



Article

Discovery of Obacunone as a TGR5 Agonist from *Rhizoma coptidis*: Affinity Screening, Functional Verification and Transcriptional Analysis

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Abstract

Rhizoma coptidis (RC), known as Huang Lian, is widely used for treating diabetes in Traditional Chinese Medicine. G protein-coupled bile acid receptor 1 (TGR5) is a potential therapeutic target for glucose-lipid metabolic disorders due to its capacity to stimulate glucagon-like peptide-1 (GLP-1) secretion. However, whether RC contains ingredients targeting TGR5 remains unclear. In this study, we screened 330 secondary metabolites of RC via molecular docking and identified obacunone as a top candidate. We confirmed binding using Cell Membrane Chromatography (CMC) and quantified affinity via Surface Plasmon Resonance (SPR), determining a KD of 101 μ M. cAMP assays identified obacunone as an activator of TGR5. Subsequently, concentration-dependent cAMP assays characterized it as a partial agonist with an EC₅₀ of 9.6 μ M. Finally, transcriptomic profiling revealed a stress adaptation response, identifying Heme Oxygenase 1 (*HMOX1*) as the most significantly upregulated gene ($\text{padj} = 9.99 \times 10^{-72}$, $\log_2\text{FoldChange} = 1.65$). These findings contribute to understanding the pharmacological profile of RC and provide a screening example for identifying components with GPCR activity in RC.

Keywords: TGR5; *Rhizoma coptidis*; obacunone; molecular docking; CMC; SPR; *HMOX1*

1. Introduction

Rhizoma coptidis (RC), commonly referred to as ‘Huanglian’ in China, is a widely used herb of Traditional Chinese Medicine (TCM). It is officially documented in the Pharmacopoeia of the People’s Republic of China for its primary therapeutic actions of ‘clearing heat, drying dampness, purging fire, and detoxifying’ [1]. It serves as the “Monarch” drug (principal herb) in classic therapeutic prescriptions for glycolipid metabolic disorders such as Huanglian Jiedu Decoction and Gegen Qinlian Decoction [2]. Historically, it has been utilized for over a millennium in the treatment of “Xiao Ke” (Wasting-Thirst Syndrome), the TCM equivalent of diabetes mellitus [3]. Modern clinical practice has validated its efficacy through standardized formulations, most notably Jinqi Jiangtang Tablets (JQJT), in which *C. chinensis* serves as the principal ingredient. A landmark multicenter, randomized, double-blind, placebo-controlled trial (RCT) published demonstrated the stabileness axis of JQJT in metabolic intervention [4]. This evidence not only led to JQJT becoming the



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first TCM approved by the NMPA for pre-diabetes intervention but also substantiated the critical role of *C. chinensis* in improving insulin sensitivity and glucose homeostasis. Modern pharmacological assessments have corroborated its broad therapeutic spectrum, confirming significant hypoglycemic, lipid-lowering, anti-inflammatory, neuroprotective effects and antibacterial activities [5,6]. Berberine, a primary bioactive constituent of RC, has been confirmed to possess pharmacological activities such as anti-diabetic, lipid-regulating and multi-target neuroprotective effects [7,8]. However, crude extracts of RC often exhibit superior hypoglycemic effects compared to Berberine alone, hinting at the presence of other synergistic bioactive compounds [9].

G protein-coupled bile acid receptor 1 (*GPBAR1*), also known as TGR5, is a membrane receptor activated by bile acids that transduces these stimuli into complex downstream signaling cascades. Upon ligand binding, TGR5 undergoes a conformational change that triggers the dissociation of the heterotrimeric G protein; the $G_{\alpha s}$ subunit subsequently binds GTP and activates adenylyl cyclase (AC), which catalyzes the conversion of ATP into the second messenger cyclic AMP (cAMP) [10].

