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Article

Biomimetic Total Synthesis and the Biological Evaluation of Natural Product (−)-Fargesone A as a Novel FXR Agonist

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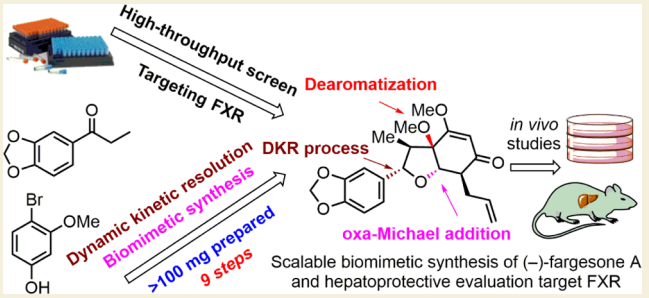
ABSTRACT: Farnesoid X receptor (FXR), a member of the nuclear receptor superfamily, plays an important role in maintaining or reversing metabolic homeostasis during the development of liver diseases. However, developing FXR modulators to intervene in FXR-related diseases is still an unmet clinical need. Therefore, it is significant to develop novel small-molecule agonists for drug discovery targeting FXR. Through a high-throughput chemical screen and follow-up biological validations, we first identified the natural product Fargesone A (FA) as a potent and selective FXR agonist. The limited, variable supply of FA from natural product isolation, however, has impeded its biological exploration and potential drug development. Accordingly, we have developed a biomimetic and scalable total synthesis of FA in nine steps that provides a solution to the supply of FA. Enabled by chemical synthesis, the *in vivo* efficacy of FA has been further investigated. The results showed that FA alleviates hepatocyte lipid accumulation and cell death in an FXR-dependent manner. Moreover, treatment of bile duct ligation (BDL)-induced liver disorder with FA ameliorates pathological features in mice. Therefore, our work lays the foundation to develop new small-molecule FXR agonists as a potential therapy for liver diseases.



KEYWORDS: *Fargesone A, FXR, liver disorders, total synthesis*

INTRODUCTION

Farnesoid X receptor (FXR, NR1H4), also known as bile acid receptor, is one of the members of the nuclear receptor superfamily and is highly expressed in the mammalian liver, kidney, intestine, and adrenal gland.[1−3] As a ligand-regulated nuclear receptor, the activity of FXR is mediated through the ligand-dependent recruitment or release of co-regulators, including coactivators, such as the steroid receptor coactivators (SRCs), and corepressors, such as the nuclear corepressor (NCoR).[4] Without ligand binding or antagonist binding into the pocket, FXR interacts with corepressors and binds to the response element of DNA; the downstream target genes are not expressed. Agonist binding will induce FXR transcriptional function, leading to a series of biological homeostasis regulations through the diversity of downstream target genes, such as those related to bile acid metabolism,[5,6] lipid balance,[7,8] inflammatory response,[9] and organ fibrosis.[10] In recent years, increasing evidence has indicated that activation of FXR by small-molecule ligands, such as the well-known endogenous bile acids[11] and GW4064[12] show critical roles in regulating metabolic balance. Obeticholic acid (OCA, also named 6E-CDCA or INT-747), a potent FXR agonist derived from chenodeoxycholic acid (CDCA), has been approved by FDA for primary biliary cirrhosis (PBC) treatment in clinics,[13] and also been evaluated in phase III clinical trials for nonalcoholic steatohepatitis (NASH).[14,15]

FXR has been confirmed as a vital regulator in metabolism diseases. Much evidence showed that bile acids or their derivatives are FXR agonists with biological efficacy but also activate other receptors besides FXR, indicating their poor selectivity as FXR agonists.[16] Cafestol is reported to be an agonist of FXR, but also can activate pregnane X receptor (PXR), with poor FXR selectivity.[17] Fexaramine was identified as an intestinal-restricted FXR agonist with limited direct FXR.[18,19] Ivermectin, an antiparasitic drug, was revealed as a novel FXR modulator and showed the ability to regulate metabolic profiles *in vivo*.[20] Other synthetic FXR agonists have also been reported, but either low *in vivo* efficacy or potential side effects have limited their further advances in drug discovery and development.[21,22] For example, GW4064, the most widely used full agonist of FXR exhibits ambiguous efficacy in regulating metabolic hemostasis, including bile acid,

