Schisandrin B for treatment of male infertility

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Abstract

The decline of male fertility and its consequences on human populations are important public-health issues. However, there are limited choices for treatment of male infertility. In an attempt to identify a compound that could promote male fertility, we identified and characterized a library of small molecules from an ancient formulation Wuzi Yanzong-Pill, which was used as a folk medicine since the Tang dynasty of China. We found that SB enabled evident repairs in oligoasthenospermia-associated testicular tissue abnormality and in spermatogenesis disruption, resulting in significant improvements of sperm count, mobility, and reproductive ability in oligoasthenospermia mice. Furthermore, SB could alter substantial testicular genes (2033), among which, upregulation of *Fst* while downregulation of *Inhba* involved in reproductive signaling pathway could explain its role in enhancing spermatogenesis. The encouraging preclinical data with pharmacokinetics warranted a rapid

development of this new class of therapeutic agent. Our finding provides a strong potent drug for treatment of male infertility.

Introduction

Infertility is a failure to conceive despite 1 year of regular unprotected intercourse. Based on assessments by the World Health Organization (WHO) and European healthcare bodies over recent years, approximately 8–15% of couples experience infertility. Of these, in 20% of cases the man will be solely responsible; men contribute in an additional 30% of infertility cases [1,2]. The causes of male infertility vary widely, but oligoasthenospermia is a common cause [3]. Treatment choices for male infertility are limited [4–6]. The ancient formulation Wuzi Yanzong-Pill (WP) has been documented as a medication of male infertility in the book *Xuanjielu* since the Tang dynasty of China. However, its active component(s) are not known. In addition, scientific evaluation of its efficacy and mechanism of action are still lacking. Here, we find that schisandrin B (SB) from WP can be used to treat male infertility, and we further uncover its underlying mechanism of action.

Results and Discussion

Identification of schisandrin B for treatment of oligoasthenospermia

To find active components, we had detected >120 types of compounds in WP [7]. We further identified 106 major compounds, and their druggability was evaluated using MedChem Studio (SimulationsPlus, Lancaster, CA, USA) (Supplementary Dataset S1-2; Fig. 1A). Twenty-two compounds had high scores for druggability: 14 phenylpropanoids (including SB), 3 alkaloids, 3 flavonoids and 3 organic acids.

To screen a drug candidate, the relative content of these components was assessed by factor analysis using SPSS v20 (IBM, Armonk, NY, USA) (Supplementary Dataset S3; Fig. 1B). SB had the highest druggability and was then selected as a candidate.

To determine its oral availability according to site of action, the SB levels in the plasma and in testicular tissues of normal male mice were measured by ultra-performance liquid

chromatography-tandem mass spectrometry (UPLC-MS/MS). For the purpose of identification, pure SB was used as the standard reference. The $[M+H]^+$ of pure SB had a mass/charge (*m/z*) of 401.19434 ($C_{23}H_{29}O_6$) and eluted at 2.40 min. The representative fragment ions of multistage MS were displayed at *m/z* 401 $[M+H]^+$, 386 $[M+H\text{-}CH_3]^{+\cdot}$, 370 $[M+H\text{-}OCH_3]^{+\cdot}$, 331 $[M+H\text{-}C_5H_{10}]^+$, 316 $[M+H\text{-}C_5H_{10}\text{-}CH_3]^{+\cdot}$, and 300 $[M+H\text{-}OC_6H_{13}]^{+\cdot}$ (Fig. 1C–E).

Three hours after oral administration, SB was identified in the plasma (Fig. 1F, G) and testicular samples (Fig. 1H, I) of mice. SB structure in plasma and testicular samples was identified further by multistage MS as compared with that of pure SB (Supplementary Fig. S1). These results demonstrated the SB availability in plasma and testicular tissue of mice upon oral administration.

Inspired by gene-profiling studies used to screen new chemical chaperones [8–11], we investigated SB involvement in regulation of testicular gene (TG) expression by comparing it with that of WP in an established model of OM [12,13]. In mice, expression of 100 of the most upregulated and downregulated TGs (50:50) by WP was compared with the corresponding TGs regulated by SB. Heatmap analyses revealed that SB had a similar role in regulation of TG expression as that of WP (Fig. 1J; Supplementary Dataset S4).

Subsequently, Pearson's correlation analysis was used to assess this similarity quantitatively. The roles of SB and WP were highly correlated: both were involved in regulation of TG expression (r = 0.735) (Fig. 1K). Among the multiple active components of WP, SB had a major role, suggesting that SB could be used to treat male infertility.

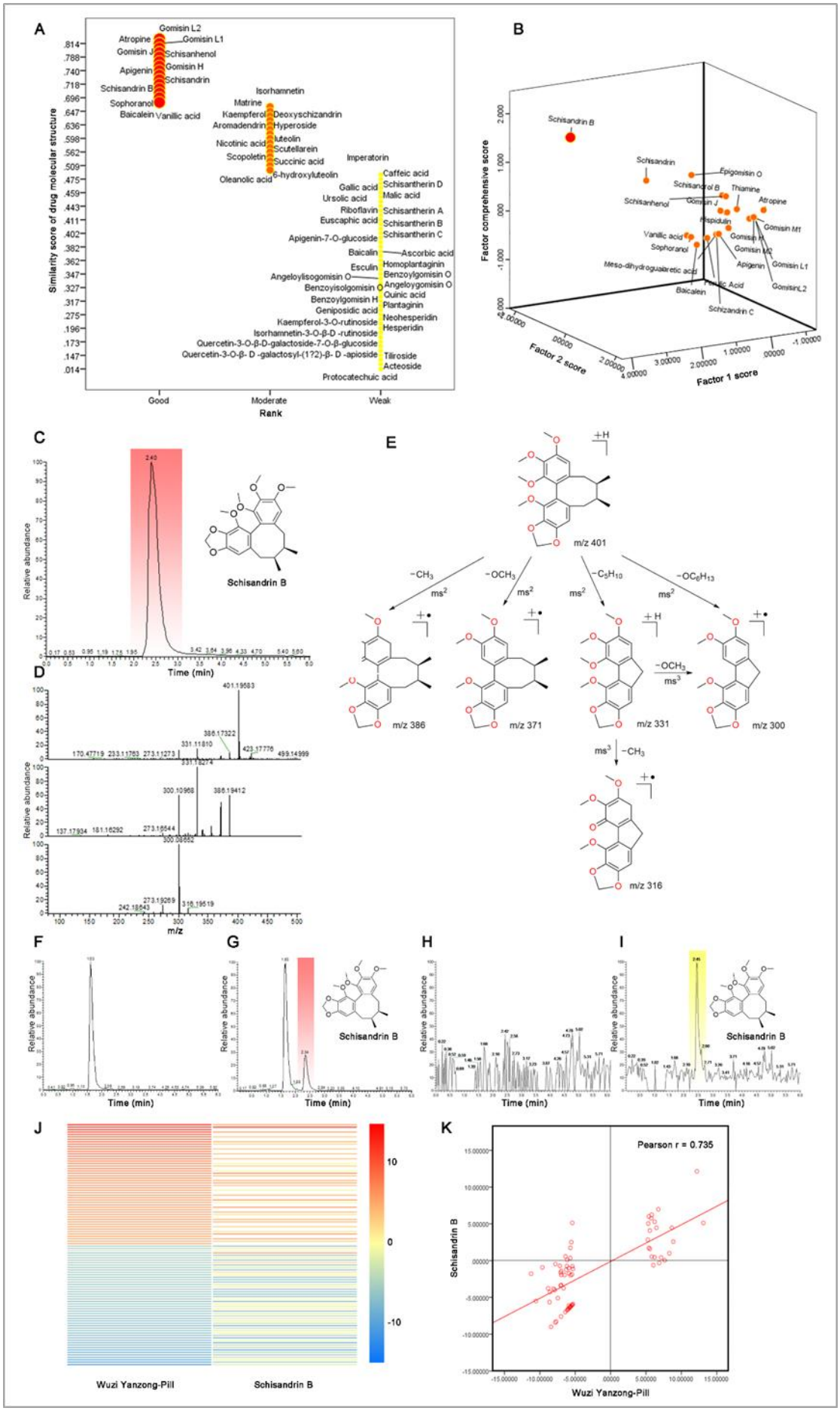


Fig. 1. Schisandrin B is identified as a potent agent for treatment of male fertility

Notes:

The studies (A-B) were performed by simulation and statistical analyses in accordance with measurements on the methanol extract of Wuzi Yanzong-Pill (WP) by UPLC-ESI-LTQ-Orbitrap-MS.

A. Similarity scores of drug molecular structures for 106 major compounds extracted from WP. The study was performed for evaluating the druggability for each component by software of Medchem Studio v3.0 (Simulations Plus, Inc., Lancaster, CA). The result reveals that schisandrin B (SB) along with 21 other components has been listed in the higher score in evaluating the druggability.

B. Factor comprehensive score of SB among 22 compounds which have higher similarity scores of drug molecular structures. The study was performed for further screening the drug candidate with the Factor Analysis with software of SPSS v 20 (IBM, Armonk, NY). Factor 1, the similarity scores of drug molecular structures; Factor 2, the relative abundances of a compound among 22 compounds extracted from WP. The result indicates that SB has the highest druggability among them in evaluating the factor comprehensive score.

The studies (C-I) were analyzed by UPLC-ESI-LTQ-Orbitrap-MS:

C. Typical total ion chromatogram (TIC) of pure SB;

D. Triple fragment spectra of pure SB;

E. Fragmentation pathways of SB;

F. Typical TIC chromatogram of blank mouse plasma;

G. Typical TIC chromatogram of mouse plasma after oral administration of SB (20mg/kg) at 3 h;

H. Typical TIC chromatogram of blank mouse testis;

I. Typical TIC chromatograms of mouse testis after oral administration of SB (20mg/kg) at 3 h.

The studies (J-K) were performed by gene sequence profiling on the testicular samples of oligoasthenospermia mice (OM) after oral administration of SB (20mg/kg/d for 2 weeks; n = 3) or WP (1.56g/kg/d for 2 weeks; n = 3):

J. Gene heatmaps for the most significant up- and downregulated genes (each 50 genes) in the testicular samples from OM after oral treatment with WP (1.56g/kg/d for 2 weeks; n = 3) or SB (20mg/kg/d for 2 weeks; n = 3). Red color indicates the upregulated genes; Blue color indicates the downregulated genes.

K. Pearson correlation of the regulated gene log-folds between WP and SB. r represents correlation coefficient.

Schisandrin B enhances male fertility

To observe the effect of SB on the target tissue, testicular tissues from OM were sampled after oral administration, and histology slices investigated by microscopy. As a pathologic control, the seminiferous tubules of OM were distributed disorderly, and their spermatogenic cells shed severely. In contrast, the seminiferous tubules were distributed uniformly, and injured spermatogenic cells (spermatogonia, spermatocytes, spermatids) were repaired, by treatment with SB or WP (Fig. 2A). As a positive control (testosterone propionate (TP) treatment), the repairing effect on seminiferous tubules and spermatogenic cells was also observed, but to a moderate degree. These results demonstrated that SB and WP could repair damaged seminiferous tubules and spermatogenic cells.

To observe the effect of SB on sperm, sperm samples were collected from OM after oral administration, and the number and movement of sperm investigated by a computer-aided

sperm-analysis system. As a pathologic control, the sperm number of OM was decreased significantly, and sperm movement was inactive or less motile. In contrast, sperm number was increased significantly and sperm movement was very active in OM after oral treatment with SB or WP, indicating a similar number and motility of sperm to those of normal mice (Fig. 2B, C; Supplementary Video 1–5). As a positive control after TP treatment, the repairing effects on the number and movement of sperm were exhibited to a moderate degree. These results revealed that SB and WP could increase the number and motility of sperm.

Subsequently, five visual fields from each video were recorded for quantitative evaluation of sperm parameters. The established OM model met the diagnostic criteria set by the WHO [14] for oligozoospermia (sperm concentration $<20\times10^6$/mL) and asthenospermia (sperm mobility <40%; progressive mobile sperm <32%) (Fig. 2D1–3). Furthermore, the sperm-activity parameters of OM were also decreased significantly, *i.e.*, sperm-motion velocities (curvilinear velocity (VCL) (Fig. 2D4); straight-line velocity (VSL) (Fig. 2D5); average path velocity (VAP) (Fig. 2D6), sperm-motion locus (straightness (STR) (Fig. 2D7)), and dynamic parameters (beat cross frequency (BCF) (Fig. 2D9); amplitude of lateral head displacement (ALH) (Fig. 2D10)), but not an increase in linearity (LIN) (Fig. 2D8). After treatment with SB or WP, all sperm parameters were increased significantly to the levels observed in normal mice (Fig. 2D1–10; Supplementary Dataset S5). As a positive control (TP treatment), the repairing effect on OM was moderate. These results provided robust evidence that SB and WP could increase the number and quality of sperm in OM.

To investigate the reproductive ability, male mice were mated with female mice at a 1:2 ratio (Supplementary Dataset S6). For verification purposes, various experimental and control groups were designed. Normal female mice were included in all groups. Male mice were normal mice, OM, OM after treatment with SB, WP or TP for 2 weeks, respectively. The number of pups in the first litter of OM was diminished significantly as compared with that of normal male mice. In contrast, the number of pups in the first litter was increased markedly in the group of OM after treatment with SB or WP (Fig. 2E1). Furthermore, the number of pups

in the first litter of OM after treatment with SB or WP was very close to that of normal mice. In addition, TP exhibited only slight efficacy upon treatment in OM. Treatment of OM with SB or WP increased the average number of births (ANB) significantly, showing fertility close to that of normal mice, respectively (Fig. 2E2). Hence, SB could be used to treat to treat infertility in OM.

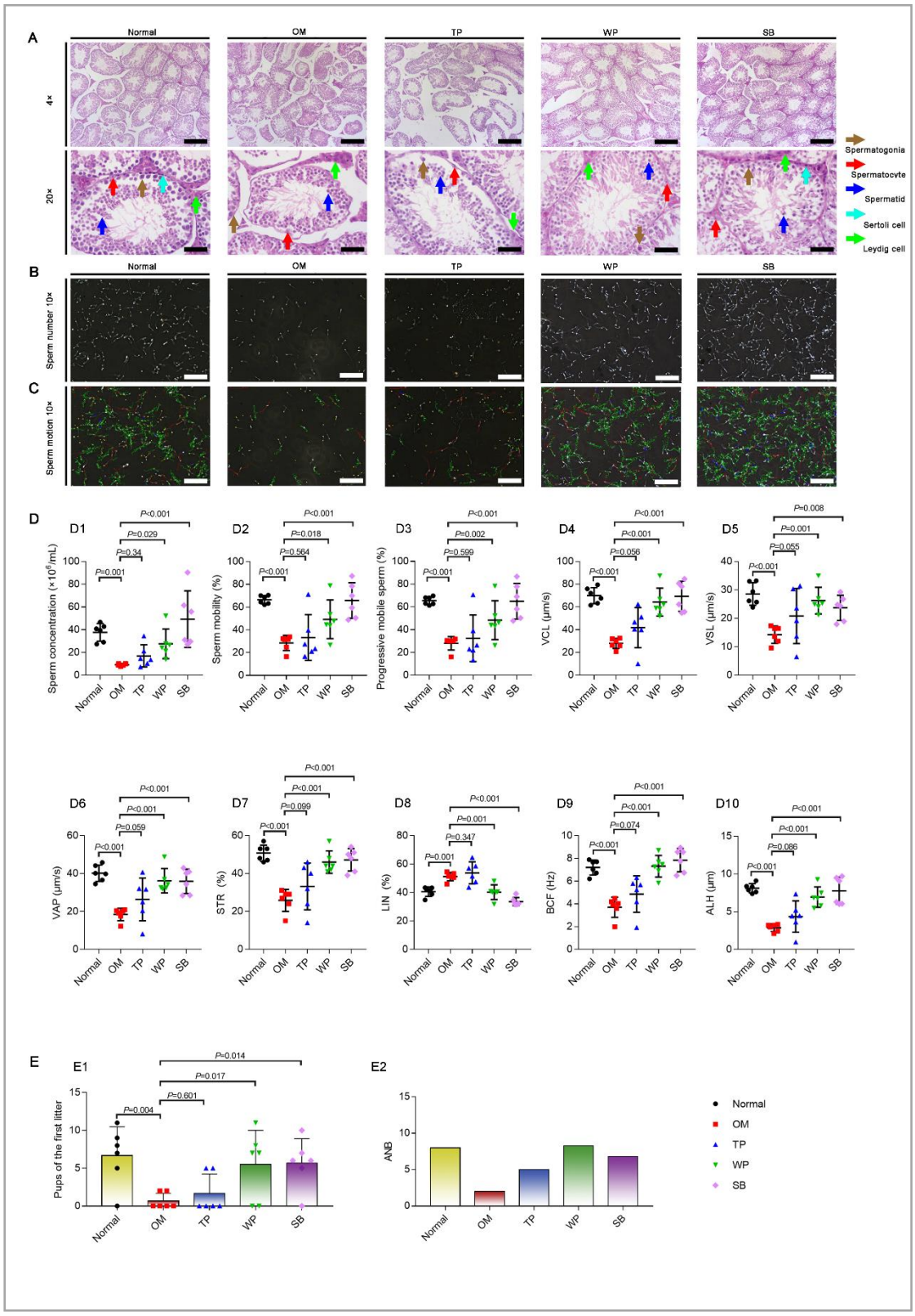


Fig. 2. Schisandrin B enables to enhance male fertility in oligoasthenospermia mice

Notes:

The studies (**A-C**) were performed to verify the efficacy of SB in treatment of male fertility, including spermatogenesis, sperm number, and sperm activity in Balb/c mice.

A. Hematoxylin and eosin staining images of mouse testicular samples. The samples were obtained from normal mice (n=6), OM (n=6), and TP-treated OM (n=6; *i.p.*TP 0.2mg/kg /twice a week for 2 weeks), WP-treated OM (n=6; *i.g.* WP 1.56g/kg/d for 2 weeks) or SB-treated OM (n=6; *i.g.* SB 20mg/kg/d for 2 weeks). *i.g.*, intragastric administration; OM, oligoasthenospermia mice; SB, schisandrin B; TP, testosterone propionate; WP, Wuzi Yanzong-Pill. Scale bar, 200 μm. Brown arrow indicates spermatogonia; red arrow indicates spermatocyte; blue arrow indicates spermatid; cyan arrow indicates sertoli cells; and green arrow indicates leydig cells. The results demonstrate that SB enables to repair the disrupted spermatogenesis of OM.

B. Sperm number images of mouse cauda epididymidis samples under Suiplus Semen Analysis Automatic Detection System (Suiplus, BeiJing, China). The samples were obtained from normal mice (n=6), OM (n=6), and TP-treated OM (n=6; *i.p.* TP 0.2mg/kg /twice a week for 2 weeks), WP-treated OM (n=6; *i.g.* WP 1.56g/kg/d for 2 weeks) or SB-treated OM (n = 6; *i.g.* SB 20mg/kg/d for 2 weeks). The results directly demonstrate that SB enable to increase the sperm number of OM. The dynamic videos of this study are available in the **Supplementary Video 1- Video 5**.

C. Sperm motion track images of mouse cauda epididymidis samples under Suiplus Semen Analysis Automatic Detection System (Suiplus). The samples were obtained from the same as above (**Fig. 2B**). The observation displays that SB increases the sperm mobile activity of OM.

The analyses were performed for evaluating the quality of sperms in OM after oral treatment with SB.

D. Quality of spermatogenesis. **D1,** sperm concentrations; **D2,** sperm mobility; **D3,** progressive mobile sperms; **D4,** curvilinear velocity (VCL); **D5,** straight-line velocity (VSL); **D6,** average path velocity (VAP); **D7,** straightness (STR); **D8,** linearity (LIN); **D9,** beat cross frequency (BCF); **D10**, amplitude of lateral head displacement (ALH).