Recent studies have shown that TGR5 is widely recognized as a promising therapeutic target for metabolic diseases [11], a potential underscored by its expression in tissues crucial for metabolic regulation, including the intestine, brown adipose tissue, and skeletal muscle. [12] Activation of TGR5 on the basolateral membrane of enteroendocrine L cells potently stimulates the secretion of the incretin hormone GLP-1 [13]. GLP-1 plays an important role in postprandial glucose homeostasis by enhancing glucose-dependent insulin secretion, inhibiting glucagon release, and delaying gastric emptying [14]. Crucially, TGR5-mediated GLP-1 secretion remains effective even under conditions of insulin resistance, distinguishing it as a promising target for metabolic intervention [15]. Beyond the gut, TGR5 signaling in brown adipose tissue (BAT) and skeletal muscle triggers the cAMP-PKA pathway, thereby inducing the expression of type 2 iodothyronine deiodinase (DIO2). DIO2 catalyzes the conversion of the pro-hormone thyroxine (T4) into the metabolically active triiodothyronine (T3), which locally amplifies mitochondrial oxidative phosphorylation and energy expenditure [16].

Given the established role of TGR5 as a key metabolic regulator and the well-documented clinical efficacy of RC in treating metabolic disorders, we hypothesized that RC may contain constituents that directly activate TGR5. However, despite this potential connection, there is currently a lack of research systematically screening the chemical constituents of RC with TGR5 as the direct target. Questions such as whether RC contains novel TGR5 agonists beyond its known constituents, as well as the nature of their agonist effects and downstream signaling pathways, remain unanswered. To address this gap, we employed a comprehensive screening strategy that integrates virtual prediction with physical validation. Leveraging the Gene-encoded Natural Diverse Components Repository (GNDC) [17], we accessed a vast chemical space to systematically analyze the secondary metabolites of RC. Subsequently, we employed AutoDock Vina to conduct a high-throughput virtual screening of the GNDC database, aiming to identify ligands with high binding affinity for the TGR5 orthosteric pocket.

Cell membrane chromatography (CMC) represents a bioaffinity chromatographic methodology wherein membrane proteins are immobilized onto a chromatographic stationary phase, thereby simulating the physiological process of drug-ligand interactions and providing an efficacious and expedient instrument for pharmaceutical discovery. Due to this unique biomimetic feature, CMC has emerged as a high-efficiency platform for elucidating the pharmacodynamic material basis of TCM [18,19]. In this study, CMC was used to screen candidate compounds from RC that bind to TGR5.

Surface Plasmon Resonance (SPR) is widely recognized by regulatory authorities, including the US FDA and the Pharmacopoeia of the People's Republic of China (ChP), as a standard and robust label-free technology for characterizing molecular interactions [1,20]. In this study, SPR was subsequently employed to confirm and characterize the binding kinetics and affinity of the interaction between TGR5 and candidate compounds. However, receptor binding does not inherently guarantee functional activation. Furthermore, we assessed downstream cAMP assay using the Promega GloSensor™ technology (Madison, WI, USA) to evaluate the TGR5 agonist activity of obacunone.

RNA seq technology can be used to quantify transcriptome, differentially expressed genes, and determine transcriptional regulatory mechanisms, thus providing a more complete genetic picture than DNA sequencing [21]. To elucidate the underlying molecular effect and signaling networks, high-throughput RNA sequencing (RNA-seq) was performed on TGR5-overexpressing cells. This transcriptome analysis enabled us to map the precise gene regulatory network modulated by obacunone. In conclusion, this study systematically reports the screening, identification, and transcriptional regulatory effects of obacunone—a natural agonist of TGR5—thereby expanding our understanding of the pharmacological basis of RC.