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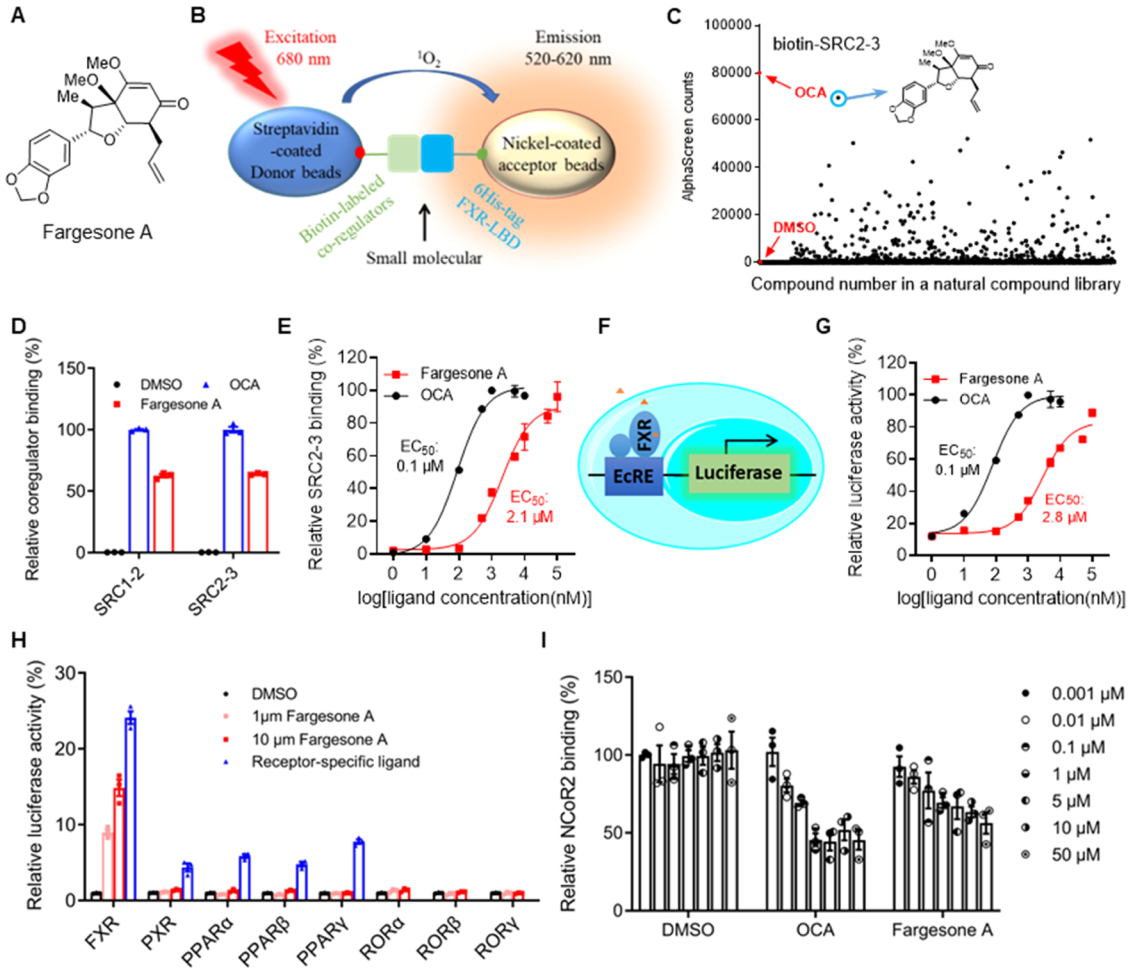

Figure 1. Identification of fargesone A as a novel FXR agonist. (A) Chemical structure of fargesone A (FA). (B) AlphaScreen-based high-throughput screening assay for the discovery of FXR modulators. (C) Results of screening a natural product library. Work concentration of compounds, 10 μM. (D) SRC1-2 and SRC2-3 coactivator binding motifs bind to FXR in response to 10 μM FA or positive control OCA in biochemical-based AlphaScreen assay. (E) Dose−response curve of FA and OCA in inducing FXR to recruit coactivator motif SRC2-3 in AlphaScreen assay. (F) Cell-based Dual-Luciferase reporter assay schematic diagram (see the Methods section for details). (G) Dose curve of FA and OCA in transactivating FXR in Dual-Luciferase reporter assay. (H) Receptor-selective transactivation by FA. HEK293T (ATCC) cells were co-transfected with plasmids encoding full-length nuclear receptors and their cognate response reporters, respectively (see the Methods section). After 6 h of transfection, cells were treated with DMSO, 1 or 10 μM FA or specific agonists for each receptor: FXR, 1 μM OCA; Pregnane X receptor (PXR), 10 μM rifampicin; peroxisome proliferator-activated receptor α (PPARα), 1 μM GW590735; PPARβ, 1 μM GW0742; PPARγ, 1 μM rosiglitazone. For (G) and (H), the luciferase response was normalized with the renilla response by co-transfection. (I) Recruitment of NCoR-2 to FXR in response to FA and OCA by AlphaScreen assay. *P* values were determined by one-way ANOVA with Tukey's multiple comparison post hoc test; values are the mean ± SEM of three independent experiments.

lipid, glucose, insulin metabolism, and energy expenditure.[16,23] Indeed, multiple FXR ligands have entered the clinic trials, such as OCA (intercept), GS9674 (Gilead), EDP305 (Enanta), and LJN452 (Novartis), as therapies for FXR-mediated diseases, including PBC, primary sclerosing cholangitis (PSC), biliary atresia, and NASH. These examples strongly demonstrate the feasibility of FXR as a target for metabolic diseases. However, despite some encouraging progress in clinical trials for applying FXR ligands, only OCA has been approved by FDA for PBC treatment, and OCA has shown significant side effects such as causing severe pruritus and raising the LDL level in many patients.[13] Therefore, given the important role of FXR in metabolic diseases and the lack of approved drugs directly targeting FXR, developing novel small-molecule FXR modulators for further drug discovery with better efficacy and safety profiles to meet the clinical need is urgent and significant.