The studies (**E-F**) were performed for evaluating the male reproductive ability by comparing the number of pups in the first litter of female mice, and the average number of births (ANB; = total number of births/birth females). Each male mouse was placed in one cage, and mated with two females.

E. Efficacy in enhancing reproductive ability (n=3). **E1,** pups in the first litter of female mice; **E2,** average number of births (ANB; = total number of births/birth females).

These data demonstrate that SB significantly increase male reproductive ability, leading to an enhanced ability of male mice to make female mice pregnant and the mean number of offspring.

Schisandrin B regulates testicular genes in the reproductive signaling pathway

We wished to reveal the mechanism of action of SB. Hence, RNA sequencing was done on the testicular tissues of OM and after oral treatment with SB for 2 weeks. Volcano plots showed that SB treatment resulted in significantly changed expression of 2033 genes (836 upregulated; 1197 downregulated) (Fig. 3A).

To further identify the relevant biologic pathways, the Gene Ontology (GO) database was applied. Three reproductive pathways were involved in the top-10 pathways: gamete generation, meiotic cell cycle, and spermatid development (Fig. 3B; Supplementary Dataset S7).

In these three pathways, 137 genes whose expression was regulated significantly were plotted into heatmaps. We found an obvious difference in the TG signature between OM and SB-treated OM (Fig. 3C; Supplementary Dataset S8).

Of the enriched 137 genes in the three reproductive pathways, the predicted genes and

pseudogenes were assigned “low priority” by searching gene databases in the public domain (National Center for Biotechnology Information, Ensembl, Gene Cards) and, finally, 19 candidate genes were obtained.

Oligoasthenospermia can manifest as upregulation or downregulation of TG expression, which correspond to positive or negative fold-changes. Hence, TGs with the most significant absolute fold-changes were studied. Accordingly, the absolute fold-changes of the 19 TGs mentioned above were calculated by comparing SB-treated mice with untreated mice, and processed by statistical analyses with adjusted *P*-values. *Fst* was the most regulated TG, showing remarkable upregulation of expression upon oral administration of SB (Supplementary Dataset S8).

We further investigated expression of *Inhba* in testicular tissues in OM treated and un-treated by SB. As a pathologic control, mRNA sequencing showed that *Fst* was expressed at a low level whereas *Inhba* was expressed at a high level in the testicular tissues of OM. Conversely, *Fst* expression was upregulated significantly whereas *Inhba* expression was downregulated markedly in the testicular tissue of OM after SB treatment (Fig. 3D, Supplementary Dataset S9). Furthermore, upregulation of *Fst* expression and downregulation of *Inhba* expression were verified by quantitative reverse transcription-polymerase chain reaction (RT-qPCR) (Fig. 4E, Supplementary Dataset S10).

These results indicated that SB could treat oligoasthenospermia by regulating expression of *Fst and Inhba*. We postulated a mechanism. Briefly, overexpression of activin-A protein (encoded by *Inhba*) can inhibit the growth of testicular spermatogenic cells and induces apoptosis in spermatogenic cells. Conversely, follistatin protein (encoded by *Fst*) promotes the growth and development of spermatogenic cells by blocking the action of activin-A protein [15–19]. There is serious disorder in the testicular spermatogenic cells of patients with oligoasthenozoospermia, and the major reason is activin-A overexpression, which leads to spermatogenic blockage [20–22]. Therefore, upregulated *Fst* expression would increase follistatin expression, which enables blockade of the action of overexpressed activin-A,

thereby repairing spermatogenic blockage [23–25]. Moreover, downregulation of *Inhba* expression could contribute directly to the decrease in activin A-expression, thereby attenuating spermatogenic blockage. Therefore, we revealed that upregulation of *Fst* expression and downregulation of *Inhba* expression could promote spermatogenesis by inhibiting apoptosis of spermatogenic cells.

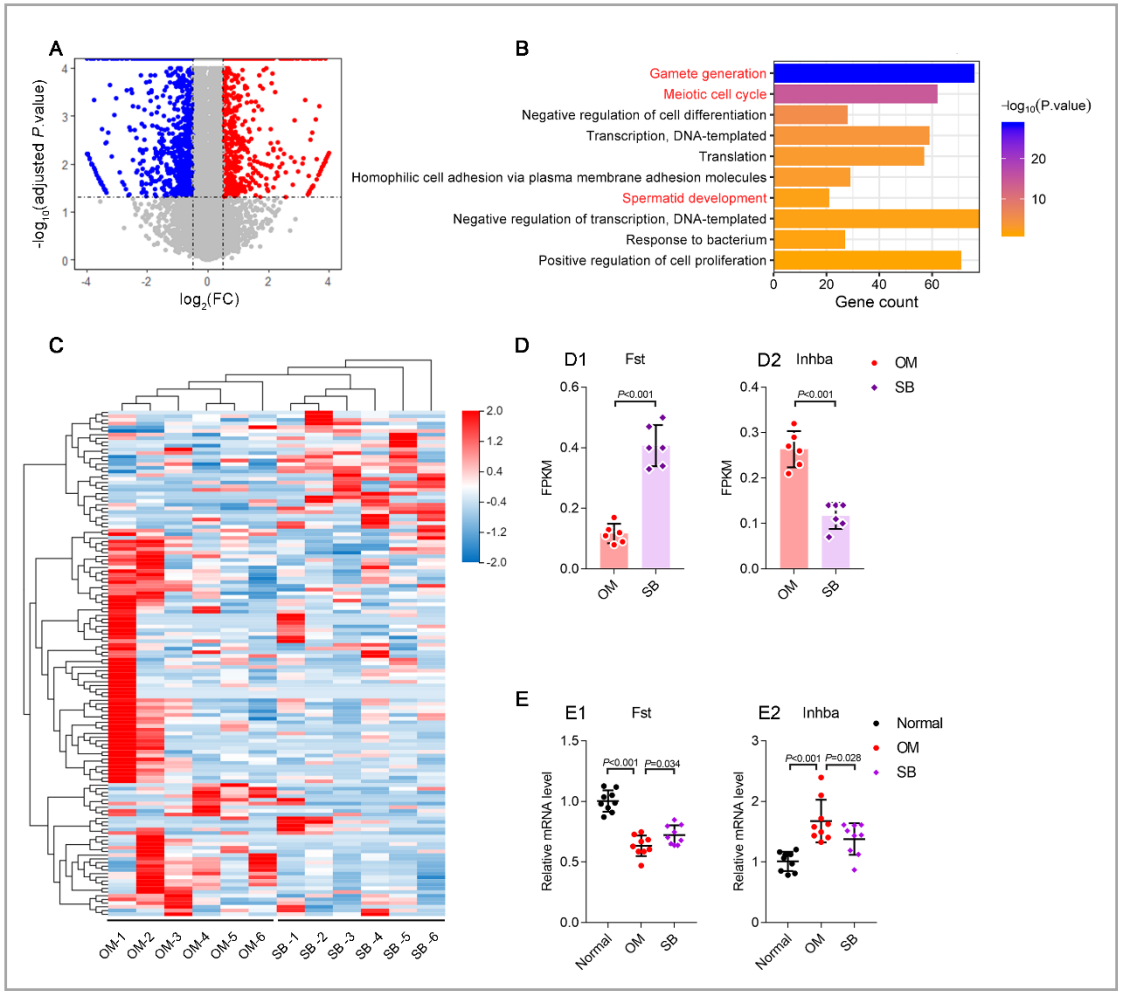


Fig. 3. Schisandrin B regulates testicular gene expressions of *Fst* and *Inhba* in the reproductive pathway

Notes:

The studies (**A-D**) were performed to reveal the regulated functional genes by schisandrin B (SB) by using gene sequencing on testicular samples of oligoasthenospermia mice (OM) and on those of SB-treated OM (*i.g.* SB 20mg/kg/d for 2 weeks; n = 6):

A. Volcano plot of SB-mediated changes of testicular genes by software of Dr Tom (v2.0, Beijing Genomics Institute, BGI Shenzhen, China).The samples were obtained from OM (n=6), and SB-treated OM (*i.g.* SB 20mg/kg/d for 2 weeks; n = 6). SB-changed genes were identified with two threshold criteria: fold up- or down regulation in SB -treated mice of $|\log_2^{(FC)}| > 0.58$, and adjusted *P* value of less than 0.05. The results reveal that, after oral administration of SB in OM, it significantly up-regulates 836 genes, while down-regulates 1197 genes.

B. Top ten GO pathways involved in above changed genes by GO analysis. GO enrichment was performed on above regulated-genes (totally 2033 genes) by using software of Dr Tom. The results indicate that, among top ten GO pathways, three reproductive pathways, including gamete generation, meiotic cell cycle, and spermatid development, are involved in the gene regulations by SB. Besides, a number of 137 genes are included in the reproductive pathways.

C. Gene heatmap for SB-regulated testicular genes (n=137 genes) by using software of Dr Tom. The results indicate that oral administration of SB significantly alters testicular gene signature in OM. Furthermore, it reveals that *Fst* gene is the mostly regulated functional gene in viewing the absolute fold change or adjusted *P* value.

D. *Fst* and *Inhba* gene expressions in testicular samples of OM after oral treatment of SB. **D1,** *Fst* gene expression level; **D2,** *Inhba* gene expression level. FPKM represents the fragments per kilobase per million mapped fragments. The results reveal that SB significantly up-regulates *Fst* gene while down-regulates *Inhba* gene in OM after oral treatment of SB.

The studies were performed for verifying the regulated mRNA levels of *Fst* and *Inhba* gene expressions by RT-qPCR in testicular samples of OM after oral treatment of SB:

E. mRNA levels of *Fst* and *Inhba* in testicular samples of OM after oral treatment of SB. **E1,** *Fst* mRNA expression; **E2,** *Inhba* mRNA expression. The samples were obtained from normal mice (n=3), OM (n=3), and SB treated-OM (*i.g.* SB 20mg/kg/d for 2 weeks; n = 3).The results exhibit that oral treatment of SB significantly increases *Fst* mRNA expression while decreases *Inhba* mRNA expression in testicular tissue of OM, indicating that SB could treat oligoasthenospermia by regulating expressions of *Fst* and *Inhba* genes.

Preclinical pharmacokinetic evaluation on schisandrin B

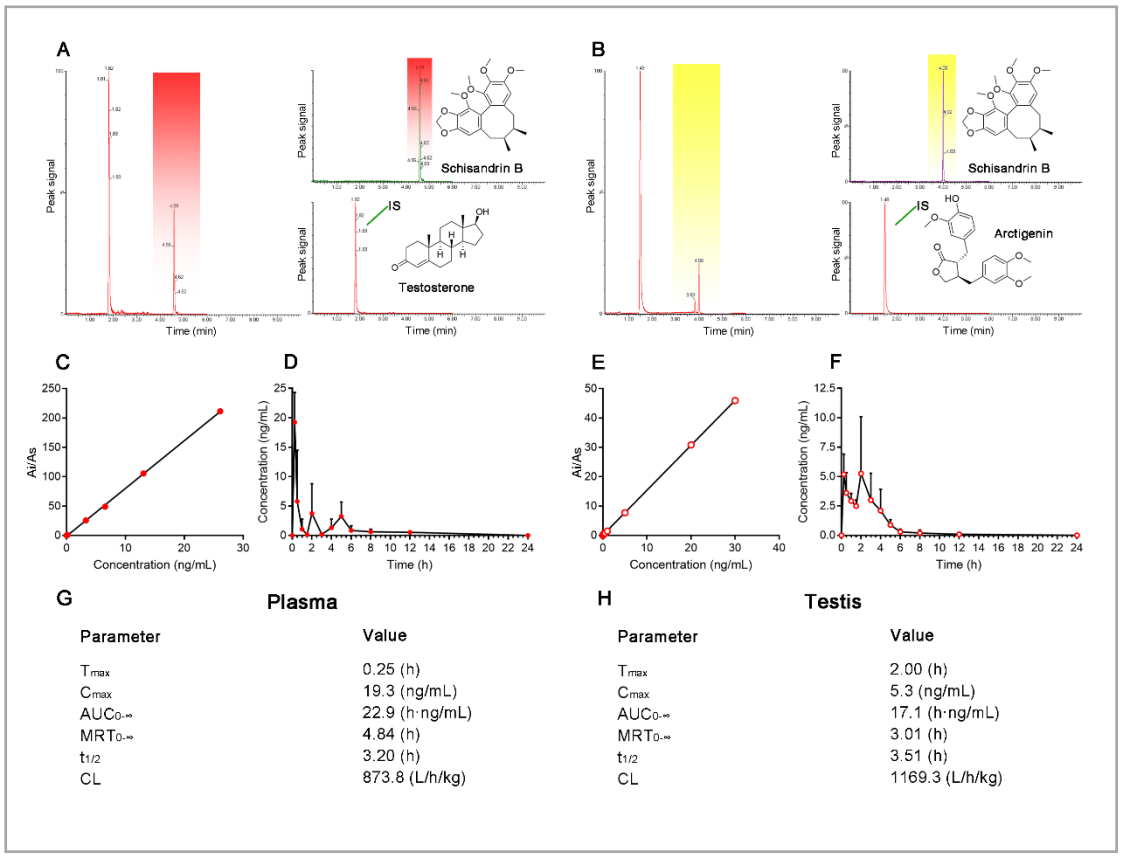
With regard to potential clinical use [26-28], we investigated the pharmacokinetics of SB in normal mice after oral administration. Plasma and testicular concentrations of SB were measured by LC-MS/MS. SB and the internal standard (IS) in plasma were eluted at 4.59 min and 1.82 min (Fig. 4A), and those in testicular samples were eluted at 4.00 min and 1.48 min (Fig. 4B), respectively. There were no interfering peaks in the chromatograms of plasma and testicular samples, indicating suitable specificity for measurement. The calibration curves of SB in plasma and testicular samples exhibited appropriate linearity in a wide concentration range, respectively (Fig. 4C, E). Besides, the measurement was validated [29，30] and consisted of: correlation coefficients, linear ranges, and lower limit of quantifications (LLoQs)(Supplementary Dataset S11); intra-/inter-day precisions and accuracies (Supplementary Dataset S12); recovery stability; measurement stability (Supplementary Dataset S13 and S14).

After oral administration, plasma and testicular concentration–time profiles for SB were plotted (Fig. 4D, F; Supplementary Dataset S15, S16), and the corresponding pharmacokinetic parameters calculated (Supplementary Dataset S17 and S18), respectively. The major pharmacokinetic parameters of SB were evaluated: time to reach maximum concentration (T_{max}), maximum concentration (C_{max}), area under the concentration–time curve ($AUC_{0-\infty}$), mean residence time ($MRT_{0-\infty}$), half-life of elimination ($t_{1/2}$), and clearance

(CL).

Accordingly, SB parameters in plasma were: T_{max}= 15 min; C_{max}= 19.3 ng/mL; $AUC_{0-\infty}$= 22.9 h.ng/mL; $MRT_{0-\infty}$= 4.84 h; CL = 873.8 L/h/kg; $t_{1/2}$ = 3.2 h (Fig. 4G). Plasma concentration–time profiles revealed re-absorption in the gastrointestinal tract due to three concentration peaks, suggesting a hepatointestinal circulation. Assessment of plasma parameters demonstrated that oral administration led to rapid absorption and an effective exposure of SB in blood. Furthermore, SB could be eliminated from blood within 1 day (7-fold half-life washing-out period about 21 h).

SB parameters in testicular tissue were: T_{max}= 2.0 h; C_{max} = 5.3 ng/mL; $AUC_{0-\infty}$ = 17.1 h.ng/mL; $MRT_{0-\infty}$ = 3.0 h; CL = 1169.3 L/h/kg; $t_{1/2}$ = 3.5 h (Fig. 4H). Concentration–time profiles from testicular tissue showed two concentration peaks (minor peak at 15 min and maximum peak at 3 h) suggesting that, after absorption, SB was distributed effectively into testicular tissue but with a delay. Nonetheless, there was effective testicular exposure of SB as well. Hence, SB in testicular tissue had comparable pharmacokinetic behavior to that in blood.



G **Plasma**

Parameter	Value
T_{max}	0.25 (h)
C_{max}	19.3 (ng/mL)
$AUC_{0-\infty}$	22.9 (h·ng/mL)
$MRT_{0-\infty}$	4.84 (h)
$t_{1/2}$	3.20 (h)
CL	873.8 (L/h/kg)

H **Testis**

Parameter	Value
T_{max}	2.00 (h)
C_{max}	5.3 (ng/mL)
$AUC_{0-\infty}$	17.1 (h·ng/mL)
$MRT_{0-\infty}$	3.01 (h)
$t_{1/2}$	3.51 (h)
CL	1169.3 (L/h/kg)

Fig. 4. Pharmacokinetics of schisandrin B in plasma and in testicular tissue in normal mice after oral administration

Notes:

The studies (**A-F**) were performed to establish analysis method for the separation and determination of schisandrin B (SB) in plasma and testicular tissue of normal mice.

A. Typical multiple reaction monitoring (MRM) chromatogram of mouse plasma after oral administration of SB (20mg/kg) at 3 h. Internal standard (IS) added in plasma, testosterone. The results display that the chromatogram peaks of SB and IS in plasma appear at 4.59 min and 1.82 min, respectively, and there are no interfering peaks in the chromatogram, demonstrating a good specificity of analysis method for plasma samples.

B. Typical MRM chromatogram of mouse testes after oral administration of SB (20mg/kg) at 3 h. IS added in testicular tissue, arctigenin. The results display that the chromatogram peaks of SB and IS in testicular tissue appear at 4.00 min and 1.48 min, and there are no interfering peaks in the chromatogram, demonstrating a good specificity of analysis method for testicular samples.

C. Calibration curve of SB in mice plasma. Ai indicates peak area of SB; and As indicates peak area of IS (testosterone) in plasma samples. The results show that the peaks and concentration of SB are linearly correlated in the range of 0.07 to 26.09 ng/mL in plasma.

D. Mean plasma concentration-time curve of SB. The sampling was performed at 0 min, 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h and 24h before and oral administration of SB (20mg/kg) (n= 5). The results show that SB can be rapidly absorbed into blood, and has triple absorption peaks, suggesting a hepato-intestinal circulation pathway during absorption and metabolism.

E. Calibration curve of SB in testicular tissue. Ai indicates peak area of SB; and As indicates peak area of IS in testicular samples. The results show that the peaks and concentration of SB are linearly correlated in the range of 0.10 to 30.00 ng/mL in testicular tissue.

F. Mean testicular concentration-time curve of SB. The sampling was performed at 0 min, 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h and 24h before and oral administration of SB (20mg/kg) (n= 5). The results show that SB reaches the testicular tissue rapidly after absorption, demonstrating that SB is able to reach the action site.

The studies (**G-H**) were performed to calculate the pharmacokinetic parameters in mice plasma and in testicular tissues by software of DAS v3.2 (China State Drug Administration, Shanghai, China).