2. Results

2.1. Preliminary Screening of Potential TGR5 Ligands in RC by Molecular Docking

To systematically explore the components of RC for potential TGR5 agonists, we initiated our study with a virtual screening approach. A specialized library comprising 330 small-molecule constituents of RC was constructed by retrieving chemical structures from the GNDC repository. These compounds were classified based on their biosynthetic pathways into alkaloids, fatty acids, terpenoids, polyketides, and shikimates/phenylpropanoids (Figure 1A). The cryo-EM structure of the TGR5-Gs complex bound with the synthetic agonist INT-777 (PDB ID: 7CFN) served as the structural template for docking simulations. Using AutoDock Vina, we evaluated the binding affinity of each compound to the TGR5 orthosteric pocket. The endogenous ligand, cholic acid (CA), was used as a reference standard, exhibiting a binding affinity of -9.026 kcal/mol. The screening results revealed a subset of 14 natural compounds that displayed higher theoretical binding affinities than cholic acid (Figure 1B). Notably, two limonoid triterpenes stood out: limonin (-11.21 kcal/mol) and obacunone (-10.94 kcal/mol). These compounds demonstrated marked high binding scores, suggesting a strong potential for receptor interaction. In contrast, berberine, the most abundant and well-studied alkaloid of RC, exhibited a lower binding affinity of -8.45 kcal/mol, suggesting it may not be a high-affinity ligand for the TGR5 orthosteric site.

Detailed structural analysis of the ligand-receptor complexes provided mechanistic insights into these binding disparities (Figure 1C). Detailed parameters of hydrogen bonds and hydrophobic interactions are provided in (Tables S1 and S2). Obacunone was observed to fit within the hydrophobic binding cavity of TGR5. A critical hydrogen bond was identified between the A-ring lactone oxygen of obacunone and the amide nitrogen of residue Gln236 (Chain A), with a bond length of approximately 3.5 Å. Furthermore, the obacunone-TGR5 complex was stabilized by an extensive network of hydrophobic interactions involving residues Leu57, Tyr72, Phe144, Leu227, Leu245, and Leu249. Comparatively, limonin, despite its structural similarity to obacunone, adopted a slightly different pose within the pocket. While it engaged in hydrophobic interactions with residues Tyr72, Phe144, Leu227, Leu246, and Leu249, it failed to form the stabilizing hydrogen bond with Gln236 or other key polar residues. This absence of polar anchoring suggested that, despite the high docking score driven by hydrophobic complementarity, the binding of limonin

might be less stable than that of obacunone. Berberine formed a hydrogen bond with Asn76 and interacted hydrophobically with Tyr72 and Phe144, but its planar structure appeared less optimized for the deep hydrophobic pocket compared to the triterpenoid core of the limonoids. These *in silico* findings prioritized obacunone and limonin as the primary candidates for subsequent biophysical and functional validation.