Natural products are one of the primary sources of drug molecules; many approved drugs are derived from natural products and their secondary derivatives.[24,25] Fargesone A (FA) is a natural product isolated from the flower buds of *Magnolia fargesii* that has been used in traditional Chinese medicine, named Xin Yi.[26] Sporadic studies reported that FA showed weak Ca^{2+} antagonistic and anti-inflammatory activities.[26,27] However, the poor accessibility from natural product isolation (68 mg out of 12.7 kg dried leaves) significantly hinders its more extensive biological evaluations *in vitro* and *in vivo*.[28] Here, through a high-throughput chemical screening of a natural product library and follow-up biological validations, we have identified FA as a potent and selective FXR novel agonist (Figure 1A). We have further developed a biomimetic and scalable total synthesis of FA in nine steps that provides a solution to the supply of FA. Enabled by chemical synthesis, the *in vivo* efficacy of FA has been

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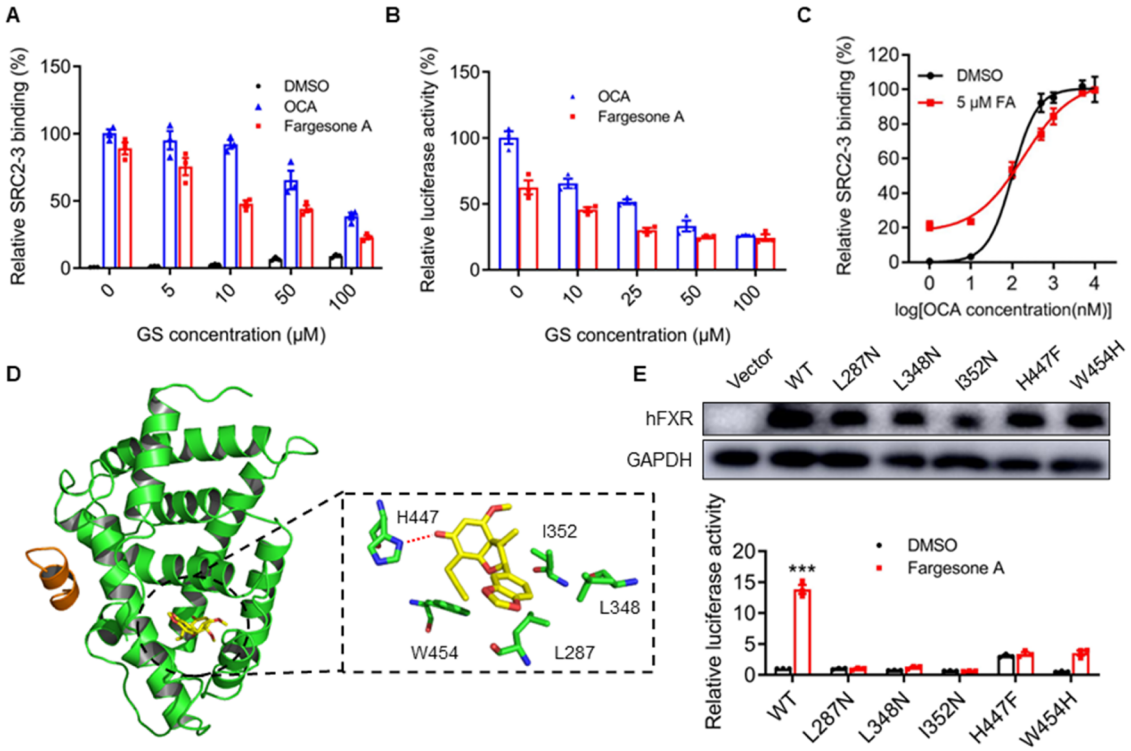

Figure 2. Fargesone A activities as an agonist through direct interaction with FXR. (A) Binding of FXR-LBD with the SRC2-3 coactivator motif in response to FA was blocked by gradient Guggulsterone E&Z (GS) (the first reported compound with the antagonistic activity of FXR) in AlphaScreen assays. OCA, 1 μM. FA, 10 μM. (B) GS inhibited the FA-induced transcriptional activity of FXR in the Dual-Luciferase reporter assay. HEK293T cells were co-transfected with plasmids encoding full-length FXR and EcRE-luciferase reporter, then treated with FA or OCA with gradient GS concentration after 6 h transfection and determined luciferase activity 24 h later, normalized with Renilla response. (C) OCA curve inducing FXR to recruit coactivator motif SRC2-3 with or without 5 μM FA in AlphaScreen assay. (D) Molecular docking structure of FA (yellow in the sticks) in the pocket of FXR-LBD protein (green cartoon) based on the reported OCA/FXR-LBD co-crystal structure (PDB ID, 1OSV) by MOE software. Hydrogen-bonding (dashed red lines) and hydrophobic interactions of FA with surrounding residues in docked FXR-LBD pocket. (E) Expression of FXR mutants with indicated key FXR residues (L287, L348, I352, H447, W454) and their transcriptional activity in response to FA in cell-based Dual-Luciferase reporter assay in HEK293T cell. *P* values were determined by one-way ANOVA with Tukey's multiple comparison post hoc test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Values are the mean ± SEM of three independent experiments.

further investigated. The results showed that FA alleviates hepatocyte lipid accumulation and cell death in an FXR-dependent manner in hepatocytes. Moreover, treatment of bile duct ligation (BDL)-induced liver disorder with FA ameliorates pathological features in mice. Therefore, our work lays the foundation to develop novel small-molecule FXR modulators as a potential therapy for liver diseases.