G. Major pharmacokinetic parameters in mice plasma after oral administration of SB.

H. Major pharmacokinetic parameters in mice testicular tissue after oral administration of SB.

Conclusion

In summary, SB could be used to treat infertility in male mice. The present study involved four main stages. First, SB was detected from 106 compounds of an ancient formulation (WP) to treat male infertility by study of: similarity in drug structure; SB availability after oral administration; regulation of TG expression by comparing SB with WP. Second, the efficacy of SB was assessed to treat male infertility by studying: repair of damaged seminiferous tubules and spermatogenic cells by pathologic staining; enhancement of sperm-number and sperm-motility parameters using a computer-aided sperm-analysis system; and improvement of reproductive ability. Third, 2033 differentially expressed genes induced by SB were revealed by RNA sequencing. Use of the GO database showed that three reproductive pathways were enriched in gene regulation: gamete generation, meiotic cell cycle and spermatid development. We found that upregulation of *Fst* expression and downregulation of *inhba* expression interacted to repair spermatogenesis. This phenomenon could be explained by the fact that follistatin (encoded by *Fst*) promotes the growth and development of spermatogenic cells by blocking the induced apoptosis of spermatogenic cells by activin-A

(encoded by *Inhba*). Fourth, pharmacokinetic studies demonstrated that SB could be absorbed rapidly after oral administration, and became fully available at the intended action site, indicating a remarkable potential for clinical application.

In conclusion, SB enables the repairs of spermatogenesis arrest and male infertility. The action mechanism could be explained by the repaired spermatogenesis via upregulation of *Fst* while downregulation of *Inhba* genes involved in the reproductive signaling pathway. Our study provides a promising drug for treatment of male infertility and a novel strategy for discovery of new small-molecule drugs from vast plant-based medicinal resources.

Materials and Methods

Ethical approval of the study protocol

All procedures involving the care and handing of animals were carried out with approval of the Authorities for Laboratory Animal Care of Peking University (Beijing, China; LA2018330).

Reagents

Busulfan was purchased from Macklin Biochemicals (Shanghai, China). Pure SB was obtained from the National Institutes for Food and Drug Control (Beijing, China; purity >98%; HPLC grade). TP (injection) was purchased from Shanghai General Pharmaceuticals (Shanghai, China). *Fructus lych, semen cuscutae* (fried), *fructus rubi*, *fructus Schisandrae chinensis* (steamed), and *semen plantaginis* (fried with salt) were from Beijing Tong Ren Tang Group (Beijing, China). Acetonitrile, methanol, ethanol, and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA; LC-MS grade). Water was purified by a Milli-Q™ ultraviolet purification system (Millipore, Bedford, MA, USA). Dimethyl sulfoxide was obtained from Sigma–Aldrich (Saint Louis, MO, USA). Polyethylene glycol $(PEG)_{400}$ was purchased from Harveybio Gene Technology (Beijing, China). Sperm Culture Medium 199 (M199) was obtained from Thermo Scientific (Waltham, MA, USA). Bovine serum albumin was purchased from Solarbio (Beijing, China). PCR primers were obtained from Tsingke Biological Technology (Beijing, China). All other chemicals were from commercial sources.

Animals

Male and female Balb/c mice (10 weeks; 20.0 ± 2 g) were obtained from the Department of Laboratory Animal Science, Peking University Health Science Center (order ID: SCXK (jing) 2016-0010). Each animal was housed in an individual cage at controlled temperature (25±1°C) and humidity (55±5%) and exposed to a 12-h light–dark cycle (7 pm to 7am). Animals had free access to food (regular chow comprising 5% fat, 53% carbohydrate and 23% protein) and water unless indicated otherwise.

Ancient formulation for treatment of male infertility

The ancient formulation consisted of *Fructus lych*, *semen cuscutae* (fried), *fructus rubi*, *fructus Schisandrae chinensis* (steamed), and *semen slantaginis* (fried with salt). The medicinal materials were weighed, mixed (8:8:4:2:1, *w/w*) and crushed to powder (mesh size = 40). Then, they were immersed in a 10-fold volume of water for 1 h at 100°C. After boiling, heating was continued until the volume was reduced to fivefold volume as compared with the original one. The mixture was filtered immediately through gauze, concentrated to 1 g of crude drug per mL, and freeze-dried to become powders. Finally, refined honey (85 g) was mixed with freeze-dried powders (100 g) to make pellets of WP for experimental use.

Extraction of compounds and prediction of druggability

WP (250 mg) were extracted with 50 mL of methanol with the aid of ultrasound for 60 min. Extracts were centrifuged at 10,000 revolution per minute (rpm) for 15 min at 4°C. The supernatant was collected and passed through a filter (0.22 μm). The filtrate was collected for UPLC coupled with electrospray ionization-linear ion trap-Orbitrap tandem mass spectrometry (UPLC-ESI-LTQ-Orbitrap-MS) measurement. Based on chromatographic data, 106 major compounds were identified in the WP extract, including organic acids, flavonoids, phenylpropanoids, alkaloids and terpenoids. To predict the most promising drug candidate, the druggability was evaluated on the 106 compounds extracted from WP using MedChem Studio v3.0 (Simulations Plus). Compounds with a good drug-similarity score (i.e., druggability) were selected for further consideration by combination with drug contents in the extract. Based on the druggability and drug content (which was indicated in the corresponding peak relative abundance in the chromatogram of UPLC-ESI-LTQ-Orbitrap-MS),

drug candidates were selected preliminarily in accordance with the highest factor comprehensive score using factor analysis employing SPSS v20.0 (IBM).

Availability of SB by action site

Measurement: To ascertain if the drug candidate (SB) could be absorbed in blood or reach the action site, SB in plasma and testicular tissue (action site of drugs for male infertility) was measured by UPLC-MS/MS after oral administration of SB in male mice. Analyses were undertaken on a UPLC system (Acquity™ UPLC I-Class system; Waters, Milford, MA, USA) consisting of an auto-sampler, quaternary pump, and column oven. A C18 reverse-phase column (Acquity UPLC BEH, 100 × 2.1 mm, 1.7 μm, 130 Å) was used to separate samples. The mobile phase was 0.1% formic acid in water (A) and acetonitrile (B). The gradient elution was: 0 min 78% B, 1 min 78% B, 4 min 60% B, and 6 min 78% B. Samples were kept in the autosampler at 4°C until measurement. The column was maintained at 40°C, the flow rate was 0.3 mL/min, and injection volume was 5 μL. The UPLC was connected to a mass spectrometer (LTQ/Orbitrap; Thermo Scientific) *via* an ESI interface. The effluent was split at a ratio of approximately 3:1 (*v/v*) before entering the ESI source. Positive-ion mode was used, and operation parameters were: capillary voltage, 25 V; electrospray voltage, 4.0 kV; capillary temperature, 350°C; sheath gas, 30 (arbitrary units); auxiliary gas, 5 (arbitrary units); tube lens, 110 V. High-resolution full scan was used to scan samples with a resolution of 30,000 and a scanning mass range of 100 to 500 amu. Data-dependent scan was used to scan secondary and tertiary mass spectra, and the three peaks with the highest abundance in the upper MS level were selected for collision-induced fragmentation scanning. The normalized collision energy was set to 35%. To avoid many repeated data acquisitions on the same sample, dynamic exclusion was used for data collection with an exclusion duration of 60 s and the repeat count was set at 5 with a dynamic repeat time at 30 s. An external calibration for mass accuracy was carried out before the analysis. The measured masses were within 5 ppm of the theoretical masses. Data analyses were processed using a Xcaliber 2.1 workstation (Thermo Fisher Scientific). Meanwhile, pure SB (5 mg) was dissolved in 10 mL of methanol, passed through a filter (0.22 μm), and used as the reference for analyses.

Dosing: Pure SB (1 mg/mL) was dissolved in a mixture of ethanol, PEG_{400} and 0.5% sodium

carboxymethyl cellulose (CMC-Na) (1:1:1, *v/v/v*) for oral administration. Male mice were divided randomly into two groups of three. In the treatment group, each mouse was administered SB (20 mg/kg, i.g.). In the blank control group, each mouse was given physiologic saline (PS).

Sampling: Three hours after dosing, venous blood (0.75 mL) was sampled and centrifuged at 5000 rpm for 10 min at 4°C. Then,+ 200 μL plasma was transferred, added to 600 μL of acetonitrile, vortex-mixed (120 s), and centrifuged (13,000 rpm, 10 min, 4°C) to remove proteins. The supernatant was evaporated at 25°C by a CentriVap™ centrifugal thickener (Labconco, Kansas City, MO, USA). The residues were dissolved in 200 μL of methanol, and centrifuged (13,000 rpm, 10 min, 4°C). The resultant supernatant was injected into the UPLC-MS/MS system.

The animals were sacrificed. The testicular tissues were collected on an ice plate at the same time as blood sampling. Next, they were washed with PS, drained with filter paper and weighed. PS (1:4, *w/v*) was added and the testicular tissue homogenized. One milliliter of testicular-tissue homogenate was centrifuged at 5,000 rpm for 10 min at 4°C. The supernatant (200 μL) was collected, and 400 μL of acetonitrile added, followed by vortex-mixing (120 s) and centrifugation at 13,000 rpm for 15 min at 4°C. Finally, the resultant supernatant was injected into the UPLC-MS/MS system.

Involvement of SB in regulation of TG expression

To investigate SB involvement in regulating TG expression by comparing it with that of WPs, OM models were induced by intraperitoneal injection of busulfan (20 mg/kg dissolved in sterile dimethyl sulfoxide). OM were divided into three groups of three, and treated once daily with PS, SB (20 mg/kg/d, i.g.) or WP (1.56 g/kg/d, dissolved in 0.5% CMC-Na). After 2-week treatment, the testes of all animals were dissected, frozen immediately in liquid nitrogen, and stored at −80°C for gene sequencing.

To extract total RNA, 200 mg of the testicular sample was processed using TRIzol by following manufacturer (Invitrogen, Carlsbad, CA, USA) protocols and its expression determined using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, VCA, USA). Only qualified RNAs from testicular samples were used for construction of cDNA libraries. Preparation and sequencing of cDNA libraries were undertaken by the BGI Genomics Co., Ltd. (BGI, Shenzhen, China) using the BGISEQ-500

platform.

To analyze RNA-sequencing data, initially raw reads were excluded if they contained >10% nitrogen, or were adapter or low-quality reads, using SOAPnuke v1.5.2 by BGI. High-quality reads were aligned to the reference genome (mouse) using HISAT v2.0.4 and gene expression was normalized to fragments per kilobase of exon model per million mapped reads (FPKM) using RSEM v1.2.12 by BGI. Normalized FPKM expression was analyzed using Dr Tom v2.0 by BGI to identify differentially expressed genes. The 100 most-regulated genes in the testes of WP-treated OM, and their corresponding gene expression fold-changes in the testes of SB-treated OM, were selected as typical gene signatures to compare the gene profile. The 100 most-regulated TGs consisted of 50 upregulated genes and 50 downregulated TGs. The comparison of gene heatmaps between WP and SB was made by Dr Tom v2.0 by BGI. Besides, Pearson's correlation analysis was applied to quantitatively analyze the similarity in gene expressions in the testes of OM after oral treatment with SB or WP.

Spermatogenesis repair by SB

Dosing: OM were divided into four groups of six and treated with SB (20 mg/kg/d, once daily, i.g.), WP (1.56 g/kg/d, once daily, i.g.), TP (0.2 mg/kg/twice a week, i.p.) or PS (14 mL/kg/d, i.g.), respectively. Normal mice (n = 6) were given PS (14 mL/kg/d, i.g.). All animals were given these agents consecutively for 2 weeks and the observations shown below made.

Sampling of testicular tissue: After 2-week treatment, each mouse was anesthetized with diethyl ether. Tissue from the left testes was harvested, stored in 10% formalin, and paraffin-embedded for staining (hematoxylin and eosin).

Sperm sampling: After 2-week treatment, the limbs of each mouse (under anesthesia) was fixed on a thermostatic hot plate (37°C). The left epididymis was dissected promptly, cleaned with PS (37°C) and transferred immediately to 0.5 g of bovine serum albumin per μL of medium 199 (1 mL, 37°C). Tissue was cut into pieces by scissors. Sperm was allowed to flow out of the tissue, and then placed in an incubator in an atmosphere of 5% CO_2 for 3 min at 37°C. After incubation, the suspension was mixed homogeneously by a pipette, then 10 μL of sperm suspension was placed on a

semen-counting slide (Yulu Optics, Nanjing, China). This slide had a depth of 0.01 mm, and enabled unimpeded movement of sperm.

Microscopic observation of sperm: The sperm-counting slide was placed under a phase-contrast microscope (E200; Nikon, Tokyo, Japan). A video was recorded by a semen analysis automatic detection system (Suiplus; Beijing, China). Five visual fields were taken from each counting slide for observation. The movement track, morphology, concentration and number of sperm were observed, and recorded for qualitative evaluations and parameter evaluations.

Quality parameters of sperm: IVOS software (Hamilton Thorne Biosciences, Beverly, MA, USA) in the semen analysis automatic detection system (Suiplus) was used to evaluate the quality parameters of sperm. The parameters were sperm concentration, sperm mobility, progressive mobile sperm, sperm motion velocity (VCL, VSL, VAP), sperm-motion locus (STR, LIN) and dynamic parameters of sperm movement (BCF, ALH).

Efficacy of SB in enhancing male reproductive ability

Dosing: OM were divided into four groups of three and treated with SB (20 mg/kg/d, once daily, i.g), WP (1.56 g/kg/d, once daily, i.g.), TP (0.2 mg/kg, twice a week, i.p.) and PS (14 mL/kg/d, once daily, i.g.), respectively. Normal mice (n = 3) were given PS (14 mL/kg/d, i.g.). All animals were given these agent consecutively for 2 weeks.

Reproductive ability: After 2-week treatment, each male mouse was mated with females at a 1:2 ratio. Mating mice were placed in one cage for 10 days (two sex cycles of females). Females were examined for pudendal embolus each morning at 8:30. The plugged female was removed from the cage immediately. If there was no sign of intercourse, the female(s) and male mice were placed in the same cage continuously until the end of the tenth day. After 10 days, female mice were separated from the male mouse, and observed for 40 days. The total number of pups in the first litter for a pregnant female, and the number of non-pregnant females, was recorded. The ANB was calculated using the formula:

ANB = total number of births/number of females who gave birth

Gene profiling and biologic pathways regulated by SB

Dosing and sampling: OM were divided into two groups of six and treated once daily with SB (20 mg/kg/d, i.g.) or PS (14 mL/kg/d, i.g.), respectively. After 2-week treatment, each mouse was anesthetized with diethyl ether. Testicular tissue was frozen immediately in liquid nitrogen and stored at −80°C for further analyses. Normal male mice were included as a blank control (n = 6). After experimentation, mice were sacrificed by cervical dislocation.

Gene profiling and GO analyses: Frozen testicular samples (n = 6) from SB-treated or non-SB-treated OM were used for RNA sequencing. Extraction of total RNA and data analyses were done as described above. Furthermore, functional annotation of differentially expressed genes in the GO database was applied using Dr Tom v2.0 by BGI.

RT-qPCR verification

Frozen testicular samples (n = 3) from normal mice, OM, or SB-treated OM were used for RT-qPCR. Total RNA was extracted using a TRIzol Plus RNA Purification kit (Invitrogen), and analyzed (excitation wavelength =260 nm, emission wavelength = 280 nm) using a spectrophotometer (Nano300; Allsheng, Hangzhou, China).

cDNA was reverse-transcribed from 1 μg of total RNA using PrimeScript RT reagent (TaKaRa Biotechnology, Shiga, Japan), and 10 ng of cDNA was analyzed using SYBR Premix Ex Taq II (TaKaRa Biotechnology) on a CFX Connect TM Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Each sample was tested in triplicate. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. Relative quantification of genes of interest was done using the $2^{-\Delta\Delta ct}$ method. Primer sequences used for RT-qPCR (forward and reverse, respectively) were 5′- TGCTCTTCTGGCGTGCTTCTTG-3′ and 5′- TGTAGTCCTGGTCTTCCTCCTCCT-3′ for the *Fst* primer; 5′- GTCCTCGCTCTCCTTCCACTCAA-3′ and 5′- AGCAGCCACACTCCTCCACAAT-3′ for the *Inhba primer*; 5′- AGAAGGTGGTGAAGCAGGCATCT-3′ and 5′- CGGCATCGAAGGTGGAAGAGTG-3′ for the *GAPDH* primer.

Pharmacokinetics

Working solutions: Pure SB was weighed accurately and dissolved in methanol to prepare

working standard solutions (0.05–30.0 ng/mL). The IS solution of testosterone (25.0 ng/mL) and arctigenin (25.0 ng/mL) was prepared similarly for SB measurements in plasma and testicular tissue, respectively. All solutions were stored at 4°C before use.

Sampling of blank plasma and testicular tissue: Normal mice (n = 15) were anesthetized with diethyl ether. Aliquots of venous blood (0.75 mL) were sampled, centrifuged at 5000 rpm for 10 min at 4°C to obtain plasma, and stored at −80°C until use. Animals were sacrificed, testicular tissues were collected on an ice plate at the same time of blood sampling, and frozen immediately at −80°C for use.

Calibration curves and quality control (QC): Calibration curves and QC samples for SB in plasma and testicular tissue were prepared in duplicate to evaluate the precision, accuracy, stability and recovery of our analytical method. The handling procedures are described below.

Plasma handling: Plasma was thawed at 4°C for ~30 min and vortex-mixed for 30 s. Plasma (200 μL) was vortex-mixed with 60 μL of a working solution of SB for 30 s, added to 60 μL of IS solution (testosterone) and vortex-mixed for 30 s. Then, 600 μL of acetonitrile was added, followed by vortex-mixing for 120 s, and centrifugation at 13,000 rpm for 10 min at 4°C. The resultant supernatant was injected into the UPLC-MS/MS system. SB concentrations for calibration curves were prepared at 0.05, 0.10, 3.0, 6.0, 12.0 and 25.0 ng/mL in plasma, whereas those for QC analyses were prepared at 1.0, 10.0 and 20.0 ng/mL in plasma. In these samples, the IS concentration was 25.0 ng/mL.

Handling of testicular tissue: Testicular tissue was thawed at 4°C for ~30 min, washed with PS, drained with filter paper and weighed accurately. Then, PS (1:4, *w/v*) was added, and the tissue homogenized. The homogenate (200 μL) was added to 60 μL of SB, and 60 μL of IS (arctigenin) working solution. The mixture was vortex-mixed for 30 s, followed by addition of 400 μL of acetonitrile, vortex-mixing for 120 s, and centrifugation at 13,000 rpm for 15 min at 4°C. The resultant supernatant was injected into the UPLC-MS/MS system. SB concentrations for calibration curves were 0.10, 0.20, 0.50, 1.0, 5.0, 20.0 and 30.0 ng/mL in testicular tissue, whereas those for QC analyses were 1.0, 10.0 and 20.0 ng/mL in testicular tissue. In these samples, the IS concentration was 25.0 ng/mL.

Analytical conditions: After oral administration of SB in male mice, concentrations of SB in

plasma and testicular tissues were detected by UPLC-MS/MS. Analyses were undertaken on a UPLC system (Acquity UPLC I-Class system; Waters) consisting of an auto-sampler, quaternary pump, and a column oven. A C18 reverse-phase column (Acquity UPLC BEH, 100 × 2.1 mm, 1.7 μm, 130 Å) was used to separate samples. The mobile phase comprised 0.1% formic acid in water (A) and acetonitrile (B). Gradient elutions were: 0 min 50% B, 0.5 min 50% B, 1.5 min 80% B, and 6 min 50% B. Samples were kept in the autosampler at 4°C until measurement. The column was maintained at 40°C, the flow rate was 0.3 mL/min, and injection volume was 2 μL. Detection was carried out on a Xevo triple quadrupole mass spectrometer (Waters). High-purity nitrogen served as the nebulizing gas and drying gas. Optimal MS conditions were: positive ion mode, source temperature = 110°C, desolvation-gas temperature = 450°C, cone gas flow = 50 h, desolvation gas flow = 600 L/h, capillary voltage = 3.0 kV, sampling cone voltage = 25 V, and extraction cone voltage = 3.0 V. Multiple-reaction monitoring data were acquired in centroid mode between *m* 50 and *m/z* 1000 using MassLynx v4.1 (Waters), and the scan time and interscan time were set at 0.4 s and 0.1 s, respectively. Leucine-enkephalin (*m/z* 556.2771) was used as the external reference of LockSpray infused at a constant flow of 5 μL/min. The mass spectrometer was calibrated over a range of 50–1000 Da with sodium formate. The following precursors to product ions were monitored: *m/z* 401.2843→300.3354 for SB (collision energy, 24 eV; dwell time, 25 ms); *m/z* 289.4323→253.3991 for testosterone (14 eV; 25 ms); *m/z* 373.3807→355.3415 for arctigenin (48 eV; 25 ms).