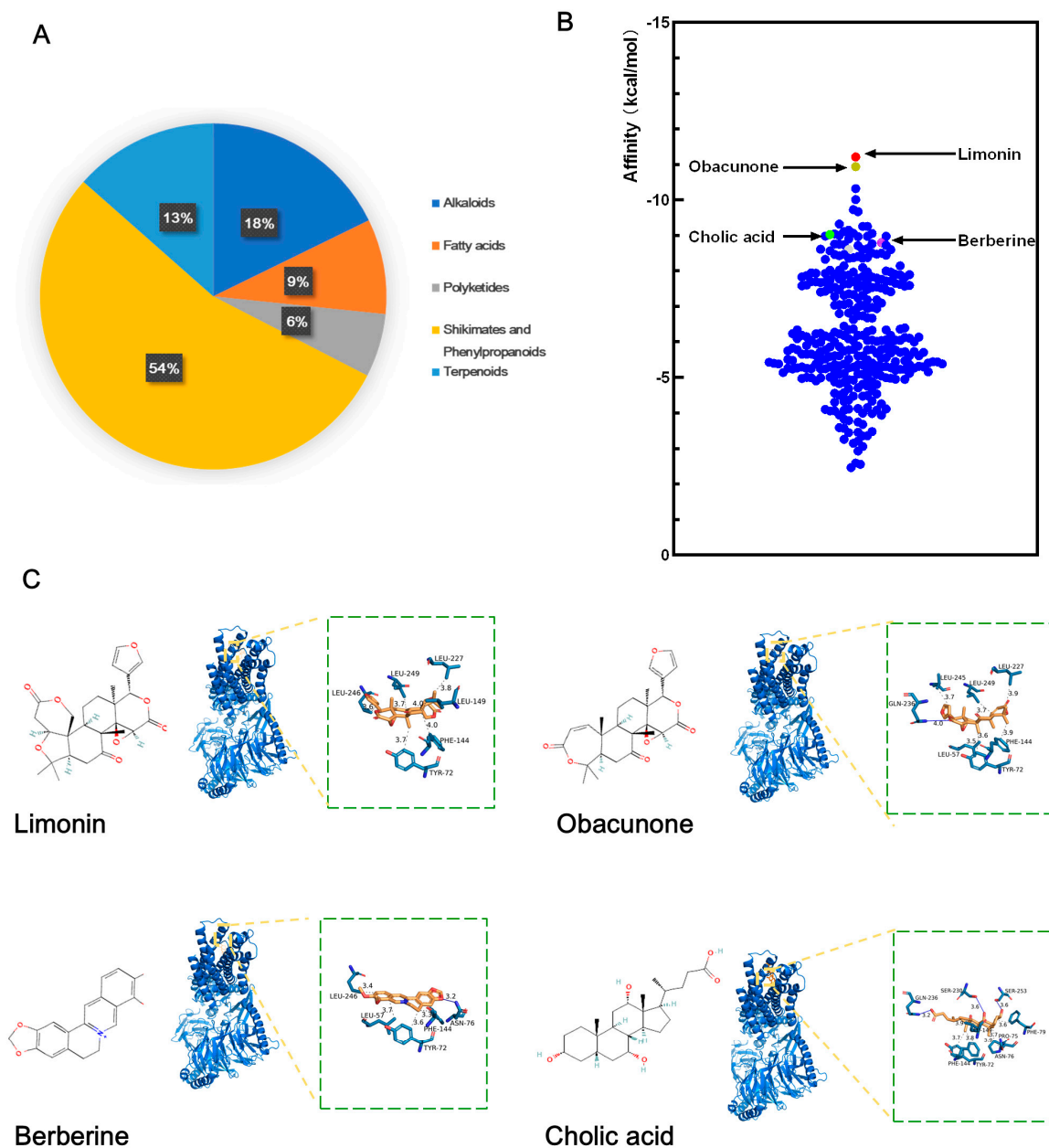


Figure 1. Virtual screening and molecular docking analysis of bioactive compounds from *Rhizoma coptidis* (RC) targeting TGR5. **(A)** Classification of identified chemical constituents from RC. **(B)** Binding affinity profiles obtained from virtual screening, highlighting obacunone as a top candidate with high affinity. In the binding affinity profile, blue circles represent ordinary compounds; green represents the positive control Cholic Acid; red represents Limonin; yellow represents Obacunone; and purple represents Berberine. **(C)** Chemical structures and comparative 3D binding modes of cholic acid, obacunone, limonin, and berberine within the TGR5 orthosteric pocket predicted by molecular docking. Key amino acid residues are labeled. The TGR5 homology model was constructed based on [PDBID:7CFN] in RCSB. Hydrogen bonds are shown as blue lines.

2.2. Identification of Obacunone as a Potential TGR5 Ligand via CMC

To bridge the gap between computational prediction and physical reality, we employed CMC. We constructed a TGR5-CMC column by immobilizing cell membrane fragments from TGR5-overexpressing HEK293T cells onto a silica support. The chromatographic retention behavior of a compound on the CMC column is directly proportional to its affinity for the immobilized receptor. When the candidate compounds were injected into the TGR5-CMC system, distinct retention profiles were observed. Obacunone exhibited a marked and prolonged retention, with a retention time (t_R) of 64.69 min (Figure 2A). This substantial delay compared to the solvent front indicates a distinct interaction with the stationary phase TGR5 receptors.