RESULTS AND DISCUSSION

Identification of Natural Product Fargesone A as a Novel FXR Agonist

In search of FXR agonists with novel chemical scaffolds, we have initiated a research program using combined efforts of high-throughput screening, chemical synthesis, and follow-up biological evaluations. We first used 6His-tagged FXR ligand-binding domain (LBD) protein as a bait to set up a biochemical AlphaScreen assay[9,20] (Figure 1B), which could rapidly and effectively determine the potency of small-molecule agonist for regulating the binding affinity of 6His-labeled FXR-LBD protein with biotin-labeled co-regulator peptides. Natural products and their derivatives have long been used as medicinal agents, and they still make up a significant fraction of clinically approved drugs.[24,25] With this high-throughput screening assay established, we screened a natural product library (~2700 compounds) (Figure 1C). Interestingly, fargesone A (FA), with a significantly different chemical scaffold compared to the known FXR agonists, such as obeticholic acid (OCA) and GW4064 (Figures S1 and 1A), was identified as a positive FXR agonist with the ability to induce the recruitment of SRC1-2 and SRC2-3 motifs to FXR-LBD (Figure 1D). Furthermore, the binding affinity determined by the AlphaScreen assay was dose-dependent (Figure 1E). We further performed the cell-based Dual-Luciferase reporter assay (Figure 1F) to validate the results from the above-mentioned AlphaScreen system. Cells were co-transfected with plasmids encoding full-length FXR and cognate EcRE response reporter. Like the positive control compound OCA, FA activates the transcriptional activity of FXR in a dose-dependent manner (Figure 1G), which is consistent with the AlphaScreen results. To confirm the specificity of FA in activating FXR, cells were co-transfected with plasmids encoding several other well-known full-length nuclear receptors and their cognate response reporters. Then, the cells were stimulated with specific ligands based on the reporter assay. In agreement with the initial AlphaScreen and reporter assay results, FA treatment significantly activated FXR with an EcRE reporter, but had no substantial agonistic activities on other nuclear receptors, including the peroxisome proliferator-activated receptor families (α, β, and γ), pregnane X receptor (PXR) and the retinoid-related orphan receptor families (α, β, and γ) (Figure 1H). As a typical ligand-regulated

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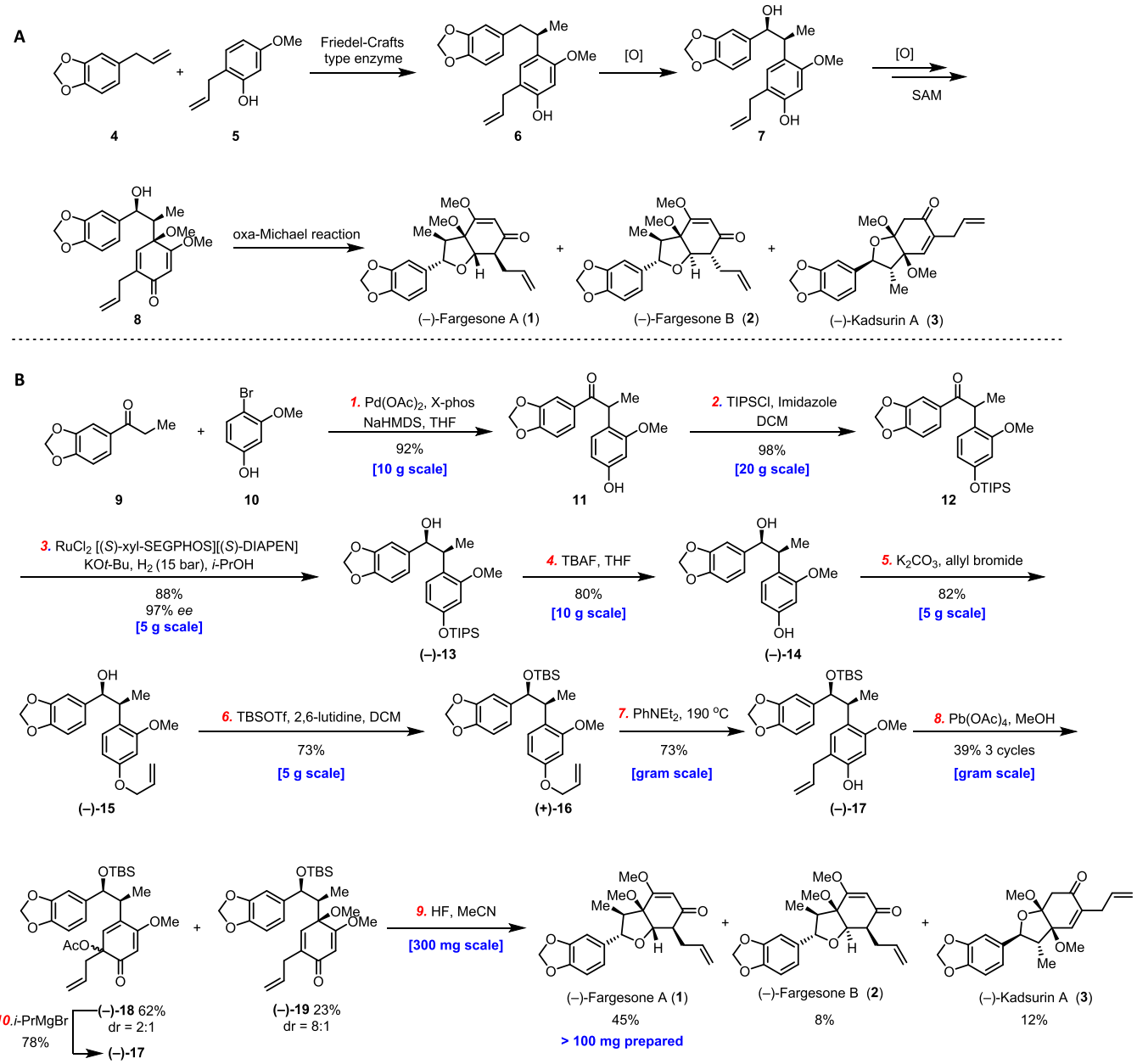
Scheme 1. Scalable Biomimetic Syntheses of (−)-Fargesones A, B and (−)-Kadsurin A[a]