Specificity: Blank plasma, blank plasma with addition of working solutions of SB and IS, and plasma samples after oral administration of SB were analyzed by UPLC/MS/MS for exclusion of interference at the peak concentration of SB or IS. Similarly, specificity for measurement of SB in testicular tissue was also validated.

LoQ: The LLoQ was determined as the lowest concentration that the instrument could quantify accurately (i.e., the lowest concentration point on the standard curve).

Precision and accuracy: The precision and accuracy were validated by measuring QC samples at 1.0, 10.0 and 20.0 ng/mL of SB in plasma (n = 3) or in testicular tissue (n = 3), respectively. During measurements in 3 consecutive days, the intra- and inter-day variations were calculated. Precision was

expressed as the relative standard deviation (RSD)% and accuracy was expressed as the relative error (RE)% by comparing the SB concentration measured with the SB concentration added. The criterion for acceptability was: precision, <15%, accuracy, 85%–115%; LLoQ ± 20% accuracy.

Extraction recovery: SB recovery from plasma or testicular tissue was calculated by comparing the SB concentration measured with the SB concentration added.

Sample stability: SB stability was assessed on the QC samples mentioned above at three concentrations after three freeze–thaw cycles (−20°C to 25°C) on 3 consecutive days, storage at 25°C for 24 h, and storage at −80°C for 1 month, respectively. Sample stability was expressed as the RSD for the SB concentration measured.

Dosing: Normal male mice (n = 65) were fasted 12 h but had free access to water. Then, each mouse was administered (p.o.) a single dose of SB (20 mg/kg, i.g.) for subsequent experiments.

Sampling: Blood sampling was done at 0 min (before dosing), 15 min, 30 min, as well as 1, 1.5, 2, 3, 4, 5, 6, 8, 12 and 24 h (five mice at each time point) under anesthesia. Aliquots of venous blood (0.75) were sampled, centrifuged at 5,000 rpm for 10 min at 4°C to obtain plasma, and stored at −80°C until use. After each blood sampling, animals were sacrificed, testicular tissues were collected on an ice plate, and frozen immediately at −80°C for use.

Plasma handling: Plasma was thawed at 4°C for ~30 min and vortex-mixed for 30 s. Plasma (200 μL) was vortex-mixed with 60 μL of IS solution (testosterone) for 30 s, followed by addition of 60 μL of methanol. After vortex-mixing for 30 s, 600 μL of acetonitrile was added, followed by vortex-mixing for 120 s, and centrifugation at 13,000 rpm for 10 min at 4°C. The resultant supernatant was injected into the UPLC-MS/MS system.

Handling of testicular tissue: Testicular tissue was thawed at 4°C for ~30 min, washed with PS, drained with filter paper, and weighed accurately. Then, PS (1:4, *w/v*) was added and the tissue homogenized. The homogenate (200 μL) was added to 60 μL of methanol and 60 μL of IS (arctigenin) working solution. The mixture was vortex-mixed for 30 s, followed by addition of 400 μL of acetonitrile, vortex-mixing for 120 s, and centrifugation at 13,000 rpm for 15 min at 4°C. The resultant supernatant was injected into the UPLC-MS/MS system.

Pharmacokinetic analyses: Pharmacokinetic parameters in plasma and testicular tissue were calculated using a non-compartmental approach employing DAS v3.2 (China State Drug Administration, Shanghai, China).

Statistical analyses

Statistical analyses were conducted by Prism v7.0 (GraphPad, La Jolla, CA, USA) and SPSS v20.0 (IBM). No data were excluded from analyses. The Student's *t*-test (two-tailed) or one-way analysis of variance was used for statistical analyses. $p < 0.05$ was considered significant. Data are the mean ± standard deviation.

Supplementary information

All data needed to understand and assess the conclusions of this research are available in the main text and supplementary materials. Raw datasets supporting the findings of this study are available online or from the corresponding author.

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Author contributions

Lu W.-L., Lin R.-C. and Yang W.-P. designed the study and supervised the analyses.

Zou D.-X., and Meng X.-D. completed the major research work. Xie Y., Liu R., Duan J.-L., Bao C.-J., and Liu Y.-X. undertook experiments under the direction of Lu W.-L., Lin R.-C. and Yang W.-P.

Du Y.-F., Xu J.-R., Luo Q., Zhang Z. and Ma S. helped with data analyses.

Zou D.-X., Meng X.-D., Yang W.-P., Lin R.-C. and Lu W.-L. wrote the manuscript with input from all authors.

All authors approved the final version for submission.

Competing interests

The authors declare no competing interests in relation to publication of this study.

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Supply Figure

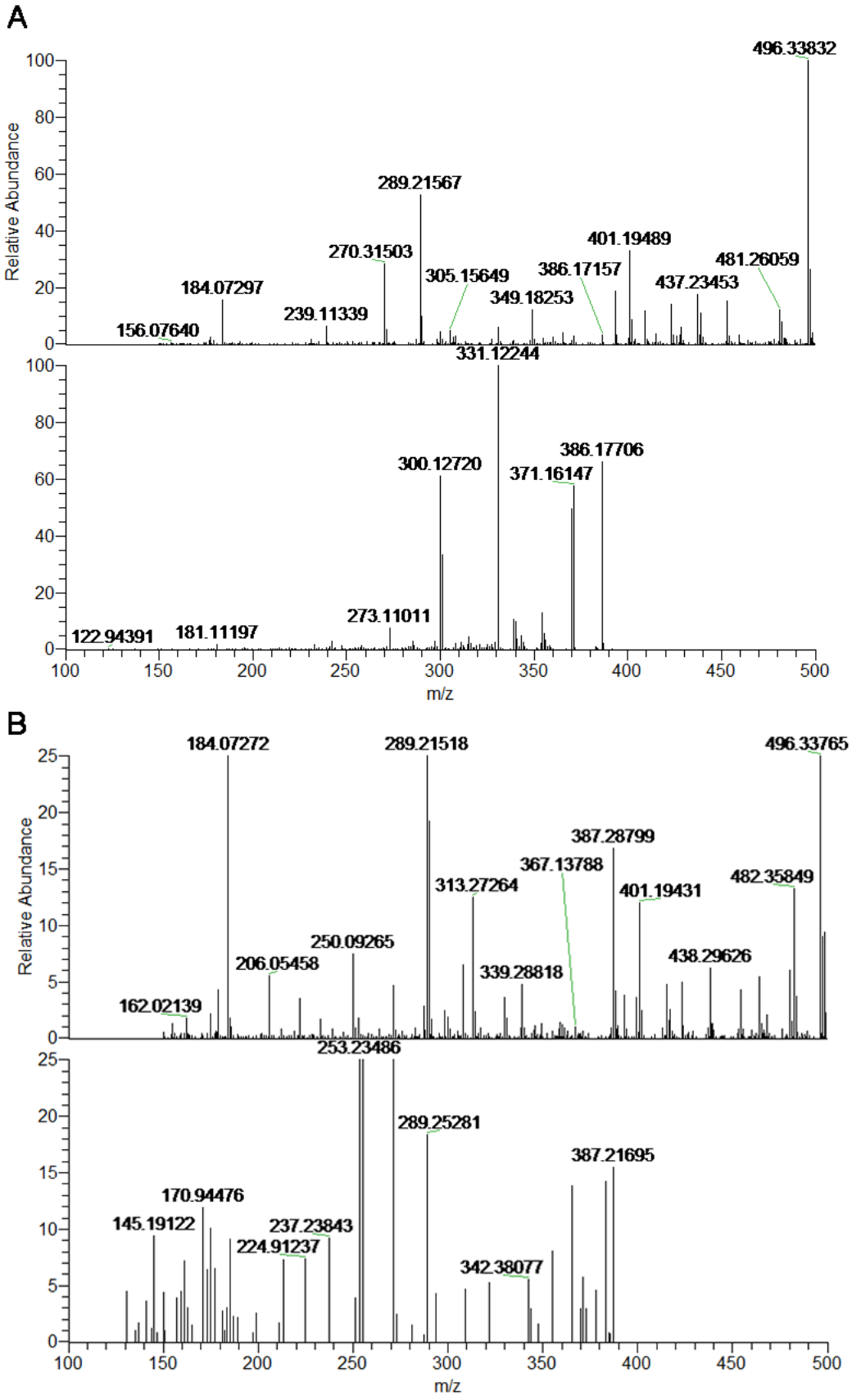

Figure S1. Identification of schisandrin B in mouse plasma and testicular tissue

Notes:

L. Typical mass spectra of mouse plasma after oral administration of schisandrin B (20mg/kg) at 3 h in **Fig.1G**, which was used as identifying schisandrin B structure in plasma.

M. Typical mass spectra of mouse testicular tissue after oral administration of schisandrin B (20mg/kg) at 3 h in **Fig.1I**, which was used for identifying schisandrin B structure in testicular tissue.

Supply Dataset S1

No.	tR (min)	Molecular formula	Caculated mass (m/z)	Experimental mass (m/z)				MS/MS fragments
				N-ion	ppm	P-ion	ppm	
1	0.81	$C_5H_{11}NO_2$	117.08626	—	—	118.08569	-0.6	235$[2M+H]^+$;118$[M+H]^+$; 59$[C_3H_9N]^+$;58$[C_3H_8N]^+$
2	0.87	$C_6H_8O_6$	176.02371	175.02289	-4.7	—	—	175$[M-H]^-$; 115$[C_4H_3O_4]^-$;113$[C_5H_5O_3]^-$
3	1.12	$C_6H_8O_7$	192.01863	191.0189	1.4	—	—	191$[M-H]^-$;173$[M-H-H_2O]^-$; 129$[M-H-H_2O-CO_2]^-$; 111$[M-H-H_2O-COOH-OH]^-$
4	1.12	$C_4H_6O_5$	134.01315	133.01372	4.3	—	—	133$[M+H]^+$;115$[M+H-H_2O]^+$
5	1.28	$C_6H_5NO_2$	123.03931	122.02417	4.2	124.03876	-4.3	122$[M-H]^-$;124$[M+H]^+$; 106 $M+H-H_2O]^+$
6	1.58	$C_{12}H_{16}N_4OS$	264.11176	—	—	265.11071	-3.9	265$[M+H]^+$;156$[C_7H_{10}NOS]^+$; 144$[C_6H_{10}NOS]^+$;122$[C_6H_8N_3]^+$
7	1.97	$C_4H_6O_4$	118.01824	117.01791	-2.8	—	—	117$[M-H]^-$;99$[M-H-H_2O]^-$; 73$[M-H-CO_2]^-$
8	2.68	$C_7H_6O_5$	170.01315	169.01301	-0.8	—	—	169$[M-H]^-$;125$[M-H-CO_2]^-$
9	3.08	$C_4H_6O_6$	150.00806	149.00808	0.1	—	—	149$[M-H]^-$;131$[M-H-H_2O]^-$; 103$[M-H-HCOOH]^-$
10	3.28	$C_{17}H_{20}N_4O_6$	376.12991	375.12988	-0.1	—	—	375$[M-H]^-$;255$[C_{13}H_{11}N_4O_2]^-$; 212$[C_{12}H_{10}N_3O]^-$
11	4.48	$C_7H_6O_3$	138.02332	137.02365	2.4	—	—	137$[M-H]^-$;93$[C_6H_5O]^-$
12	4.66	$C_7H_6O_4$	154.01824	153.01862	2.5	—	—	153$[M-H]^-$;109$[M-H-CO_2]^-$
13	4.74	$C_7H_6O_3$	138.02332	137.02373	3	—	—	137$[M-H]^-$;93$[C_6H_5O]^-$
14	7.2	$C_2H_7NO_3S$	125.00629	124.00638	0.7	—	—	124$[M-H]^-$;80$[SO_3]^-$
15	7.38	$C_8H_8O_4$	168.03389	167.0342	1.9	—	—	167$[M-H]^-$;152$[M-H-CH_3]^-$; 123$[M-H-CO_2]^-$;108$[M-H-CH_3-CO_2]^-$
16	8.35	$C_{16}H_{22}O_{10}$	374.11293	373.11377	2.3	397.10892	-4	373$[M-H]^-$;329$[M-H-CO_2]^-$; 211$[M-H-Glc]^-$;193 $[M-H-Glc-H_2O]^-$; 167$[M-H-Glc-CO_2]^-$; 149$[M-H-Glc-CO_2-H_2O]^-$; 397$[M+Na]^+$;353$[M+Na-CO_2]^+$; 235$[M+Na-Glc]^+$; 217$[M+Na-Glc-H_2O]^+$; 149$[C_7H_{10}O_2Na]^+$

17	8.79	$C_7H_{12}O_6$	192.05502	191.05503	0.1	193.06981	-4.4	191[M-H]$^-$;173[M-H-H_2O]$^-$; 155[M-H-2H_2O]$^-$; 127[M-H-2H_2O-CO]$^-$; 193 [M+H]$^+$;175[M+H-H_2O]$^+$; 157[M+H-2H_2O]$^+$
18	9.68	$C_9H_8O_4$	180.03389	179.03429	2.2	181.04889	-3.5	179[M-H]$^-$;135[M-H-CO_2]$^-$; 181[M+H]$^+$;163[M+H-H_2O]$^+$; 145[M+H-2H_2O]$^+$
19	10.2	$C_{17}H_{23}NO_3$	289.17507	—	—	290.17578	2.4	290[M+H]$^+$;272[M+H-H_2O]$^+$; 260[M+H-HCHO]$^+$; 242[M+H-HCHO-H_2O]$^+$; 124[$C_8H_{14}N$]$^+$;95[C_7H_{11}]$^+$
20	11.91	$C_{10}H_{10}O_4$	194.04954	193.05001	2.4	195.06447	-3.7	193[M-H]$^-$;175[M-H-H_2O]$^-$; 149[M-H-CO_2]$^-$; 134[M-H-CH_3-CO_2]$^-$; 195[M+H]$^+$; 177[M+H-H_2O]$^+$; 145[M+H-H_2O-CH_3-OH]$^+$
21	12.37	$C_{16}H_{18}O_9$	354.08671	353.08731	1.7	355.10097	-3.9	353[M-H]$^-$;191[$C_7H_{11}O_6$]$^-$; 173[$C_7H_9O_5$]$^-$;127[$C_6H_7O_3$]$^-$; 355[M+H]$^+$;163[$C_9H_7O_3$]$^+$; 117[C_8H_5O]$^+$
22	13.1	$C_9H_8O_3$	164.03897	163.03905	0.5	165.05393	-4.2	163[M-H]$^-$;119[M-H-CO_2]$^-$; 165 [M+H]$^+$;147[M+H-H_2O]$^+$
23	13.64	$C_7H_{10}O_5$	174.04445	173.04381	-3.7	—	—	173[M-H]$^-$;155[M-H-H_2O]$^-$; 137[M-H-2H_2O]$^-$;128[M-H-COOH]$^-$
24	13.97	$C_{27}H_{30}O_{17}$	626.24908	625.25148	3.8	—	—	625[M-H]$^-$;463[M-H-Gal]$^-$; 301[M-H-Gal-Glc]$^-$; 300[M-H-Gal-Glc-H]$^{\cdot-}$; 273[M-H-Gal-Glc-CO]$^-$; 255[M-H-Gal-Glc-CO-H_2O]$^-$
25	15.6	$C_9H_6O_4$	178.01824	177.01847	1.3	179.03317	-4	177[M-H]$^-$;149[M-H-CO]$^-$; 133[M-H-CO_2]$^-$;121[M-H-2CO]$^-$; 105[M-H-CO_2-CO]$^-$; 93[M-H-3CO]$^-$;179[M+H]$^+$; 151[M+H-CO]$^+$; 133[M+H-CO-H_2O]$^+$
26	17.17	$C_{22}H_{22}O_{11}$	462.10784	461.10962	3.9	—	—	461[M-H]$^-$;299[M-H-Glu]$^-$; 271[M-H-Glu-CO]$^-$;181[$C_8H_5O_5$]$^-$
27	19.43	$C_{10}H_8O_4$	192.03387	191.03419	1.6	193.04933	-1.1	191[M-H]$^-$;193[M+H]$^+$; 178[M+H-CH_3]$^+$; 165[M+H-CO]$^+$; 161[M+H-CH_3OH]$^+$; 133[M+H-CH_3OH-CO]$^+$
28	19.6	$C_{21}H_{20}O_{12}$	464.08711	463.08829	2.6	—	—	463[M-H]$^-$;301[M-H-Glc]$^-$; 273[M-H-Glc-CO]$^-$;

29	20.93	$C_{22}H_{20}O_{12}$	476.0871	475.08633	-1.6	—	—	257[M-H-Glc-CO_2]$^-$; 167[$C_7H_3O_5$]$^-$ 475[M-H]$^-$;457[M-H-H_2O]$^-$; 433[M-H-CO=CH_2]$^-$; 415[M-H-CH_3COOH]$^-$; 285[M-H-$C_7H_{10}O_6$]$^-$
30	21.62	$C_{15}H_{10}O_7$	302.03428	301.03396	-1.1	—	—	301[M-H]$^-$;273[M-H-CO]$^-$; 257[M-H-CO_2]$^-$;167[$C_7H_3O_5$]$^-$
31	21,78	$C_{26}H_{28}O_{16}$	596.14463	595.14543	1.4	—	—	595[M-H]$^-$;505[M-H-Api+C_2H_2O]$^-$; 463[M-H-Api]$^-$; 445[M-H-Api-H_2O]$^-$; 301[M-H-Api-Gal]$^-$; 300[M-H-Api-Gal-H]$^-$; 273[M-H-Api-Gal-CO]$^-$; 257[M-H-Api-Gal-2CO]$^-$; 179[$C_8H_3O_5$]$^-$
32	21.88	$C_{14}H_6O_8$	302.01355	—	—	303.01321	-1.1	303[M+H]$^+$;285[M+H-H_2O]$^+$; 275[M+H-CO]$^+$; 257[M+H-CO-H_2O]$^+$; 247[M+H-2CO]$^+$;201[$C_{11}H_5O_4$]$^+$
33	22.26	$C_{29}H_{28}O_9$	520.18061	—	—	521.18042	-0.4	521[M+H]$^+$; 399[M+H-C_6H_5COOH]$^+$; 355[M+H-C_6H_5COOH-C_2H_4O]$^+$
34	22.9	$C_{21}H_{20}O_{12}$	464.08711	463.08887	3.8	—	—	463[M-H]$^-$;301[M-H-Gal]$^-$; 255[M-H-Gal-CO-H_2O]$^-$; 151[$C_7H_3O_4$]$^-$;107[$C_6H_3O_2$]$^-$
35	23.02	$C_{16}H_{12}O_6$	300.05502	299.05453	-1.6	—	—	299[M-H]$^-$;271[M-H-CO]$^-$; 255[M-H-CO_2]$^-$; 227[M-H-CO-CO_2]$^-$;181[$C_8H_5O_5$]$^-$
36	23.1	$C_{27}H_{30}O_{16}$	610.14501	609.1479	4.7	—	—	609[M-H]$^-$;343[$C_{17}H_{11}O_8$]$^-$; 301[M-H-Rha-Glc]$^-$; 300[M-H-Rha-Glc-H]$^-$; 271[M-H-Rha-Glc-H-CO-H]$^-$; 255[M-H-Rha-Glc-H-CO-OH]$^-$; 179[$C_8H_3O_5$]$^-$;151[$C_7H_3O_4$]$^-$
37	23.74	$C_{21}H_{20}O_{12}$	464.08711	463.0874	0.6	—	—	463[M-H]$^-$;301[M-H-Glc]$^-$; 255[M-H-Glc-CO-H_2O]$^-$; 151[$C_7H_3O_4$]$^-$;107[$C_6H_3O_2$]$^-$
38	24.2	$C_{29}H_{36}O_{16}$	640.19197	639.19263	1	—	—	639[M-H]$^-$;477[M-H-$C_9H_6O_3$]$^-$; 315[M-H-$C_9H_6O_3$-Glc]$^-$; 153[M-H-$C_9H_6O_3$-2Glc]$^-$; 135[M-H-$C_9H_6O_3$-2Glc-H_2O]$^-$
39	24.95	$C_{27}H_{30}O_{15}$	594.15009	593.1507	1	—	—	593[M-H]$^-$;327[M-H-$C_{10}H_{18}O_8$]$^-$; 285[M-H-Rha-Glc]$^-$; 284[M-H-Rha-Glc-H]$^-$;