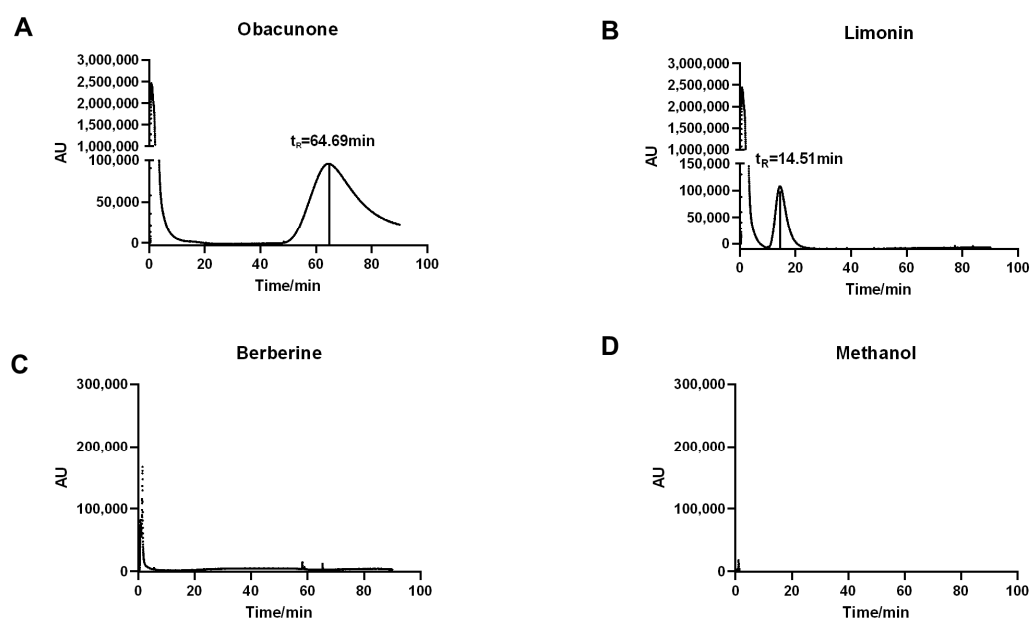


Figure 2. Characterization of ligand-receptor interactions using CMC. (A–C) Representative chromatograms showing the retention behaviors of (A) obacunone, (B) limonin, (C) berberine and (D) methanol (solvent control) on the TGR5-CMC stationary phase. Retention times were recorded and compared against methanol. Ligand-receptor interactions were identified by observing marked shifts in retention time on the TGR5 column compared to the control, where a delayed retention indicates binding affinity.

In contrast, limonin eluted much earlier, with a t_R of 14.51 min (Figure 2B). Although this retention is greater than the void volume, indicating some degree of interaction, it is markedly weaker than that of obacunone. This finding aligns with the structural analysis which suggested that the lack of critical hydrogen bonding might compromise limonin's binding stability. Most notably, berberine showed no marked retention on the TGR5 column (t_R) similar to solvent control (Figure 2C,D), confirming the docking prediction that berberine does not specifically bind to the TGR5 receptor. These CMC results provided the first line of experimental evidence identifying obacunone as a physical ligand for TGR5.

2.3. Verification of Direct Binding and Affinity Quantification via SPR

To further validate the direct interaction and rigorously quantify the binding kinetics, we performed SPR analysis. SPR is recognized as the widely accepted label-free technology for characterizing molecular interactions in real-time. Recombinant human TGR5 protein was purified from the HEK293T expression system and immobilized on a CM5 sensor chip via amine coupling. We first validated the functional integrity of the immobilized TGR5 using the endogenous ligand, cholic acid (CA). Injection of CA resulted in a rapid, concentration-dependent increase in Response Units (RU) (Figure 3C), followed by a fast

dissociation phase. Kinetic analysis yielded an equilibrium dissociation constant (KD) of 200 μM ($2.00 \times 10^{-4}\text{M}$) (Figure 3D). This KD value falls within the typical affinity range for natural product screening hits [20], confirming the reliability of our SPR platform.