[a](A) Proposed biosynthesis of (−)-fargesones A. (B) Scalable biomimetic syntheses of (−)-fargesones A and related natural products. Reagents and conditions are as follows; (1) $Pd(OAc)_2$, NaO*t*-Bu, X-phos, THF, 80 °C. (2) TIPSCl, imidazole, DCM, r.t. (3) $RuCl_2$ [(*S*)-xyl-SEGPHOS][(*S*)-DIAPEN], KO*t*-Bu, H_2 (15 bar), *i*-PrOH, r.t., 48 h. (4) TBAF, THF, 0 °C. (5) K_2CO_3, allyl bromide, acetone, reflux. (6) TBSOTf, 2,6-lutidine, DCM, 0 °C. (7) $PhNEt_2$, 190 °C. (8) $Pb(OAc)_4$, MeOH, r.t. (9) aq. HF, MeCN, r.t. (10) *i*-PrMgBr, diethyl ether/dioxane = 7:3, r.t.

nuclear receptor, the activity of FXR is mediated through ligand-dependent recruitment or release of different co-regulators, including coactivators and corepressors. Besides the coactivators SRC1-2 and SRC2-3, we also tested the corepressor NCoR2 recruitment condition of FXR induced by FA. Similar to OCA, FA decreased the binding affinity between FXR and NCoR2 (Figure 1I), corresponding to the activity of inducing the coactivator's recruitment for FXR. These results provide solid evidence to demonstrate that FA is a potent and selective FXR agonist.

Agonistic Activity of Fargesone A Is through Direct Interaction with FXR

Guggulsterone E&Z (GS) is the first compound with reported antagonistic activity against FXR (Figure S1).[29] As indicated by the AlphaScreen test, GS blocked the OCA- and FA-induced recruitment of SRC2-3 to FXR in a dose-dependent manner (Figure 2A). Furthermore, OCA- or FA-elevated FXR transcriptional activity was also reversed by GS, based on the cell-based Dual-Luciferase reporter assay (Figure 2B). As a full agonist of FXR, OCA potently activates FXR in both the AlphaScreen and the reporter assay. FA showed a slightly reduced FXR activation activity compared to OCA, suggesting

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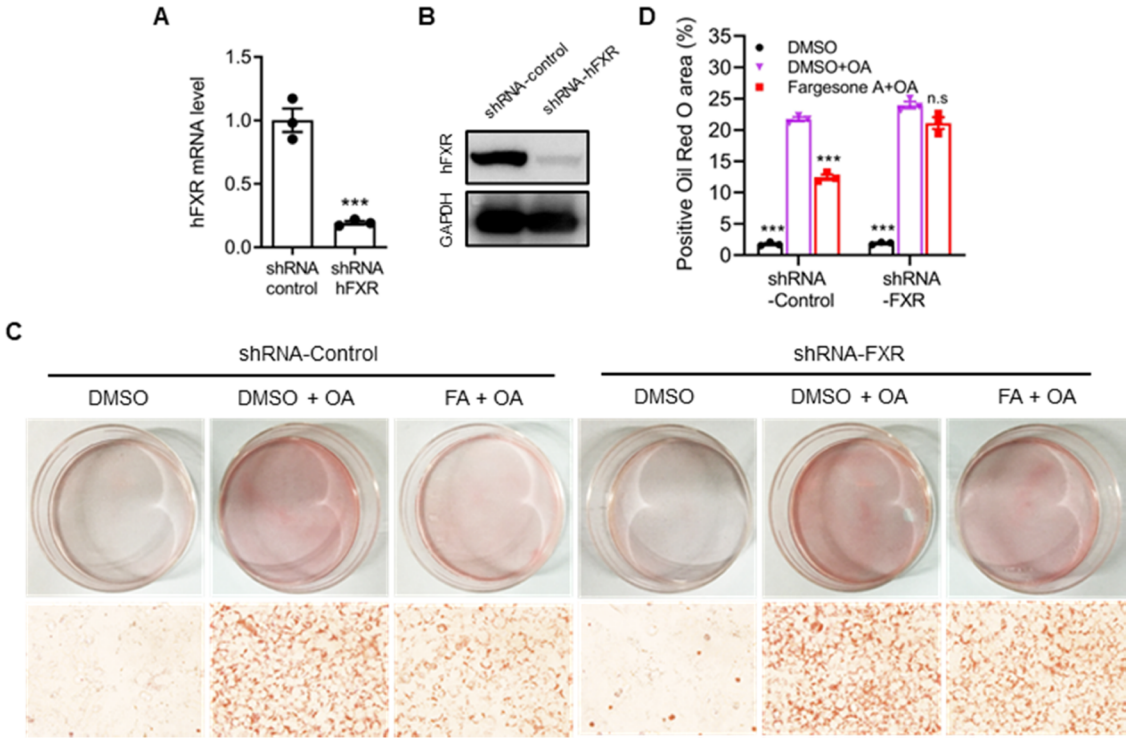

Figure 3. Fargesone A reverses hepatocyte injury in a partial FXR-dependent manner in the human WRL68 cell line. Lentivirus-mediated FXR knockdown (shRNA-FXR) in the human WRL68 cell line and scrambled shRNA was used as control (shRNA-Control). (A, B) mRNA and protein levels of FXR were confirmed by QPCR and western blot to verify the effectiveness of FXR stable silencing in cells. (C, D) shRNA-Control and shRNA-FXR human liver WRL68 cells were incubated with 400 μM oleic acid (OA) and treated with DMSO or 10 μM FA for 24 h. Lipid accumulation in cells was determined by Oil Red O staining (C) and the corresponding quantifications of positive Oil Red O staining area (D). *P* values were determined by one-way ANOVA with Tukey's multiple comparison post hoc test, ***$P < 0.001$. Values are the mean ± SEM of three independent experiments.

that it might be a partial agonist. Therefore, if FA competitively binds to the same OCA-binding pocket of FXR, the presence of FA will interfere with the response of FXR to OCA treatment. Indeed, FXR is more sensitive at low OCA concentrations because a small amount of OCA can not fully compete with FA for FXR binding. FXR is less sensitive at higher OCA concentrations, as expected (Figure 2C). These data confirmed that the agonistic effect of FA on FXR is through direct binding to the same OCA-binding pocket of FXR.

