. Wortham, K. Zamani, D.P. Conner, J.C. Mullin, and L.R. Cantilena, *J. Am. Med. Assoc.* **269**, 1513 (1993).
[19] M.H. Ju and Y. Li, *Acta Pharm. Sin.* **40**, 111 (2005).
[20] M.M. Bradford, *Anal. Biochem.* **72**, 248 (1976).
[21] D. Smith, N. Sadagopan, M. Zientek, A. Reddy, and L. Cohen, *J. Chromatogr. B* **850**, 455 (2007).
[22] M. Turpeinen, U. Jouko, J. Jorma, and P. Olavi, *Eur. J. Pharm. Sci.* **24**, 123 (2005).
[23] D. Vaishali, H. Niresh, D. Pankaj, and D.U. Jashvant, *Biopharm. Drug Dispos.* **28**, 257 (2007).
[24] H. Jin, S.J. Li, and W.J. Xiong, *Pharm. Care Res.* **7**, 431 (2007).
[25] Y.J. Moon, A.K. Lee, H.J. Chung, E.J. Kim, S.H. Kim, D.C. Lee, I. Lee, S.G. Kim, and M.G. Lee, *Drug Metab. Dispos.* **31**, 776 (2003).