								257[M-H-Rha-Glc-CO]$^-$; 255[M-H-Rha-Glc-H-CO-H]$^-$; 229[M-H-Rha-Glc-2CO]$^-$; 227[M-H-Rha-Glc-H-2CO-H]$^-$	
40	25.34	$C_{28}H_{36}O_8$	500.24829	—	—	501.25002	3.4	501[M+H]$^+$;484[M+H-OH]$^{+\cdot}$; 483[M+H-H_2O]$^+$; 384[M+H-OH-C_4H_7COOH]$^{+\cdot}$; 357[M+H-C_4H_7COOH-C_2H_4O]$^+$	
41	25.95	$C_{21}H_{20}O_{11}$	448.09219	447.09378	3.6	—	—	447[M-H]$^-$;285[M-H-Glc]$^-$; 284[M-H-Glc-H]$^{-\cdot}$;257[M-H-Glc-CO]$^-$; 243[M-H-Glc-C_2H_2O]$^-$;241[M-H-Glc-CO_2]$^-$; 197[M-H-Glc-2CO_2]$^-$; 151[$C_7H_3O_4$]$^-$	199[M-H
42	26.1	$C_{28}H_{36}O_8$	500.24829	—	—	501.25071	4.8	501[M+H]$^+$; 484[M+H-OH]$^{+\cdot}$; 483[M+H-H_2O]$^+$; 384[M+H-OH-C_4H_7COOH]$^{+\cdot}$; 357[M+H-C_4H_7COOH-C_2H_4O]$^+$	
43	26.57	$C_{29}H_{36}O_{15}$	624.19707	623.19604	-1.6	—	—	623[M-H]$^-$;461[M-H-$C_9H_6O_3$]$^-$; 315[M-H-$C_9H_6O_3$-Rha]$^-$; 179[$C_9H_7O_4$]$^-$; 153[M-H-$C_9H_6O_3$-Rha-Glc]$^-$; 135[M-H-$C_9H_6O_3$-Rha-Glc-H_2O]$^-$	
44	27.19	$C_{21}H_{20}O_{11}$	448.09219	447.09225	0.1	449.10532	-2.5	447[M-H]$^-$;285[M-H-Glc]$^-$; 151[$C_7H_3O_4$]$^-$;449[M+H]$^+$; 287[M-H-Glc]$^+$; 241[M-H-Glc-CH_2O_2]$^+$; 213[M+H-Glc-$C_2H_2O_3$]$^+$	
45	27.19	$C_{21}H_{20}O_{11}$	448.09219	447.09225	0.1	449.10699	-1.9	447[M-H]$^-$;285[M-H-Glc]$^-$; 267[M-H-Glc-H_2O]$^-$; 255[M-H-Glc-CH_2O]$^-$; 239[M-H-Glc-H_2O-CO]$^-$; 167[$C_7H_3O_5$]$^-$;449[M+H]$^+$; 287[M+H-Glc]$^+$;269[M+H-Glc-H_2O]$^+$; 169[$C_7H_5O_5$]$^+$	
46	27.48	$C_{29}H_{36}O_{15}$	624.19705	623.19794	1.4	625.21005	-4.2	623[M-H]$^-$;477[M-H-Rha]$^-$; 315[M-H-Rha-Glc]$^-$; 300[M-H-Rha-Glc-CH_3]$^{-\cdot}$; 297[M-H-Rha-Gldc-H_2O]$^-$; 285[M-H-Rha-Glc-2CH_3]$^-$; 282[M-H-Rha-Glc-H_2O-CH_3]$^{-\cdot}$; 272[M-H-Rha-Glc-CH_3-CO]$^{-\cdot}$; 257[M-H-Rha-Glc-2CH_3-CO]$^-$; 229[M-H-Rha-Glc-2CH_3-2CO]$^-$; 625[M+H]$^+$;479[M+H-Rha]$^+$; 317[M+H-Rha-Glc]$^+$; 299[M+H-Rha-Glc-H_2O]$^+$;	

47	27.72	$C_{21}H_{20}O_{11}$	448.09219	447.09402	4.1	—	—	281[M+H-Rha-Glc-2H_2O]$^+$; 193[$C_{10}H_9O_4$]$^+$;165[$C_9H_9O_3$]$^+$ 447[M-H]$^-$;301[M-H-Rha]$^-$; 300[M-H-Rha-H]$^{\cdot-}$; 273[M-H-Rha-CO]$^-$; 255[M-H-Rha-CO-H_2O]$^-$
48	27.86	$C_{22}H_{22}O_{12}$	478.10275	477.10461	3.9	—	—	477[M-H]$^-$;315[M-H-Glc]$^-$; 287[M-H-Glc-CO]$^-$; 271[M-H-Glc-CO_2]$^-$;181[$C_8H_5O_5$]$^-$
49	27.93	$C_{21}H_{20}O_{10}$	432.09727	431.09805	1.8	433.11093	-4.6	431[M-H]$^-$;413[M-H-H_2O]$^-$; 285[M-H-Rha]$^-$;267[M-H-Rha-H_2O]$^-$; 169[$C_{12}H_9O$]$^-$; 155[$C_{11}H_7O$]$^-$;433[M+H]$^+$
50	27.97	$C_{21}H_{20}O_{10}$	432.09727	431.09866	3.2			431[M-H]$^-$;269[M-H-Glc]$^-$; 201[M-H-Glc-C_3O_2]$^-$; 151[$C_7H_3O_4$]$^-$
51	27.99	$C_{15}H_{16}O_9$	340.07106	339.07058	-1.4	—	—	339[M-H]$^-$;177[M-H-Glc]$^-$; 133[M-H-Glc-CO_2]$^-$; 105[M-H-Glc-CO_2-CO]$^-$;89[C_7H_5]$^-$
52	28.28	$C_{27}H_{30}O_{14}$	578.15518	577.15662	2.5	579.17073	-0.2	577[M-H]$^-$;457[M-H-$C_4H_8O_4$]$^-$; 431[M-H-Rha]$^-$;413[M-H-Rha-H_2O]$^-$; 269[M-H-Rha-Glc]$^-$; 225[M-H-Rha-Glc-CO_2]$^-$; 183[$C_{12}H_7O_2$]$^-$;579[M+H]$^+$; 433[M+H-Rha]$^+$;415[M+H-Rha-H_2O]$^+$;397[M+H-Rha-2H_2O]$^+$;271[M+H-Rha-C
53	28.28	$C_{28}H_{32}O_{16}$	624.16066	623.16248	2.9	625.17896	4.2	243[M+H-Rha-Glc-CO]$^+$; 225[M+H-Rha-Glc-H_2O-CO]$^+$; 211[M+H-Rha-Glc-H_2O-C_2H_2O]$^+$; 153[$C_7H_5O_4$]$^+$; 119[C_8H_7O]$^+$;91[C_7H_7]$^+$ 623[M-H]$^-$;477[M-H-Rha]$^-$; 315[M-H-Rha-Glc]$^-$; 300[M-H-Rha-Glc-CH_3]$^{\cdot-}$; 272[M-H-Rha-Glc-CH_3-CO]$^{\cdot-}$; 271[M-H-Rha-Glc-CH_3-CO-H]$^-$; 243[M-H-Rha-Glc-CH_3-CO-H-CO]$^-$; 227[M-H-Rha-Glc-CH_3-CO-H-CO_2]$^-$; 151[$C_7H_3O_4$]$^-$;625[M+H]$^+$; 479[M+H-Rha]$^+$;317[M+H-Rha-Glc]$^+$; 302[M+H-Rha-Glc-CH_3]$^{+\cdot}$; 285[M+H-Rha-Glc-CH_3OH]$^+$; 274[M+H-Rha-Glc-CH_3-CO]$^{+\cdot}$; 257[M+H-Rha-Glc-CH_3OH-CO]$^+$; 246[M+H-Rha-Glc-CH_3-2CO]$^{+\cdot}$; 229[M+H-Rha-Glc-CH_3OH-2CO]$^+$; 153[$C_7H_5O_4$]$^+$

54	28.31	$C_{21}H_{18}O_{11}$	446.07654	445.07493	-3.6	—	—	445[M-H]$^-$;269[M-H-GluA]$^-$; 225[M-H-GluA-CO_2]$^-$;167[$C_7H_3O_5$]$^-$
55	29.09	$C_{20}H_{26}O_4$	330.19039	—	—	331.18914	-3.8	331[M+H]$^+$;313[M+H-H_2O]$^+$; 301[M+H-2CH_3]$^+$
56	29.58	$C_{15}H_{10}O_5$	270.0601	269.09566	-1.6	271.05966	-1.7	269[M-H]$^-$;241[M-H-CO]$^-$;225[M-H-CO2]-;197[M-H-CO-CO2]-; 167[C7H3O5 271[M+H]+;253[M+H-H_2O]$^+$;169[$C_7H_5O_5$]$^+$
57	30.7	$C_{28}H_{34}O_{15}$	610.1814	609.18229	1.5	611.1994	3.9	609[M-H]$^-$;505[M-H-$C_4H_8O_3$]$^-$; 463[M-H-Rha]$^-$;445[M-H-Rha-H_2O]$^-$; 427[M-H-Rha-2H_2O]$^-$; 301[M-H-Rha-Glc]$^-$; 286[M-H-Rha-Glc-CH_3]$^{\cdot-}$; 283[M-H-Rha-Glc-H_2O]$^-$; 268[M-H-Rha-Glc-H_2O-CH_3]$^{\cdot-}$; 258[M-H-Rha-Glc-CH_3-CO]$^{\cdot-}$; 257[M-H-Rha-Glc-CO_2]$^-$; 242[M-H-Rha-Glc-CH_3-CO_2]$^{\cdot-}$; 239[M-H-Rha-Glc-H_2O-CO_2]$^-$; 199[$C_{12}H_7O_3$]$^-$;125[$C_6H_5O_3$]$^-$; 611[M+H]$^+$;303[M+H-Rha-Glc]$^+$; 285[M+H-Rha-Glc-H_2O]$^+$; 179[$C_9H_7O_4$]$^+$;177[$C_{10}H_9O_3$]$^+$; 151[$C_8H_7O_3$]$^+$
58	31.48	$C_{23}H_{28}O_8$	432.1857	—	—	433.18538	-0.7	433[M+H]$^+$;415[M+H-H_2O]$^+$; 361[M+H-C_4H_8O]$^+$; 372[M+H-H_2O-CH_3CO]$^+$; 343[M+H-H_2O-C_4H_7OH]$^+$
59	31.54 39.44	$C_{15}H_{10}O_5$	270.04445	269.04353	-3.4	271.06145	4.9	269[M-H]$^-$;241[M-H-CO]$^-$; 227[M-H-C_2H_2O]$^-$; 225[M-H-CO_2]$^-$;201[M-H-C_3O_2]$^-$; 151[$C_7H_3O_4$]$^-$;271[M+H]$^+$; 253[M+H-H_2O]$^+$; 225[M+H-H_2O-CO]$^+$
60	31.71	$C_{28}H_{34}O_{15}$	610.1814	609.18351	3.5	611.20001	4.8	609[M-H]$^-$;463[M-H-Rha]$^-$; 301[M-H-Rha-Glc]$^-$; 286[M-H-Rha-Glc-CH_3]$^{\cdot-}$; 283[M-H-Rha-Glc-H_2O]$^-$; 268[M-H-Rha-Glc-H_2O-CH_3]$^{\cdot-}$; 258[M-H-Rha-Glc-CH_3-CO]$^{\cdot-}$; 257[M-H-Rha-Glc-CO_2]$^-$; 242[M-H-Rha-Glc-CH_3-CO_2]$^{\cdot-}$; 239[M-H-Rha-Glc-H_2O-CO_2]$^-$; 199[$C_{12}H_7O_3$]$^-$;125[$C_6H_5O_3$]$^-$; 611[M+H]$^+$;303[M+H-Rha-Glc]$^+$; 285[M+H-Rha-Glc-H_2O]$^+$;

								179[$C_9H_7O_4$]$^+$;177[$C_{10}H_9O_3$]$^+$;
								151[$C_8H_7O_3$]$^+$
								301[M-H]$^-$;273[M-H-CO]$^-$;
								255[M-H-CO-H_2O]$^-$;
61	32.13	$C_{15}H_{10}O_7$	302.03428	301.03549	4	303.0488	-1.1	151[$C_7H_3O_4$]$^-$;303[M+H]$^+$;
								285[M+H-H_2O]$^+$;257[M+H-H_2O-CO]$^+$;247[M+H-2CO]$^+$;
								229[M+H-H_2O-2CO]$^+$
								593[M-H]$^-$;447[M-H-$C_9H_6O_2$]$^-$;
								285[M-H-$C_9H_6O_2$-Glc]$^-$;
								257[M-H-$C_9H_6O_2$-Glc-CO]$^-$;
62	33.02	$C_{30}H_{26}O_{13}$	594.12897	593.13049	2.6	595.14142	-3.2	229[M-H-$C_9H_6O_2$-Glc-2CO]$^-$;
								151[$C_7H_3O_4$]$^-$;617[M+Na]$^+$;
								595[M+H]$^+$;449[M+H-$C_9H_6O_2$]$^+$;
								287[M+H-$C_9H_6O_2$-Glc]$^+$
								287[M-H]$^-$;269[M-H-H_2O]$^-$;
								259[M-H-CO]$^-$;243[M-H-CO_2]$^-$;
								225[M-H-H_2O-CO_2]$^-$;
63	34.12	$C_{15}H_{12}O_6$	288.05502	287.05549	1.7	289.07002	-2.2	215[M-H-CO-CO_2]$^-$;
								151[$C_7H_3O_4$]$^-$;107[$C_6H_3O_2$]$^-$;
								289[M+H]$^+$;271[M+H-H_2O]$^+$;
								195[$C_9H_7O_5$]$^+$;145[$C_9H_5O_2$]$^+$
								285[M-H]$^-$;257[M-H-CO]$^-$;
								229[M-H-2CO]$^-$;151[$C_7H_3O_4$]$^-$;
64	35.18	$C_{15}H_{10}O_6$	286.03936	285.03995	2.1	287.05359	4.9	309[M+Na]$^+$;287[M+H]$^+$;
								241[M+H-CH_2O_2]$^+$;
								213[M+H-$C_2H_2O_3$]$^+$
								571[2M-H]$^-$;285[M-H]$^-$;
								243[M-H-C_2H_2O]$^-$;241[M-H-CO_2]$^-$;
65	35.31	$C_{15}H_{10}O_6$	286.03936	285.04019	2.9	—	—	199[M-H-C_2H_2O-CO_2]$^-$;
								197[M-H-2CO_2]$^-$;
								171[M-H-C_2H_2O-CO_2-CO]$^-$;
								151[$C_7H_3O_4$]$^-$
								315[M-H]$^-$;287[M-H-CO]$^-$;
66	35.58	$C_{16}H_{12}O_7$	316.04993	315.05117	3.9	—	—	271[M-H-CO_2]$^-$;
								243[M-H-CO-CO_2]$^-$; 181[$C_8H_5O_5$]$^-$
								285[M-H]$^-$;257[M-H-CO]$^-$;
67	35.61	$C_{15}H_{10}O_6$	286.03937	285.03989	1.8	287.05627	4.4	241[M-H-CO_2]$^-$;167[$C_7H_3O_5$]$^-$;
								287[M+H]$^+$;269[M+H-H_2O]$^+$;
								241[M+H-H_2O-CO]$^+$;169[$C_7H_5O_5$]$^+$
								315[M-H]$^-$;300[M-H-CH_3]$^{\cdot-}$;
								272[M-H-CH_3-CO]$^{\cdot-}$;
68	35.61	$C_{16}H_{12}O_7$	316.04993	315.05027	1.1	317.06486	-2.3	271[M-H-CH_3-CO-H]$^-$;
								243[M-H-CH_3-CO-H-CO]$^-$;
								227[M-H-CH_3-CO-H-CO_2]$^-$;
								151[$C_7H_3O_4$]$^-$;107[$C_6H_3O_2$]$^-$;

								339$[M+Na]^+$;317$[M+H]^+$;
								302$[M+H-CH_3]^{+\cdot}$;285$[M+H-CH_3OH]^+$;
								274$[M+H-CH_3-CO]^{+\cdot}$;
								257$[M+H-CH_3OH-CO]^+$;
								246$[M+H-CH_3-2CO]^{+\cdot}$;
								229$[M+H-CH_3OH-2CO]^+$;
								153$[C_7H_5O_4]^+$
								469$[M-H]^-$;423$[M-H-HCOOH]^-$;
69	35.76	$C_{30}H_{46}O_4$	470.34689	469.33251	2.7	471.34509	-3.8	378$[M-H-HCOOH-HCOO]^{-\cdot}$;
								471$[M+H]^+$;456$[M+H-CH_3]^{+\cdot}$;
								453$[M+H-H_2O]^+$;390$[M+H-C_6H_9]^+$
								615$[M-H]^-$;463$[M-H-C_7H_4O_4]^-$;
70	35.89	$C_{28}H_{24}O_{16}$	616.09807	615.10097	4.7	—	—	445$[M-H-C_7H_6O_5]^-$;
								301$[M-H-C_7H_4O_4\text{-Glc}]^-$
								419$[M+H]^+$;401$[M+H-H_2O]^+$;
								384$[M+H-OH-H_2O]^{+\cdot}$;
71	36.79	$C_{23}H_{30}O_7$	418.20643	—	—	419.20477	-4	383$[M+H-2H_2O]^+$;
								369$[M+H-OH-CH_3-H_2O]^+$;
								353$[M+H-OH-OCH_3-H_2O]^+$
								499$[M+H]^+$;399$[M+H-C_4H_7COOH]^+$;
72	37.08	$C_{28}H_{34}O_8$	498.23264	—	—	499.23119	-2.9	369$[M+H-C_4H_7COOH-CH_2O]^+$;
								357$[M+H-C_4H_7COOH-C_3H_6]^+$;
								343$[M+H-C_4H_7COOH-C_4H_8]^+$
								499$[M+H]^+$;399$[M+H-C_4H_7COOH]^+$;
73	38.12	$C_{28}H_{34}O_8$	498.23264	—	—	499.23026	-4.8	369$[M+H-C_4H_7COOH-CH_2O]^+$;
								357$[M+H-C_4H_7COOH-C_3H_6]^+$;
								343$[M+H-C_4H_7COOH-C_4H_8]^+$
								417$[M+H]^+$;402$[M+H-CH_3]^{+\cdot}$;
74	39.28	$C_{23}H_{28}O_7$	416.19078	—	—	417.18903	-4.2	399$[M+H-H_2O]^+$;
								385$[M+H-CH_3OH]^+$;
								373$[M+H-C_2H_4O]^+$
								433$[M+H]^+$;415$[M+H-H_2O]^+$;
								400$[M+H-H_2O-CH_3]^{+\cdot}$;
75	39.38	$C_{24}H_{32}O_7$	432.22209	—	—	433.22103	-2.4	384$[M+H-H_2O-OCH_3]^{+\cdot}$;
								373$[M+H-H_2O-C_3H_6]^+$;
								359$[M+H-H_2O-C_4H_8]^+$
								471$[M+H]^+$;453$[M+H-H_2O]^+$;
76	39.44	$C_{30}H_{46}O_4$	470.34689	—	—	471.34551	-2.9	398$[M+H-CH_2CH_2COOH]^+$;
								370$[M+H-CH_2CH_2COOH-C_2H_4]^+$
								417$[M+H]^+$;402$[M+H-CH_3]^{+\cdot}$;
77	39.6	$C_{23}H_{28}O_7$	416.19078	—	—	417.19103	0.6	399$[M+H-H_2O]^+$;
								385$[M+H-CH_3OH]^+$;
								373$[M+H-C_2H_4O]^+$
								531$[M+H]^+$;
78	40.63	$C_{28}H_{34}O_{10}$	530.22247	—	—	531.22218	-0.6	485$[M+H-CH_2O_2]^+$;