With the previously reported X-ray crystal structure (PDB ID, 1OSV[30]) in hand, molecular docking of FA to FXR was performed. FA is compatibly bound into a hydrophobic FXR-LBD pocket formed by the H352/L348/L287/W454 residues and forms a hydrogen bond with the nitrogen atom of the histidine imidazole ring (Figure 2D). To verify the roles of FXR key residues in FA binding and FXR activation, we mutated the proposed crucial residues that may interact with different functional groups of FA through either hydrophobic or hydrogen-bonding interactions. The transcriptional activity in response to FA was tested using the cell-based Dual-Luciferase reporter assay. For example, the 447H residue of FXR may interact with FA through a hydrogen bond, playing a crucial role in stabilizing FA's binding. Accordingly, the H447F mutation was designed to prevent this hydrogen-bonding effect. As expected, this mutation abolished the FXR transcriptional activity induced by FA (Figure 2D,E). Besides, the docking model suggested that the hydrophobic side chains of 287L, 348L, 352I, and 454W of FXR stabilize FA's binding by forming several hydrophobic interactions. Therefore, L287N, L348N, I352N, and W454H mutations were designed to reduce the binding affinity. The results showed that these mutants decreased the FA-mediated FXR transcriptional activity (Figure 2D,E). The western blot also confirmed that all of the aforementioned FXR mutant plasmids could normally express in HEK293T cells (Figure 2E). Our computational and mutagenesis studies showed that the activation of FXR by FA is through direct binding in the bile acid-binding site.

Biomimetic Total Syntheses of Fargesone A, B and Kadsurin A

To further investigate FA's biological activity and *in vivo* therapeutic potential, we need a reliable material supply of this natural product. Unfortunately, FA was isolated in poor yield from the natural source (68 mg out of 12.7 kg dried leaves). Therefore, we initiated a synthetic program to develop a concise and scalable route to access FA and its congeners. From a synthetic point of view, the structure of (−)-fargesone A (**1**) is striking due to the unique polycyclic framework with five contiguous stereogenic centers. Conceivably, (−)-**1** may be biogenetically derived from an enzymatic intermolecular Friedel−Crafts reaction (Scheme 1A). Then, benzylic oxidation of **6** may provide **7**, which may undergo oxidative dearomatization to furnish **8** as the key biosynthetic intermediate. (7*R*,8*R*,3′*R*)-7-acetoxy-3′,4′-dimethoxy-3,4-methylenedi-oxy-6′-oxo-$\Delta^{-1',4',8'}$-8.3′-lignan that is a diastereomer of **8** was isolated by Boll and co-workers in 1994.[31] Subsequent oxa-Michael addition through 7β-hydroxyl group attack may eventually generate (−)-**1**. Inspired by this hypothesis, our synthesis commenced with palladium-catalyzed

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A

	$t_{1/2}$	T_{max}	C_{max}	AUC $_{0-t}$	AUC $_{0-inf}$	MRT $_{0-inf}$	F
	h	h	ng/mL	ng/mL*h	ng/mL*h	h	%
i.v. (5mpk)	0.68	~	941	469	471	0.43	~
SD	0.1	~	57	13	14	0.13	~
p.o.(10mpk)	0.33	0.25	104	101	102	0.58	10.8
SD	0.04	0.00	16	32	32	0.07	3.2

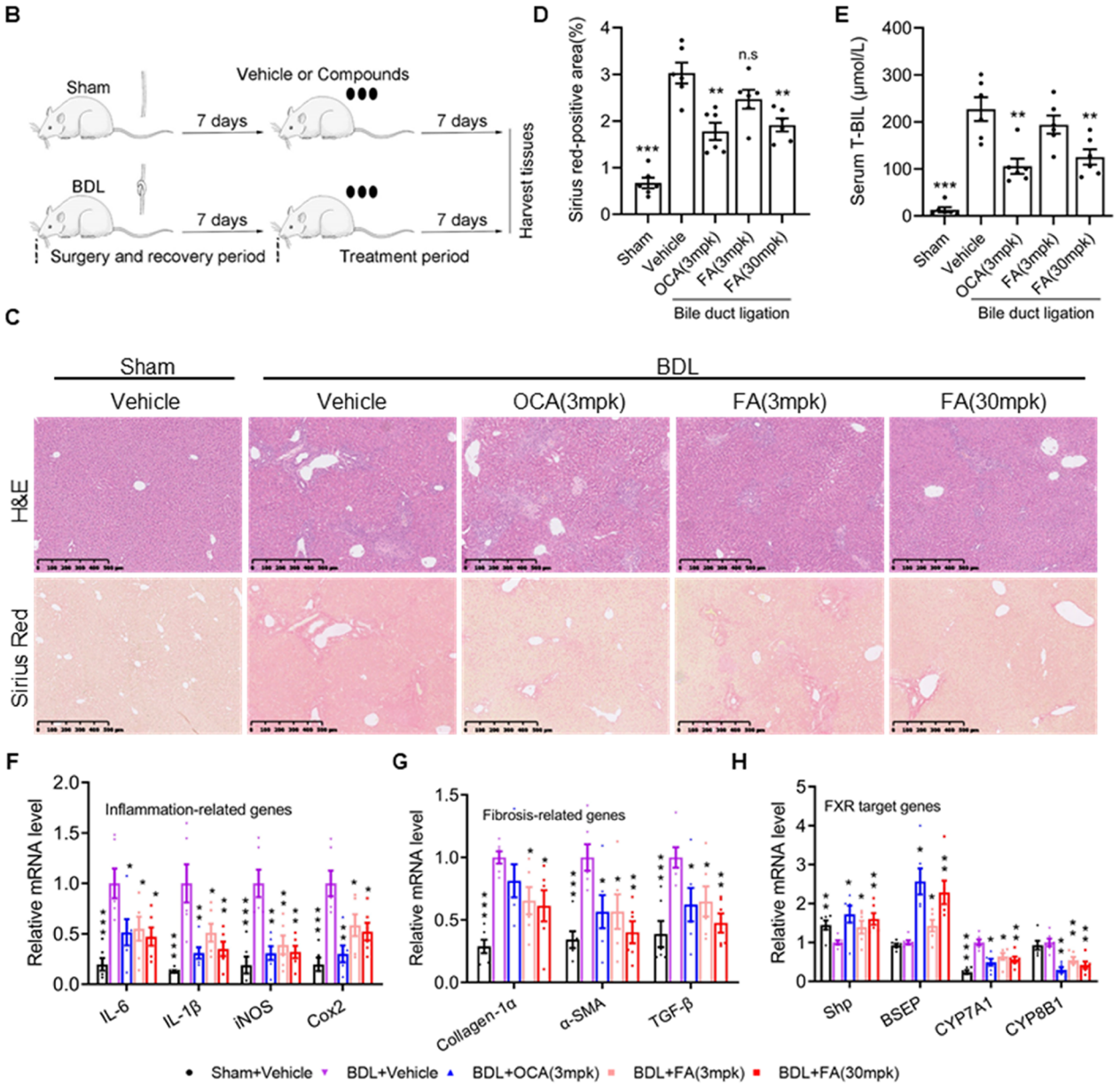

Figure 4. Fargesone A potentiates liver repair after bile duct ligation-induced liver injury. (A) Pharmacokinetics parameters of Fargesone A in mice. $t_{1/2}$, half-life; T_{max}, time of maximum plasma concentration; Cmax, maximum plasma concentration; AUC, area under the curve; MRT, mean residence time; *F*, oral bioavailability. (B) Schematic of bile duct ligation (BDL)-induced chronic liver injury and fibrosis evaluation in mice ($n = 6$). (C) Liver sections H&E and Sirius Red staining after 14 days of BDL. Pre-injured BDL for 7 days mice were treated with a vehicle, positive control OCA (3 mg/kg), FA (3 mg/kg), or FA (30 mg/kg) for another 7 days. Scale bar, 500 μm. (D) Corresponding quantification of Sirius Red staining of liver sections from (C). (E) Serum total bilirubin (T-BIL) level in different groups. (F−H) Relative mRNA levels of genes involved in inflammation (F), fibrosis (G), and FXR target genes (H) by reverse-transcription PCR in the liver. The primer pairs used in (F−H) are listed in the Supporting Information. *P* values were determined by one-way ANOVA with Tukey's multiple comparison post hoc test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Values are the mean ± SEM.

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α-arylation of ketone **9**,[34] followed by TIPS protection of the phenol group to afford ketone **12** on a 20 g scale (Scheme 1B). The resulting ketone **12** was then subjected to an asymmetric reduction via a remarkable kinetic dynamic resolution process to afford the enantiomerically pure alcohol **13** with 88% yield and 97% ee.[32] TIPS deprotection proceeded efficiently under TBAF condition with substrate **13** to furnish the phenol **14** in 80% yield. Sequential *O*-allylation and TBS protection of phenol **14** yielded **16** in 64% overall yield. After Claisen rearrangement, the intermediate **17** was prepared on a gram scale. With compound **17** in hand, we turned our attention to the crucial dearomatization reaction. After the extensive screening of different oxidative conditions (for more details, see Supporting Information), we observed that the desired product could be obtained in 23% yield with $Pb(OAc)_4$ as an oxidant.[33] The undesired product **18** could be reduced to the starting material **17** in the presence of isopropyl magnesium bromide and then re-subjected to the previous dearomatization process.[34] Compound **19** was finally obtained in a 39% yield after three cycles. With the advanced intermediate **19** in hand, we started our investigation into the final biomimetic cascade process. After careful examination, upon the treatment of **19** with HF, a two-step TBS group deprotection/oxa-Michael addition cascade was performed to furnish fargesones A and B, and kadsurin A in one pot. In summary, we have accomplished the first total synthesis of (−)-fargesone A in nine steps, starting from commercially available compounds. More than 100 mg of FA was efficiently prepared through chemical synthesis, which set the stage for the following biological evaluations.