79	40.98	$C_{22}H_{28}O_6$	388.19586	—	—	389.19503	-2.1	401[M+H-$C_6H_{10}O_3$]$^+$; 383[M+H-$C_6H_{10}O_3$-H_2O]$^+$ 389[M+H]$^+$; 374[M+H-CH_3]$^{+\cdot}$; 357[M+H-CH_3OH]$^+$; 342[M+H-CH_3OH-CH_3]$^{+\cdot}$
80	41.02	$C_{30}H_{48}O_5$	488.3418	487.3439	4.3	489.35526	-4.5	487[M-H]-;469[M-H-H_2O]$^-$; 443[M-H-CO_2]$^-$;425[M-H-CO_2-H_2O]$^-$; 407[M-H-CO_2-2H_2O]$^-$; 391[M-H-CO_2-2H_2O-CH_4]$^-$; 489[M+H]$^+$;453[M+H-2H_2O]$^+$
81	41.22	$C_{30}H_{48}O_5$	488.3418	487.34378	4	489.35544	-4.1	487[M-H]$^-$;469[M-H-H_2O]$^-$; 443[M-H-CO_2]$^-$; 425[M-H-CO_2-H_2O]$^-$; 407[M-H-CO_2-2H_2O]$^-$; 391[M-H-CO_2-2H_2O-CH_4]$^-$; 489[M+H]$^+$;453[M+H-2H_2O]$^+$
82	41.75	$C_{15}H_{24}N_2O_2$	264.10889	—	—	265.18997	-4	265[M+H]$^+$; 248[M+H-OH]$^{+\cdot}$;247[M+H-H_2O]$^+$
83	42.18	$C_{30}H_{46}O_3$	454.35198	—	—	455.35025	-3.8	455[M+H]$^+$; 409[M+H-HCOOH]$^+$; 313[M+H-$C_8H_{14}O_2$]$^+$
84	42.32	$C_{30}H_{48}O_5$	488.3418	487.34302	2.5	489.35529	-4.4	487[M-H]$^-$;469[M-H-H_2O]$^-$; 443[M-H-CO_2]$^-$; 425[M-H-CO_2-H_2O]$^-$; 407[M-H-CO_2-2H_2O]$^-$; 391[M-H-CO_2-2H_2O-CH_4]$^-$; 489[M+H]$^+$;453[M+H-2H_2O]$^+$
85	42.89	$C_{12}H_{16}N_2O$	204.13354	—	—	205.13409	2.6	205[M+H]$^+$;146[M+H-C_3H_9N]$^+$; 108[C_6H_6NO]$^+$
86	43.27	$C_{23}H_{28}O_6$	400.19587	—	—	401.19434	-3.8	401[M+H]$^+$;386[M+H-CH_3]$^{+\cdot}$; 370[M+H-OCH_3]$^{+\cdot}$; 355[M+H-CH_3-OCH_3]$^+$; 339[M+H-2CH_3-CH_3OH]$^+$
87	43.3	$C_{30}H_{34}O_8$	522.23265	—	—	523.23095	-3.2	523[M+H]$^+$;505[M+H-H_2O]$^+$; 401[M+H-C_6H_5COOH]$^+$; 383[M+H-C_6H_5COOH-H_2O]$^+$
88	45.42	$C_{23}H_{30}O_6$	402.21151	—	—	403.21011	-3.5	403[M+H]$^+$;372[M+H-OCH_3]$^{+\cdot}$; 371[M+H-CH_3OH]$^+$; 356[M+H-CH_3OH-CH_3]$^{+\cdot}$; 340[M+H-OCH_3-CH_3OH]$^{+\cdot}$
89	45.44	$C_{23}H_{28}O_7$	416.19078	—	—	417.18957	-2.9	417[M+H]$^+$;399[M+H-H_2O]$^+$; 343[M+H-C_4H_8-H_2O]$^+$;

								307[M+H-2CH_3-2OCH_3-H_2O]$^+$
								515[M+H]$^+$; 415[M+H-C_4H_7COOH]$^+$;
90	45.67	$C_{28}H_{34}O_9$	514.22755	—	—	515.22559	-3.8	385[M+H-C_4H_7COOH-CH_2O]$^+$;
								367[M+H-C_4H_7COOH-CH_2O-H_2O]$^+$;
								353[M+H-C_4H_7COOH-CH_2O-CH_3OH]$^+$
								537[M+H]$^+$;
91	46.06	$C_{30}H_{32}O_9$	536.21191	—	—	537.20981	-3.9	415[M+H-C_6H_5COOH]$^+$;
								397[M+H-C_6H_5COOH-H_2O]$^+$;
								373[M+H-C_6H_5COOH-C_3H_6]$^+$
								471[M-H]$^-$;453[M-H-H_2O]$^-$;
92	46.58	$C_{30}H_{48}O_4$	472.34689	471.34872	3.9	473.30911	-3.7	427[M-H-CO_2]$^-$;391[M-H-CO_2-2H_2O]$^-$; 473[M+H]$^+$;455[M+H-H_2O]$^+$;
								203[$C_{15}H_{23}$]$^+$;105[C_8H_9]$^+$
								271[M+Na]$^+$;249[M+H]$^+$;
93	46.73	$C_{15}H_{24}N_2O$	248.19614	—	—	249.19699	2.2	231[M+H-H_2O]$^+$;
								150[$C_{10}H_{16}N$]$^+$;148[$C_{10}H_{14}N$]$^+$
								387[M+H]$^+$; 372[M+H-CH_3]$^{+\cdot}$;
94	47.28	$C_{22}H_{26}O_6$	386.18022	—	—	387.17992	-0.8	369[M+H-H_2O]$^+$;
								357[M+H-CH_2O]$^+$;
								329[M+H-$C_2H_2O_2$]$^+$
								471[M-H]$^-$;453[M-H-H_2O]$^-$;
95	47.52	$C_{30}H_{48}O_4$	472.34689	471.34863	3.7	473.36044	-4.4	427[M-H-CO_2]$^-$;
								391[M-H-CO_2-2H_2O]$^-$; 473[M+H]$^+$;455[M+H-H_2O]$^+$;
								203[$C_{15}H_{23}$]$^+$;105[C_8H_9]$^+$
								387[M+H]$^+$;372[M+H-CH_3]$^{+\cdot}$;
96	47.53	$C_{22}H_{26}O_6$	386.18022	—	—	387.17847	-4.5	369[M+H-H_2O]$^+$;357[M+H-CH_2O]$^+$;
								329[M+H-$C_2H_2O_2$]$^+$
								515[M+H]$^+$;415[M+H-C_4H_7COOH]$^+$;
97	47.76	$C_{28}H_{34}O_9$	514.22755	—	—	515.22716	-0.7	385[M+H-C_4H_7COOH-CH_2O]$^+$;
								367[M+H-C_4H_7COOH-CH_2O-H_2O]$^+$;
								353[M+H-C_4H_7COOH-CH_2O-CH_3OH]$^+$
								455[M-H]$^-$;407[M-H-HCHO-H_2O]$^-$;
								391[M-H-HCHO-H_2O-CH_4]$^-$;
98	48.01	$C_{30}H_{48}O_3$	456.36763	455.35424	4.9	457.36636	-2.7	479[M+Na]$^+$;457[M+H]$^+$;
								439[M+H-H_2O]$^+$;
								393[M+H-HCOOH-H_2O]$^+$
								387[M+H]$^+$;372[M+H-CH_3]$^{+\cdot}$;
99	48.24	$C_{22}H_{26}O_6$	386.18022	—	—	387.17883	-3.6	369[M+H-H_2O]$^+$;
								357[M+H-CH_2O]$^+$;
								329[M+H-$C_2H_2O_2$]$^+$
								455[M-H]$^-$;407[M-H-HCHO-H_2O]$^-$;
100	48.4	$C_{30}H_{48}O_3$	456.36763	455.35394	4.3	457.36612	-3.3	391[M-H-HCHO-H_2O-CH_4]$^-$;

101	48.65	$C_{22}H_{26}O_6$	386.18022	—	—	387.17877	-3.7	479$[M+Na]^+$; 457$[M+H]^+$; 457$[M+H-H_2O]^+$; 393$[M+H-HCOOH-H_2O]^+$ 387$[M+H]^+$;372$[M+H-CH_3]^{+\cdot}$; 369$[M+H-H_2O]^+$; 357$[M+H-CH_2O]^+$; 329$[M+H-C_2H_2O_2]^+$
102	49.79	$C_{24}H_{32}O_6$	416.22714	—	—	417.22649	-1.6	417$[M+H]^+$;402$[M+H-CH_3]^{+\cdot}$; 386$[M+H-OCH_3]^{+\cdot}$; 370$[M+H-CH_3-CH_3OH]^{+\cdot}$; 347$[M+H-CH_3-C_4H_7]^+$; 316$[M+H-C_6H_{13}O]^{+\cdot}$; 286$[M+H-C_6H_{13}O-2CH_3]^{+\cdot}$; 273$[M+H-C_6H_{13}O-CH_3-CO]^+$; 227$[M+H-C_6H_{13}O-CO-OCH_3-2CH_3]^+$
103	52.02	$C_{16}H_{14}O_4$	270.09649	—	—	271.09595	-2	271$[M+H]^+$;203$[M+H-C_5H_8]^+$; 175$[M+H-C_5H_8-CO]^+$; 159$[M+H-C_5H_8-CO_2]^+$
104	53.29	$C_{30}H_{32}O_8$	520.21701	—	—	521.21559	-2.7	521$[M+H]^+$;399$[M+H-C_6H_5COOH]^+$; 369$[M+H-C_6H_5COOH-CH_2O]^+$; 357$[M+H-C_6H_5COOH-C_3H_6]^+$; 343 $M+H-C_6H_5COOH-C_4H_8]^+$
105	53.43	$C_{22}H_{24}O_6$	384.16457	—	—	385.16406	-1.3	385$[M+H]^+$;370$[M+H-CH_3]^{+\cdot}$; 355$[M+H-CH_2O]^+$
106	54.08	$C_{30}H_{32}O_8$	520.21701	—	—	521.21611	-1.7	521$[M+H]^+$;399$[M+H-C_6H_5COOH]^+$; 369$[M+H-C_6H_5COOH-CH_2O]^+$; 357$[M+H-C_6H_5COOH-C_3H_6]^+$; 343 $M+H-C_6H_5COOH-C_4H_8]^+$

Notes: Glc : -D-glucose, GluA: Glucuronic acid, Xyl:-D-xylose, Rha: L-rhamnose; Gal: D-galactose; Api: D-apiose, ppm: difference between calculated and found mass.

Supply Dataset S2

Chemical name	Molecular formular	CAS No.	Similarity score of drug molecular structure	Rank
Schisantherin D	C29H28O9	64917-82-4	0.473	Weak
Gomisin H	C23H30O7	66056-20-0	0.763	Good
Angeloylisogomisin O	C28H34O8	83864-70-4	0.342	Weak
Gomisin O	C23H28O7	72960-22-6	0.806	Good
Epigomisin O	C23H28O7	73036-31-4	0.806	Good
Gomisin D	C28H34O10	60546-10-3	0.57	Moderate
Gomisin J	C22H28O6	66280-25-9	0.782	Good
Schisanhenol	C23H30O6	69363-14-0	0.814	Good
Schisantherin B	C28H34O9	58546-55-7	0.406	Weak
Schisantherin A	C30H32O9	58546-56-8	0.415	Weak
Gomisin L1	C22H26O6	82425-43-2	0.814	Good
Gomisin L2	C22H26O6	82425-44-3	0.814	Good
Gomisin M2	C22H26O6	82425-45-4	0.814	Good

Schisantherin C	C28H34O9	58546-55-7	0.406	Weak
Deoxyschizandrin	C24H32O6	61281-38-7	0.64	Moderate
Schizandrin C	C22H24O6	61301-33-5	0.736	Good
Oleanolic acid	C30H48O3	508-02-1	0.53	Moderate
Vanillic acid	C8H8O4	121-34-6	0.696	Good
Hyperoside	C21H20O12	482-36-0	0.603	Moderate
Baicalin	C21H18O11	21967-41-9	0.362	Weak
Ursolic acid	C30H48O3	77-52-1	0.443	Weak
Succinic acid	C4H6O4	110-15-6	0.53	Moderate
Abromine	C5H11NO2	107-43-7	0.361	Weak
Ascorbic acid	C6H8O6	50-81-7	0.373	Weak
Nicotinic acid	C6H5NO2	59-67-6	0.584	Moderate
Thiamine	C12H17N4OS	70-16-6	0.806	Good
Riboflavin	C17H22N4O6	83-88-5	0.413	Weak
Taurine	C2H7NO3S	107-35-7	0.451	Weak
Caffeic acid	C9H8O4	331-39-5	0.475	Weak
Atropine	C17H23NO3	51-55-8	0.839	Good

Chlorogenic acid	C16H18O9	906-33-2	0.236	Weak
Scopoletin	C10H8O4	92-61-5	0.542	Moderate
Isorhamnetin-3-O-β-D -rutinoside	C28H32O16	no	0.18	Weak
citric acid	C6H8O7	10540-29-1	0.397	Weak
Malic acid	C4H6O5	617-48-1	0.459	Weak
Tartaric acid	C4H6O6	526-83-0	0.382	Weak
Protocatechuic acid	C7H6O4	99-50-3	0.014	Weak
Quinic acid	C7H12O6	77-95-2	0.317	Weak
Meso-dihydroguaiaretic acid	C20H26O4	66322-34-7	0.736	Good
Schizandradiol	C23H28O8	no	no	no
Angeloygomisin O	C28H34O8	83864-69-1	0.342	Weak
Schisandrin	C24H32O7	7432-28-2	0.738	Good
Kadsuric acid	C30H46O4	62393-88-8	no	no
Schizandrin B	C23H28O6	61281-37-6	0.714	Good
Benzoylgomisin H	C30H34O8	66056-23-3	0.306	Weak
Schisandrol B	C23H28O7	58546-54-6	0.811	Good
Benzoyisolgomisin O	C30H32O8	83864-71-5	0.347	Weak

Gallic acid	C7H6O5	149-91-7	0.466	Weak
Salicylic acid	C7H6O3	69-72-7	0.613	Moderate
4-Hydroxybenzoic acid	C7H6O3	99-96-7	0.613	Moderate
Ferulic Acid	C10H10O4	537-98-4	0.718	Good
Shikimic acid	C7H10O5	138-59-0	0.402	Weak
Esculetin	C9H6O4	305-01-1	0.358	Weak
Ellagic acid	C14H6O8	476-66-4	0.327	Weak
Rutin	C27H30O16	153-18-4	0.173	Weak
Kaempferol-3-O-rutinoside	C27H30O15	17650-84-9	0.196	Weak
Astragalin	C21H20O11	480-10-4	0.332	Weak
Quercitrin	C21H20O11	522-12-3	0.326	Weak
Kaempferol-7-O-α- L -rhamnoside	C21H20O10	20196-89-8	0.409	Weak
Esculin	C15H16O9	531-75-9	0.362	Weak
Neohesperidin	C28H34O15	13241-33-3	0.199	Weak
Hesperidin	C28H34O15	520-26-3	0.199	Weak
Quercetin	C15H10O7	117-39-5	0.506	Moderate
Tiliroside	C30H26O13	20316-62-5	0.147	Weak

Aromadendrin	C15H12O6	480-20-6	0.636	Moderate
Kaempferol	C15H10O6	520-18-3	0.637	Moderate
2α,3α,19α,-Trihydroxyolean-12-ene-28-oic acid	C30H48O5	no	no	no
Arjunolic acid	C30H48O5	465-00-9	no	no
Euscaphic acid	C30H48O5	53155-25-2	0.411	Weak
Ganwuweizic acid	C30H46O3	17990-42-0	0.457	Weak
Maslinic acid	C30H48O4	4373-41-5	0.177	Weak
Imperatorin	C16H14O4	482-44-0	0.486	Weak
Quercetin-3-O-β-D-galactoside-7-O-β-glucoside	C27H30O17	no	0.148	Weak
Quercetin-3-O-β- D -galactosyl-(1Ñ2)-β- D -apioside	C26H28O16	no	0.169	Weak
Isorhamnetin	C16H12O7	480-19-3	0.668	Moderate
Sophoranol	C15H24N2O2	3411-37-8	0.699	Good
N-methylcytisine	C12H16N2O	486-86-2	0.562	Moderate
Matrine	C15H24N2O	519-02-8	0.647	Moderate
Geniposidic acid	C16H22O10	27741-01-1	0.288	Weak
Homoplantaginin	C22H22O11	17680-84-1	0.364	Weak
6-Hydroxyluteolin-7-O-glucoside	C21H20O12	no	0.275	Weak

6-hydroxyluteolin	C15H10O7	18003-33-3	0.509	Moderate
Hispidulin	C16H12O6	1447-88-7	0.788	Good
Plantamajoside	C29H36O16	104777-68-6	0.08	Weak
Luteoloside	C21H20O11	20344-46-1	no	no
Acteoside	C29H36O15	61276-17-3	0.094	Weak
Plantaginin	C21H20O11	26046-94-6	0.318	Weak
Nepetin-7-O-glucoside	C22H22O12	569-90-4	no	no
Apigenin-7-O-glucoside	C21H20O10	578-74-5	0.4	Weak
Rhoifolin	C27H30O14	17306-46-6	no	no
Baicalein	C15H10O5	491-67-8	0.693	Good
Apigenin	C15H10O5	520-36-5	0.74	Good
Iuteolin	C15H10O6	491-70-3	0.598	Moderate
Scutellarein	C15H10O6	529-53-3	0.598	Moderate
Gomisin M 1	C22H26O6	82467-50-3	0.814	Good
Isoquercitrin	C21H20O12	482-35-9	0.275	Weak
p-Coumaric acid	C9H8O3	501-98-4	no	no
Benzoylgomisin O	C30H32O8	130783-32-3	0.347	Weak

Corosolic acid	C28H34O15	no	no	no
Nigranoic acid	C30H46O4	39111-07-4	no	no
Kaempferol-3-O-β-D-glucuronic acid methyl ester	C22H20O12	no	no	no
Tigloylgomisin H	C28H36O8	66069-55-4	no	no
Angeloygomisin H	C28H36O8	no	no	no
Methylhesperidin	C29H36O15	11013-97-1	no	no
Nepetin	C16H12O7	520-11-6	no	no
2”-O-Galloylhyperoside	C28H24O16	no	no	no