Fargesone A Alleviates Hepatocyte Disorders in an FXR-Dependent Manner in Human Liver WRL68 Cells

The liver is among the organs with high FXR expression. Meanwhile, FXR is an attractive target in drug discovery for various metabolic liver diseases, such as primary biliary cirrhosis (PBC) and nonalcoholic steatohepatitis (NASH). To determine whether FA could alleviate lipid accumulation and cell death in liver cells through an FXR-dependent manner, we stably silenced FXR expression in WRL68 cells (ATCC) using lentiviruses containing an shRNA-FXR construct targeting FXR. Viruses containing a scrambled shRNA construct were used as a negative control. FXR's mRNA and protein expression levels were analyzed by qPCR (Figure 3A) and western blot (Figure 3B), indicating the effective knockdown of FXR. FA treatment significantly alleviated oleic acid (OA)-induced lipid accumulation (Figure 3C,D) and also alleviated acetaminophen (APAP)-induced cell death in the WRL68 cells with shRNA-control (Figure S3A,B). Interestingly, the effects of FA were not observed in the WRL68 cells depleted of FXR. These data suggest that FA exerts its activities in an FXR-dependent manner.

Fargesone A Ameliorates Liver Inflammation and Fibrosis in the BDL Mouse Model

To check the plasma exposure and *in vivo* stability of FA, the pharmacokinetic (PK) test was performed. LC-MS/MS was used to determine the level of FA in mice plasma samples. FA showed acceptable PK profiles in general. Good oral bioavailability (10.9%), reasonable intravenous half-life (0.62 h), and maximum plasma concentration (941 ng/mL) suggest that it could be further evaluated in therapeutic animal models (Figure 4A). Bile duct ligation (BDL)-induced chronic liver injury animal model has been wildly used in preclinical studies to evaluate candidate compounds for their potential in treating liver disorders. OCA, a drug approved by FDA to treat PBC disease, showed promising activity in this animal model. Therefore, we explored the therapeutic potential of FA to alleviate liver inflammation and fibrosis associated with chronic liver injury in mice subjected to BDL. Beginning at 7 days after BDL, vehicle, the positive control OCA or FA was administered daily for an additional 7 days, before animals were sacrificed 14 days after BDL (Figure 4B). Mice treated with a vehicle showed bridging fibrosis and a large amount of collagen deposition and inflammatory infiltrates based on the H&E and Sirius red-stained tissue sections (Figure 4C,D). Treatment with FA (30 mg/kg) or OCA (3 mg/kg) resulted in a lower level of inflammatory infiltrates and a smaller amount of collagen deposition compared to the vehicle group (Figure 4C,D). Although not statistically significant, the low-dose FA group (3 mg/kg) showed a trend toward improvement in inflammatory infiltrates and collagen deposition (Figure 4C,D). Meanwhile, a BDL-induced sharp increase in total bilirubin level in the serum was also reversed in the OCA or FA treatment groups (Figure 4E). Then, we tested the mRNA levels in liver tissues and further investigated FA's molecular mechanisms for liver injury amelioration in the BDL mice model. Similar to OCA, treatment with FA significantly decreased liver mRNA expression of the inflammatory biomarkers interleukin (IL)-6, IL-1β, inducible nitric oxide synthase (iNOS), and prostaglandin-endoperoxide synthase 2 (COX2) (Figure 4F). BDL procedure also significantly induced the mRNA expression of fibrosis biomarkers collagen-1α, smooth muscle α Actin (α-SMA), and transforming growth factor-β (TGF-β) in liver tissues, which were reduced in the FA-treated group (Figure 4G). Notably, FA treatment induced the expression of small heterodimer partner (SHP) and bile salt export pump (BSEP), and reduced the levels of cytochrome P450 7A1 (CYP7A1) and cytochrome P450 8B1 (CYP8B1), several well-characterized FXR direct or indirect target genes (Figure 4H), suggesting the protective anti-inflammatory and antifibrotic effects of FA against BDL-induced liver injury is through targeting FXR.

CONCLUSIONS

In summary, by applying a high-throughput chemical screen, we have identified the natural product Fargesone A (FA) as a novel FXR agonist. FA showed good potency and selectivity to activate FXR over other nuclear receptors. We have further accomplished the first enantioselective total synthesis of FA in nine steps. The synthesis features a remarkable asymmetric reduction through a kinetic dynamic resolution to install the crucial stereogenic center and a late-stage biomimetic cascade to furnish the final natural product. The synthesis is concise and scalable to provide a solution to the supply of FA. Further *in vivo* studies using the bile duct ligation (BDL)-induced liver disorder mouse model showed that FA significantly ameliorates pathological features in mice. We can envision that adapting the chemistry reported herein to create additional derivatives with optimized structures may provide an effective means to develop new drug candidates as therapy for liver diseases.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.2c00600.

Supporting figures, materials and methods, experimental arrangements details, synthetic methods, reaction schemes, and spectroscopic data (PDF)

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Author Contributions

‖F.G. and K.C. contributed equally. X.L. conceptualized the project, and revised and finalized the manuscript. F.G. and K.C. wrote the manuscript. F.G. designed and performed the biological experiments and analyzed data. K.C. designed and performed the chemical synthesis. H.D., D.H., C.L., and S.L. assisted with the chemical synthesis. Y.G. assisted with the protein purification. CRediT: **Fusheng Guo** data curation, formal analysis, investigation, validation, writing-original draft; **Kaiqi Chen** data curation, formal analysis, investigation, validation, writing-original draft; **Haoran Dong** data curation, investigation; **Dachao Hu** formal analysis, investigation; **Yihui Gao** formal analysis, investigation; **Chendi Liu** formal analysis, investigation; **Surat Laphookhieo** data curation, investigation; **Xiaoguang Lei** conceptualization, funding acquisition, project administration, supervision, writing-original draft, writing-review & editing.

Notes

The authors declare no competing financial interest.

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