Supply Dataset S3

Chemical name	Type	Factor 1(Similarity score of drug molecular structure)	Factor 2 (Peak abundance)	FAC1_1	FAC2_1	Factor comprehensive score
Schisandrin B	Phenylpropanoids	0.714	340876	3.53928	-0.85662	1.751
Epigomisin O	Phenylpropanoids	0.806	151336	1.32027	0.88705	1.144
Schisandrin	Phenylpropanoids	0.738	183899	1.59647	-0.52995	0.731
Schisandrol B	Phenylpropanoids	0.811	82017	0.45109	0.91145	0.638
Schisanhenol	Phenylpropanoids	0.814	76773	0.39061	0.96943	0.626
Atropine	Alkaloids	0.839	13608	-0.36027	1.42935	0.368
Thiamine	Alkaloids	0.806	41147	-0.07737	0.75568	0.262
Gomisin J	Phenylpropanoids	0.782	53591	0.03218	0.2567	0.124
Hispidulin	Flavonoids	0.788	44121	-0.07587	0.37386	0.107
Gomisin M2	Phenylpropanoids	0.814	9399	-0.46396	0.8891	0.087
Gomisin M1	Phenylpropanoids	0.814	8546	-0.47478	0.88809	0.08

Gomisin L1	Phenylpropanoids	0.814	6791	-0.49704	0.886	0.066
GomisinL2	Phenylpropanoids	0.814	6283	-0.50349	0.88539	0.062
Gomisin O	Phenylpropanoids	0.806	10447	-0.46677	0.71908	0.016
Gomisin H	Phenylpropanoids	0.763	12396	-0.52858	-0.19919	-0.395
Apigenin	Flavonoids	0.74	10053	-0.60458	-0.69439	-0.641
Meso-dihydroguaiaretic acid	Phenylpropanoids	0.736	11459	-0.59479	-0.77835	-0.669
Schizandrin C	Phenylpropanoids	0.736	10777	-0.60344	-0.77916	-0.675
Ferulic Acid	Organic Acids	0.718	13077	-0.61049	-1.16178	-0.835
Vanillic acid	Organic Acids	0.696	37631	-0.34332	-1.60351	-0.856
Sophoranol	Alkaloids	0.699	30676	-0.4255	-1.54758	-0.882
Baicalein	Flavonoids	0.693	10015	-0.69964	-1.70066	-1.107

Supply Dataset S4

Gene ID	Gene Symbol	Type	log2 (Wuzi-Pills/Oligoasthenospermia)	log2 (Schisandrin B/Oligoasthenospermia)
100040714	Gm20851	mRNA	-10.54888	-5.48624
100042182	Gm16434	mRNA	5.39069	6.0042
108168641	LOC108168641	mRNA	12.19671	12.13524
BGIG10090_47792	BGIG10090_47792	mRNA	8.86181	2.58767
105244999	Gm40514	mRNA	6.73684	6.97996
BGIG10090_45257	BGIG10090_45257	mRNA	5.81453	6.24157
665301	Gm20773	mRNA	5.73883	5.76591
BGIG10090_47894	BGIG10090_47894	mRNA	5.56296	1.59608
108168516	LOC108168516	mRNA	-5.31167	-5.89289
BGIG10090_47825	BGIG10090_47825	mRNA	-5.42078	5.13063
622731	Gm6348	mRNA	-5.42444	-6.00566
108168520	LOC108168520	mRNA	-5.47526	-6.05648
20115	Rps7	mRNA	-5.57677	-6.15798
BGIG10090_47991	BGIG10090_47991	mRNA	-5.65804	-6.23926
BGIG10090_45048	BGIG10090_45048	mRNA	-5.68694	1.74709
BGIG10090_47959	BGIG10090_47959	mRNA	-5.7074	-6.28862

108168586	LOC108168586	mRNA	-5.75659	-6.3378
108168651	LOC108168651	mRNA	-5.78039	-6.3616
108168648	LOC108168648	mRNA	-5.90437	-6.48558
108168603	LOC108168603	mRNA	-5.91808	-6.12079
100040160	Gm20826	mRNA	-6.01367	-6.59489
100041375	Cyp3a41b	mRNA	-6.01944	-6.60066
108168585	LOC108168585	mRNA	-6.16543	-6.74665
108168583	LOC108168583	mRNA	-6.40926	-6.99048
102637099	Gm38495	mRNA	-6.50759	-1.95761
100043684	Amy2a4	mRNA	-6.96431	-1.94919
108168606	LOC108168606	mRNA	-6.97232	-1.41439
100040899	Gm15142	mRNA	-6.98866	-3.32956
BGIG10090_46013	BGIG10090_46013	mRNA	-7.00788	-7.5891
BGIG10090_45751	BGIG10090_45751	mRNA	-7.04943	-3.37062
230500	Efcab7	mRNA	-7.0583	-1.62561
20084	Rps18	mRNA	-7.46401	-5.08943
BGIG10090_47835	BGIG10090_47835	mRNA	-7.69446	-8.27568
BGIG10090_45898	BGIG10090_45898	mRNA	-7.82891	-8.41013

12651	Chkb	mRNA	-7.9024	-3.98664
BGIG10090_44799	BGIG10090_44799	mRNA	-8.06015	-3.82476
100039595	Gm20807	mRNA	-8.41629	-8.99751
BGIG10090_46074	BGIG10090_46074	mRNA	-8.58529	-4.278
102640368	Gm15674	mRNA	-8.62526	-5.63359
BGIG10090_45242	BGIG10090_45242	mRNA	-11.23838	-1.77414
100862314	Gm21637	mRNA	6.22448	5.2836
108168600	LOC108168600	mRNA	-5.55738	2.5138
BGIG10090_47790	BGIG10090_47790	mRNA	13.10267	5.12147
BGIG10090_47859	BGIG10090_47859	mRNA	5.3161	5.04207
624049	Gm6468	mRNA	-5.64984	-1.97858
108168524	LOC108168524	mRNA	-5.86882	-1.73195
22236	Ugt1a2	mRNA	-5.45658	-1.88561
BGIG10090_45069	BGIG10090_45069	mRNA	-6.27203	-1.24444
545758	Gm5868	mRNA	-6.64155	-3.74884
BGIG10090_48016	BGIG10090_48016	mRNA	8.65147	4.46182
394432	Ugt1a7c	mRNA	6.47738	4.46054
11946	Atp5a1	mRNA	-7.16296	-0.70526

16580	Kifc5b	mRNA	5.54562	4.08524
193322	Oog1	mRNA	-5.90371	-1.01895
100041345	Gm20870	mRNA	-7.80072	-0.45665
624931	LOC624931	mRNA	-9.645	-0.92015
BGIG10090_45072	BGIG10090_45072	mRNA	5.26726	2.83998
105244034	LOC105244034	mRNA	-8.83642	-3.77378
17842	Mup3	mRNA	-5.32889	-1.11206
666376	Gm8068	mRNA	-6.38029	0.56956
BGIG10090_47559	BGIG10090_47559	mRNA	5.39647	1.73268
BGIG10090_45524	BGIG10090_45524	mRNA	-6.18177	-0.73796
668727	Mrgpra2a	mRNA	-5.39625	-0.76957
BGIG10090_47989	BGIG10090_47989	mRNA	8.30215	0.99706
BGIG10090_48064	BGIG10090_48064	mRNA	-5.68923	0.35687
BGIG10090_36112	BGIG10090_36112	mRNA	5.8074	0.53155
58803	Pga5	mRNA	6.03846	-0.61243
545477	Bpifa6	mRNA	6.20911	0.45796
108168468	LOC108168468	mRNA	7.14693	0.42116
BGIG10090_45925	BGIG10090_45925	mRNA	6.86066	-0.30559

BGIG10090_46224	BGIG10090_46224	mRNA	-6.16324	0.09223
BGIG10090_47896	BGIG10090_47896	mRNA	7.63756	0.03853
545649	Gm13276	mRNA	6.61984	no
100040894	Gm16430	mRNA	5.07732	no
BGIG10090_47893	BGIG10090_47893	mRNA	5.35077	no
100042304	Gm38418	mRNA	5.40575	no
BGIG10090_47886	BGIG10090_47886	mRNA	5.51167	no
108169043	Gm46911	mRNA	5.55915	no
108168668	LOC108168668	mRNA	5.55014	no
BGIG10090_46858	BGIG10090_46858	mRNA	11.37959	no
619547	Rpl34-ps1	mRNA	11.11309	no
12263	C2	mRNA	9.89173	no
BGIG10090_47569	BGIG10090_47569	mRNA	8.77505	no
100039789	Gm12407	mRNA	8.45301	no
BGIG10090_47432	BGIG10090_47432	mRNA	8.39924	no
108168176	Gm10338	mRNA	8.29576	no
209324	Gm4758	mRNA	7.61984	no
19702	Ren2	mRNA	7.50748	no

100861756	Gm21191	mRNA	7.41992	no
402728	Pax6os1	mRNA	7.11171	no
BGIG10090_47571	BGIG10090_47571	mRNA	7.08045	no
100043686	Amy2a3	mRNA	6.66478	no
100034729	Gm15114	mRNA	6.14194	no
20445	St6galnac1	mRNA	6.02229	no
100039848	Gm2457	mRNA	5.92043	no
100040035	Gm2564	mRNA	5.92043	no
100041054	Gm3115	mRNA	5.7412	no
16198	Il9	mRNA	5.72368	no
BGIG10090_48074	BGIG10090_48074	mRNA	5.69943	no
108168467	Gm46683	mRNA	5.40835	no

Supply Dataset S5-1

Group	concentration(10^6/ML)	Sperm mobility(%)	Progressive mobile sperm(%)
Normal	46.67	70.484	69.776
Normal	27.395	68.584	67.994
Normal	40.83	62.113	61.293

Normal	42.86	70.125	68.125
Normal	39.5	64.531	63.087
Normal	29.095	63.312	62.683
Oligoasthenospermia	8.89	16.536	16.142
Oligoasthenospermia	9.985	32.407	29.938
Oligoasthenospermia	10.145	31.959	29.897
Oligoasthenospermia	8.71	33.811	31.805
Oligoasthenospermia	10.235	31.637	30.182
Oligoasthenospermia	7.73	24.813	30.488
Schisandrin B	61.54	70.035	69.085
Schisandrin B	27.195	50.474	50.237
Schisandrin B	29.735	48.831	47.792
Schisandrin B	90.39	87.414	86.726
Schisandrin B	31.015	59.498	58.173
Schisandrin B	55.92	78.547	77.832
Wuzi-Pills	13.905	43.956	43.956
Wuzi-Pills	24.17	53.56	52.204
Wuzi-Pills	21.285	26.751	26.212

Wuzi-Pills	27.7	47.887	46.197
Wuzi-Pills	52.14	78.838	78.285
Wuzi-Pills	26.63	44.637	43.153
Testosterone propionate	13.735	27.5	26.429
Testosterone propionate	34.46	71.243	70.686
Testosterone propionate	10.375	39.759	39.157
Testosterone propionate	14.76	23.454	22.939
Testosterone propionate	7.38	16.713	13.314
Testosterone propionate	21.285	21.122	22.066

Supply Dataset S5-2

Group	VCL(μm/s)	VSL(μm/s)	VAP(μm/s)	STR(%)	LIN(%)	BCF(Hz)	ALH(μm)
Normal	67.64	33.03	44.2	0.53	43.46625	7.71	8.3
Normal	75.99	33.23	45.84	0.57	41.804	7.89	9.11
Normal	79.49	23.89	34.54	0.46	38.86449	6.2	7.63
Normal	61.47	29.38	39.81	0.53	43.37172	7.03	7.88
Normal	63.27	26.15	36.66	0.47	41.3308	6.72	7.39
Normal	72.01	25.69	40.19	0.48	34.7618	7.66	8.37

Oligoasthenospermia	20.83	9.59	12.2	0.15	46.03937	1.99	2.11
Oligoasthenospermia	26.43	13.6	17.66	0.27	51.45668	3.59	2.98
Oligoasthenospermia	32.03	16.41	20.31	0.29	51.23322	3.94	3.21
Oligoasthenospermia	30.22	16.47	20.43	0.29	54.50033	4.19	3.21
Oligoasthenospermia	32.62	17.39	20.98	0.31	53.31085	4.48	3.33
Oligoasthenospermia	25.97	11.98	19.15	0.24	50.66102	4.03	2.43
Schisandrin B	77.91	24.81	40.22	0.51	31.84444	8.56	9.07
Schisandrin B	54.84	18.97	28.44	0.41	34.59154	6.74	6.03
Schisandrin B	55.73	17.94	28.55	0.39	32.19092	6.49	6.05
Schisandrin B	84.59	26.76	42.98	0.54	31.63495	8.95	9.71
Schisandrin B	62.27	24.38	34.51	0.48	39.15208	8.56	6.29
Schisandrin B	80.69	29.28	40.95	0.5	31.8379	7.76	9.49
Wuzi-Pills	58.95	24.1	32.66	0.42	40.8821	6.92	5.81
Wuzi-Pills	62.21	25.25	34.39	0.48	40.58833	7.26	6.78
Wuzi-Pills	51.92	24.95	31.81	0.44	48.0547	5.83	5.48
Wuzi-Pills	66.87	21.23	32.56	0.44	31.74817	7.68	6.84
Wuzi-Pills	87.3	34.94	48.85	0.57	40.02291	8.71	9.27
Wuzi-Pills	58.43	26.96	36.96	0.41	40.21053	7.47	7.49

Testosterone propionate	40.49	19.24	25.08	0.32	47.51791	4.19	4.47
Testosterone propionate	62.4	31.39	40.72	0.46	43.70419	5.67	7.34
Testosterone propionate	51.14	30.37	31.69	0.43	57.15092	6.32	3.63
Testosterone propionate	46.74	23.61	32.09	0.4	51.48774	5.79	5.21
Testosterone propionate	10.07	6.51	8.17	0.14	64.64747	1.93	0.98
Testosterone propionate	39.97	13.72	20.41	0.24	58.61544	5.25	4.44

Supply Dataset S6

Group	Male mice	Female mice	Total pups of 6 females	AOA	Pregnant mice	Nonpregnant mice	LSF(%)
Control	3	6	40	8	5	1.0	83.3
Model	3	6	4	2	2	4.0	33.3
SchisandrinB	3	6	34	6.8	5	1.0	83.3

Wuzi-Pills	3	6	33	8.25	4	2.0	66.7
Testosterone	3	6	10	5	2	4.0	33.3

Note：M, male;
F, female.
Average offspring amount (AOA) was calculated as the total number of offspring divided by the number of females that gave birth to offspring.
Litter size of female mice (LSF) was calculated as the ratio of the number of females that gave birth to offspring to total number of females.

Supply Dataset S7

GO_P Term ID	GO_P Term Desc_real	GO_P Term Level1	GO_P Term Level2	Term Candidate Gene Num	Total Candidate Gene Num	Term Gene Num	Total Gene Num	Rich Ratio	P value	Q value
GO:0007276	Gamete generation	biological_process	reproduction	76	1885	211	22257	0.3601896	4.17E-29	1.82E-25
GO:0051321	Meiotic cell cycle	biological_process	cellular process	62	1885	244	22257	0.2540984	1.9E-15	4.15E-12
GO:0045596	Negative regulation of cell differentiation	biological_process	biological regulation	28	1885	131	22257	0.2137405	4.01E-06	0.0058225
GO:0006351	Transcription, DNA-templated	biological_process	cellular process	59	1885	424	22257	0.1391509	0.000106	0.0579002

GO:0006412	Translation	biological_process	metabolic process	57	1885	437	22257	0.1304348	0.000722	0.165663
GO:0007156	Homophilic cell adhesion via plasma membrane adhesion molecules	biological_process	biological adhesion	29	1885	185	22257	0.1567568	0.000907	0.188297
GO:0007286	Spermatid development	biological_process	multicellular organismal process	21	1885	146	22257	0.1438356	0.011511	0.5971428
GO:0045892	Negative regulation of transcription, DNA-templated	biological_process	regulation of biological process	78	1885	716	22257	0.1089385	0.0127617	0.5971428
GO:0009617	Response to bacterium	biological_process	response to stimulus	27	1885	209	22257	0.1291866	0.0182366	0.5971428
GO:0008284	Positive regulation of cell proliferation	biological_process	cellular process	71	1885	682	22257	0.1041056	0.0405449	0.5971428

Supply Dataset S8

Gene ID	Gene Symbol	Type	log2 (Schisandrin B/Oligoasthenospermia)	adjusted *P* value (Schisandrin B-vs-Oligoasthenospermia)	Funcational category
108168551	LOC108168551	mRNA	-1.40886	＜0.00001	Predicted
108168586	LOC108168586	mRNA	-6.3378	＜0.00001	Predicted
100040031	Gm20823	mRNA	1.20206	＜0.00001	Predicted
102632745	Gm30737	mRNA	-0.79311	0.00051	Predicted
108168593	LOC108168593	mRNA	-4.4321	0.00271	Predicted
78803	Fbxo43	mRNA	-0.96379	＜0.00001	Known
22441	Xlr	mRNA	-0.80085	0.00221	Known
100039324	Gm10147	mRNA	-3.16871	＜0.00001	Predicted
100039585	Gm14819	mRNA	0.92643	＜0.00001	Predicted
105247287	LOC105247287	mRNA	-0.87362	0.01324	Predicted
100042417	Gm20916	mRNA	-1.18333	＜0.00001	Predicted
100039595	Gm20807	mRNA	-8.99751	＜0.00001	Predicted
100861899	Gm21310	mRNA	-0.88998	0.00008	Predicted
27084	Xlr5c	mRNA	-0.99641	0.00211	Known

100042175	Gm10230	mRNA	-1.07704	＜0.00001	Predicted
104362	Meig1	mRNA	-0.63378	＜0.00001	Known
108168555	LOC108168555	mRNA	-0.58467	0.00017	Predicted
108168585	LOC108168585	mRNA	-6.74665	＜0.00001	Predicted
108168524	LOC108168524	mRNA	-1.73195	0.00012	Predicted
100862114	Gm21489	mRNA	-1.23356	＜0.00001	Predicted
108168621	LOC108168621	mRNA	-1.07079	0.02839	Predicted
108168655	LOC108168655	mRNA	1.32563	0.00843	Predicted
20962	Sycp3	mRNA	-1.27482	＜0.00001	Known
100861730	Gm21170	mRNA	3.64156	0.03629	Predicted
105242410	LOC105242410	mRNA	-7.2714	＜0.00001	Predicted
108168661	LOC108168661	mRNA	-1.63884	＜0.00001	Predicted
101056210	Gm28576	mRNA	-0.64431	0.02825	Predicted
665301	Gm20773	mRNA	5.76591	＜0.00001	Predicted
100040509	Gm20845	mRNA	-0.67278	＜0.00001	Predicted
108168627	LOC108168627	mRNA	0.99135	0.00045	Predicted
108168600	LOC108168600	mRNA	2.5138	0.00003	Predicted

108168540	LOC108168540	mRNA	-5.24625	0.00003	Predicted
100862345	Gm21660	mRNA	1.45057	＜0.00001	Predicted
108168604	LOC108168604	mRNA	-1.26802	0.03371	Predicted
108168641	LOC108168641	mRNA	12.13524	＜0.00001	Predicted
108168671	LOC108168671	mRNA	-0.64982	0.00008	Predicted
108168595	LOC108168595	mRNA	-2.71003	＜0.00001	Predicted
100040160	Gm20826	mRNA	-6.59489	＜0.00001	Predicted
108168649	LOC108168649	mRNA	1.36934	0.00016	Predicted
108168654	LOC108168654	mRNA	-2.12355	0.00001	Predicted
108168545	Gm20855	mRNA	-1.20461	＜0.00001	Predicted
100042475	Gm20921	mRNA	-2.08854	＜0.00001	Predicted
665918	LOC665918	mRNA	-0.91675	0.02591	Predicted
108168542	LOC108168542	mRNA	1.02622	＜0.00001	Predicted
101056099	Gm21799	mRNA	-2.14146	＜0.00001	Predicted
108168520	LOC108168520	mRNA	-6.05648	＜0.00001	Predicted
74851	Spin2-ps1	mRNA	-1.62286	0.01781	pseudogene
108168546	Gm20887	mRNA	-0.9294	0.00121	Predicted

22092	Rsph1	mRNA	-0.63093	＜0.00001	Known
100504642	Gm21996	mRNA	-0.73878	＜0.00001	Predicted
108168666	LOC108168666	mRNA	-5.52635	＜0.00001	Predicted
108168624	LOC108168624	mRNA	-2.51003	0.00098	Predicted
100862025	Gm21409	mRNA	-0.85416	＜0.00001	Predicted
57749	Piwil1	mRNA	-0.8116	＜0.00001	Known
108168651	LOC108168651	mRNA	-6.3616	＜0.00001	Predicted
108168589	LOC108168589	mRNA	-1.94471	0.00097	Predicted
100862075	Gm21454	mRNA	-2.9872	＜0.00001	Predicted
108168571	LOC108168571	mRNA	-0.94995	0.00001	Predicted
100042079	Gm20901	mRNA	-0.75733	＜0.00001	Predicted
100038997	Gm2003	mRNA	-4.69611	＜0.00001	Predicted
108168623	LOC108168623	mRNA	-2.15551	0.01257	Predicted
546176	Gm5923	mRNA	-1.43674	0.00501	Predicted
108168515	LOC108168515	mRNA	-0.84671	0.01545	Predicted
108168466	LOC108168466	mRNA	-1.8828	0.0014	Predicted
108168509	LOC108168509	mRNA	0.75592	0.00113	Predicted

108168549	LOC108168549	mRNA	-0.84781	0.00829	Predicted
108168608	LOC108168608	mRNA	-1.24017	0.00043	Predicted
108168650	LOC108168650	mRNA	-5.08058	0.00008	Predicted
100043216	Gm4297	mRNA	-0.72981	0.00196	Predicted
100040022	Gm20822	mRNA	-1.86442	＜0.00001	Predicted
108168628	LOC108168628	mRNA	0.76108	0.00001	Predicted
100040429	Gm20842	mRNA	-0.69031	0.01082	Predicted
546205	Gm5926	mRNA	-1.83451	0.00425	Predicted
18005	Nek2	mRNA	-0.58245	＜0.00001	Known
100039014	Gm20793	mRNA	-2.33161	＜0.00001	Predicted
108168533	LOC108168533	mRNA	-4.23848	0.00596	Predicted
108168548	LOC108168548	mRNA	4.7768	0.00044	Predicted
108168640	LOC108168640	mRNA	-1.01929	0.00142	Predicted
666122	Gm14595	mRNA	-0.61311	＜0.00001	Predicted
100042267	Gm3757	mRNA	-4.83482	0.00037	Predicted
380994	Gm20736	mRNA	1.38201	＜0.00001	Predicted
100042201	Gm20906	mRNA	0.65644	0.00008	Predicted
108168552	LOC108168552	mRNA	-0.91034	＜0.00001	Predicted

108168583	LOC108168583	mRNA	-6.99048	＜0.00001	Predicted
100038977	Gm1993	mRNA	0.88418	0.00432	Predicted
102638610	Gm35134	mRNA	0.88907	0.00001	Predicted
108168599	LOC108168599	mRNA	-5.40181	0.00001	Predicted
100862360	Gm21672	mRNA	0.87932	＜0.00001	Predicted
108168557	LOC108168557	mRNA	0.74506	0.0008	Predicted
100862179	LOC100862179	mRNA	4.64496	0.00089	Predicted
108168510	LOC108168510	mRNA	1.1414	0.00036	Predicted
209091	Ccnb3	mRNA	-1.37539	0.01147	Known
108168563	LOC108168563	mRNA	-0.63562	0.00411	Predicted
67981	Hormad1	mRNA	-0.64861	＜0.00001	Known
108168579	LOC108168579	mRNA	-0.70943	0.00666	Predicted
108168620	Gm20856	mRNA	1.35331	0.0226	Predicted
108168516	LOC108168516	mRNA	-5.89289	＜0.00001	Predicted
108168669	LOC108168669	mRNA	1.81729	0.00001	Predicted
320558	Sycp2	mRNA	-0.63018	＜0.00001	Known
14536	Nr6a1	mRNA	0.62989	＜0.00001	Known
108168611	LOC108168611	mRNA	-5.4242	＜0.00001	Predicted

14313	Fst	mRNA	2.245756	＜0.00001	Known
108168606	LOC108168606	mRNA	-1.41439	＜0.00001	Predicted
20611	Ssty1	mRNA	1.15044	＜0.00001	Known
108168528	LOC108168528	mRNA	4.73913	0.00054	Predicted
100040262	Gm20833	mRNA	3.67984	＜0.00001	Predicted
100040335	Gm20836	mRNA	-14.16774	＜0.00001	Predicted
100862314	Gm21637	mRNA	5.2836	0.00002	Predicted
100861839	Gm21258	mRNA	-0.98255	＜0.00001	Predicted
108168648	LOC108168648	mRNA	-6.48558	＜0.00001	Predicted
108168658	LOC108168658	mRNA	-0.79393	0.00015	Predicted
108168659	LOC108168659	mRNA	-0.65247	0.01886	Predicted
101056091	Gm29024	mRNA	-1.55038	＜0.00001	Predicted
671564	Rnf212	mRNA	-0.59349	＜0.00001	Known
102637130	Gm21241	mRNA	-5.03286	0.00011	Predicted
194908	Pld6	mRNA	-0.66296	＜0.00001	Known
331416	Gm773	mRNA	-0.63319	0.01027	Predicted
622554	Majin	mRNA	-1.08032	＜0.00001	Known

73673	Rec114	mRNA	-0.73735	0.00233	Known
100862020	Gm21405	mRNA	-1.05434	＜0.00001	Predicted
108168581	LOC108168581	mRNA	-3.34553	＜0.00001	Predicted
100040714	Gm20851	mRNA	-5.48624	＜0.00001	Predicted
105247282	LOC105247282	mRNA	-0.60311	0.00001	Predicted
108168610	LOC108168610	mRNA	3.85811	0.01987	Predicted
100042578	Gm20929	mRNA	-1.73302	＜0.00001	Predicted
100041550	Gm20877	mRNA	0.87373	0.00045	Predicted
108168605	LOC108168605	mRNA	1.30515	0.01325	Predicted
108168603	LOC108168603	mRNA	-6.12079	＜0.00001	Predicted
108168626	LOC108168626	mRNA	-2.43417	＜0.00001	Predicted
100040187	Gm20828	mRNA	5.93924	＜0.00001	Predicted
108168667	LOC108168667	mRNA	-5.11578	0.00007	Predicted
100040585	Gm2854	mRNA	-2.02297	＜0.00001	Predicted
108168601	LOC108168601	mRNA	-2.50688	＜0.00001	Predicted
108168530	LOC108168530	mRNA	3.24647	＜0.00001	Predicted
75801	4930447C04Rik	mRNA	-0.82543	＜0.00001	Predicted

100039905	Gm20820	mRNA	-1.34556	＜0.00001	Predicted
380709	Spata22	mRNA	-1.61243	0.00002	Known

Supply Dataset S9

Gene ID	14313	16323
Gene Symbol	Fst	Inhba
Type	mRNA	mRNA
Oligoasthenospermia_1 Expression	0.08	0.32
Oligoasthenospermia_2 Expression	0.09	0.29
Oligoasthenospermia_3 Expression	0.11	0.27
Oligoasthenospermia_4 Expression	0.12	0.26
Oligoasthenospermia_5 Expression	0.13	0.23
Oligoasthenospermia_6	0.17	0.21

Expression		
Schisandrin B_1 Expression	1.38	0.07
Schisandrin B_2 Expression	0.47	0.1
Schisandrin B_3 Expression	0.4	0.11
Schisandrin B_4 Expression	0.4	0.14
Schisandrin B_5 Expression	0.34	0.14
Schisandrin B_6 Expression	0.33	0.14

Supply Dataset S10-1

	GAPDH	Fst			
Sample	Ct	Ct	ΔCt	ΔΔCt	$2^{-\Delta\Delta Ct}$
Normal-1	18.78	26.74	7.96	0.138	0.9089181
	18.85	26.50	7.65	-0.172	1.1267928
	18.78	26.80	8.02	0.198	0.8718925
Normal-2	18.96	26.80	7.84	0.018	0.9877530
	18.87	26.72	7.85	0.028	0.9809301
	18.88	26.65	7.77	-0.052	1.0368608
Normal-3	19.70	27.45	7.75	-0.072	1.0513348

	19.65	27.55	7.90	0.078	0.9475160
	19.75	27.41	7.66	-0.162	1.1190094
Oligoasthenospermia-1	19.57	28.04	8.47	0.648	0.6382627
	19.61	27.98	8.37	0.548	0.6840730
	19.45	28.36	8.91	1.088	0.4704855
Oligoasthenospermia-2	18.85	27.03	8.18	0.358	0.7803657
	18.84	27.02	8.18	0.358	0.7803657
	18.80	27.29	8.49	0.668	0.6294755
Oligoasthenospermia-3	18.70	27.10	8.40	0.578	0.6699950
	18.76	27.09	8.33	0.508	0.7033049
	18.66	27.25	8.59	0.768	0.5873214
Schisandrin B-1	21.15	29.59	8.44	0.618	0.6516739
	21.40	29.71	8.31	0.488	0.7131227
	21.29	29.53	8.24	0.418	0.7485768
Schisandrin B-2	20.84	29.17	8.33	0.508	0.7033049
	20.79	29.11	8.32	0.498	0.7081968
	20.79	29.03	8.24	0.418	0.7485768
Schisandrin B-3	20.85	29.00	8.15	0.328	0.7967628

	20.84	28.97	8.13	0.308	0.8078852
	20.80	28.86	8.06	0.238	0.8480506

Supply Dataset S10-2

	GAPDH	Inhba			
Sample	Ct	Ct	ΔCt	ΔΔCt	$2^{-\Delta\Delta Ct}$
Normal-1	18.78	27.95	9.17	0.349	0.7851886
	18.85	27.90	9.05	0.229	0.8532918
	18.78	27.90	9.12	0.299	0.8128782
Normal-2	18.96	27.56	8.60	-0.221	1.1656310
	18.87	27.52	8.65	-0.171	1.1259253
	18.88	27.43	8.55	-0.271	1.2067368
Normal-3	19.70	28.43	8.73	-0.091	1.0651902
	19.65	28.51	8.86	0.039	0.9734043
	19.75	28.41	8.66	-0.161	1.1181480
Oligoasthenospermia-1	19.57	27.13	7.56	-1.261	2.3968026
	19.61	27.36	7.75	-1.071	2.1010509
	19.45	27.57	8.12	-0.701	1.6257564

Oligoasthenospermia-2	18.85	26.89	8.04	-0.781	1.7184538
	18.84	27.00	8.16	-0.661	1.5813000
	18.80	27.10	8.30	-0.521	1.4350600
Oligoasthenospermia-3	18.70	26.94	8.24	-0.581	1.4960010
	18.76	27.09	8.33	-0.491	1.4055269
	18.66	27.07	8.41	-0.411	1.3297095
Schisandrin B-1	21.15	29.72	8.57	-0.251	1.1901233
	21.40	29.62	8.22	-0.601	1.5168844
	21.37	29.67	8.30	-0.521	1.4350600
Schisandrin B-2	20.84	29.49	8.65	-0.171	1.1259253
	20.73	29.75	9.02	0.199	0.8712213
	20.79	28.92	8.13	-0.691	1.6145265
Schisandrin B-3	20.85	29.14	8.29	-0.531	1.4450417
	20.84	28.97	8.13	-0.691	1.6145265
	20.80	28.94	8.14	-0.681	1.6033741

Supply Dataset S11

Sample	Analyte	Standard curve	r	Range (ng/mL)	LLoQ (ng/mL)
Plasma	Schisandrin B	y =8.03454x-0.35313	0.9997	0.065–26.087	0.065
Testis	Schisandrin B	y =1.53705x+0.177647	0.9987	0.1–30.00	0.1

Supply Dataset S12

Sample	Batch	Mean ± SD	RSD (%)	Accuracy (%)
Plasma batch (1.00 ng/mL)	Batch 1	0.98±0.04	4.08	98
	Batch 2	0.975±0.04	4.1	97.5
	Batch 3	0.96±0.015	1.56	96
	Inter-Batch	0.97±0.035	3.61	97
Plasma batch (10.00 ng/mL)	Batch 1	9.6±0.216	2.25	96
	Batch 2	10.1±0.664	6.574257426	101
	Batch 3	9.88±0.448	4.534412955	98.8
	Inter-Batch	9.86±0.496	5.030425963	98.6
Plasma batch (20.00 ng/mL)	Batch 1	19.9625±0.756	3.794614903	99.8125
	Batch 2	20.2125±0.49	2.424242424	101.0625

	Batch 3	19.9625±0.593	2.968065122	99.8125
	Inter-Batch	20±0.602	3.03125	100
Testis batch (1.00 ng/mL)	Batch 1	0.998±0.038	3.794614903	99.8125
	Batch 2	1.01±0.024	2.424242424	101.0625
	Batch 3	0.998±0.030	2.968065122	99.8125
	Inter-Batch	1.0±0.030	3.03125	100
Testis batch (10.00 ng/mL)	Batch 1	9.7±0.2	2.138613861	97
	Batch 2	9.75±0.2	6.720647773	97.5
	Batch 3	9.6±0.075	4.666666667	96
	Inter-Batch	9.8±0.175	5.030425963	98
Testis batch (20.00 ng/mL)	Batch 1	20.2±0.432	2.06185567	101
	Batch 2	19.76±0.328	2.051282051	98.8
	Batch 3	19.2±0.896	0.78125	96
	Inter-Batch	19.72±0.992	1.785714286	98.6

Supply Dataset S13

Sample	Stability	Mean ± SD	RSD (%)	Accuracy (%)
Plasma sample (1.00 ng/mL)	Three freeze–thaw cycles(-20 to 25 °C)	0.98±0.04	7.853403141	95.5
	Room temperature (25 °C) for 24 h	0.975±0.04	3.092783505	97
	Storage at −80 °C for 1 month	0.96±0.015	2.162162162	92.5
Plasma sample 10.00 ng/mL)	Three freeze–thaw cycles(-20 to 25 °C)	9.6±0.216	3.373015873	100.8
	Room temperature (25 °C) for 24 h	10.1±0.664	1.206543967	97.8
	Storage at −80 °C for 1 month	9.88±0.448	3.747534517	101.4
Plasma sample (20.00 ng/mL)	Three freeze–thaw cycles(-20 to 25 °C)	19.9625±0.756	1.245880026	94.8125
	Room temperature (25 °C) for 24 h	20.2125±0.49	0.718484651	95.6875
	Storage at −80 °C for 1 month	19.9625±0.593	3.364369957	100.6875
Testis sample (1.00 ng/mL)	Three freeze–thaw cycles(-20 to 25 °C)	0.998±0.038	1.245880026	94.8125
	Room temperature (25 °C) for 24 h	1.01±0.024	0.718484651	95.6875
	Storage at −80 °C for 1 month	0.998±0.030	3.364369957	100.6875
Testis sample (10.00 ng/mL)	Three freeze–thaw cycles(-20 to 25 °C)	9.7±0.2	89.0052356	95.5
	Room temperature (25 °C) for 24 h	9.75±0.2	30.41237113	97
	Storage at −80 °C for 1 month	9.6±0.075	102.7027027	92.5
Testis sample (20.00 ng/mL)	Three freeze–thaw cycles(-20 to 25 °C)	20.2±0.432	3.373015873	100.8

	Room temperature (25 °C) for 24 h	19.76±0.328	1.206543967	97.8
	Storage at −80 °C for 1 month	19.2±0.896	3.747534517	101.4

Supply Dataset S14

Sample	Thepretical concentration (ng/mL)	Recovery (%)	RSD (%)
Plasma sample	1	85.5±4.87	5.13
	10	88.9±3.68	3.29
	20	86.7±3.33	2.37
Testis sample	1	89.9±3.13	3.26
	10	93.1±3.87	4.06
	20	90.33±1.79	1.91

Supply Dataset S15

Time (h)	Schisandrin B's concentration in plasma (ng/ml) (n=5)					Mean	SD
0	0	0	0	0	0	0	0
0.25	25.105	22.982	19.711	13.11	15.226	19.2268	5.0574414
0.5	3.236	1.831	1.067	1.49	21.355	5.7958	8.7358798
1	0.137	4.17	0.378	0.216	0.439	1.068	1.7383045
1.5	0.104	0.268	0.197	0.062	0.182	0.1626	0.0809679
2	11.958	5.209	1.281	0.082	0.206	3.7472	5.0400899
3	0.059	0.212	0.365	0.108	0.256	0.2	0.1212951
4	3.925	1.149	0.539	0.548	0.337	1.2996	1.4987844
5	0.4	4.599	0.794	5.212	5.269	3.2548	2.4443776
6	0.372	0.364	1.463	1.932	0.174	0.861	0.7854209
8	0.332	1.352	0.3	0.737	0.4	0.6242	0.4424841
12	0.751	0.209	1.025	0.451	0.296	0.5464	0.3378458
24	0.091	0	0	0	0	0.0182	0.0406964

Supply Dataset S16

Time (h)	Schisandrin B's concentration in testis (ng/ml) (n=5)					Mean	SD
0	0	0	0	0	0	0	0
0.25	4.258	4.97	3.535	8.055	5.127	5.189	1.7225155
0.5	2.701	6.043	2.862	1.791	4.673	3.614	1.7134705
1	2.498	2.438	2.453	3.729	3.509	2.9254	0.6383089
1.5	3.108	1.763	2.455	2.281	2.859	2.4932	0.5223669
2	5.192	1.722	4.218	13.457	1.679	5.2536	4.837923
3	6.94	1.505	2.304	2.925	1.345	3.0038	2.2906123
4	5.188	0.986	2.293	0.785	1.241	2.0986	1.8221612
5	1.309	1.269	0.922	0.759	0.172	0.8862	0.4617464
6	0.495	0.573	0.289	0.175	0	0.3064	0.2335033
8	0.117	0.647	0.193	0	0	0.1914	0.2675711
12	0	0.353	0.108	0	0	0.0922	0.1531085
24	0	0	0	0	0	0	0

Supply Dataset S17

Plasma parameter of schisandrin B	Unit	Value
AUC(0-t)	pg/mL*h	22804.725
AUC(0-∞)	pg/mL*h	22889.22
MRT(0-t)	h	4.755
MRT(0-∞)	h	4.843
t1/2z	h	3.196
Tmax	h	0.25
Vz/F	mL/kg	4029909.754
CLz/F	mL/h/kg	873773.779
Cmax	pg/mL	19226.8

Supply Dataset S18

Testicular tissues parameter of schisandrin B	Unit	Value
AUC(0-t)	pg/mL*h	17062
AUC(0-∞)	pg/mL*h	17104.88
MRT(0-t)	h	2.945
MRT(0-∞)	h	3.011
t1/2z	h	3.506
Tmax	h	2
Vz/F	mL/kg	5915869.487
CLz/F	mL/h/kg	1169256.937
Cmax	pg/mL	5253.6