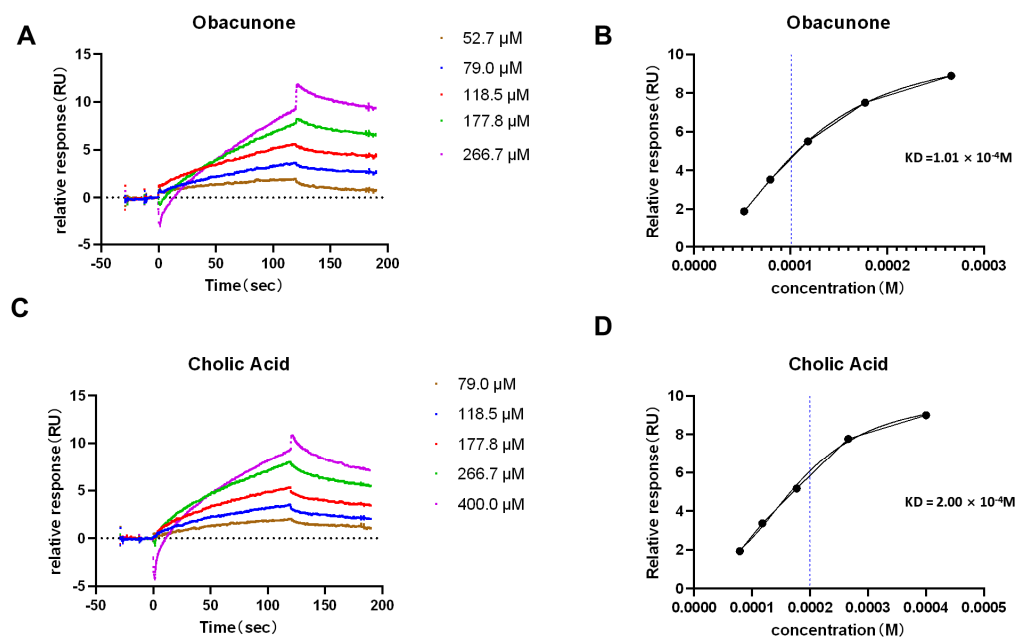


Figure 3. SPR analysis of ligand binding to the TGR5 receptor. (A,C) Representative sensorgrams showing the real-time kinetic binding profiles of (A) obacunone and (C) cholic acid (positive control) at various concentrations. (B,D) Steady-state affinity fits derived from equilibrium response values plotted against concentration for (B) obacunone and (D) cholic acid. The dissociation constants (KD) were calculated using a steady-state affinity model.

Subsequently, Obacunone was evaluated across a 1.5-fold serial dilution ranging from 52.7 μM to 266.7 μM . The resulting sensorgrams displayed clear association and dissociation phases typical of a reversible ligand-receptor interaction (Figure 3A). The data were fitted to a steady-state affinity model, determining the kinetic rate constants k_a and k_d and the affinity constant. The KD for the obacunone-TGR5 interaction was calculated to be 101 μM ($1.01 \times 10^{-4}\text{M}$) (Figure 3B), indicating a specific and measurable interaction. The SPR data confirmed that obacunone binds directly to the TGR5 receptor protein, providing a biophysical basis for its potential biological activity.

2.4. GloSensor cAMP Assay Confirms Obacunone as a TGR5 Agonist

Physical binding is a prerequisite for receptor activation, but it does not distinguish between agonists and antagonists. To determine the functional consequence of obacunone binding, we assessed its ability to trigger the canonical TGR5 signaling pathway: the $G\alpha_s$ -mediated activation of adenylyl cyclase and subsequent generation of cAMP. We utilized the Promega GloSensor™ cAMP assay, a highly sensitive live-cell biosensor system.

HEK293T cells stably overexpressing TGR5 were transiently transfected with the GloSensor plasmid. Treatment with obacunone elicited a rapid and distinct increase in intracellular cAMP levels, manifesting as a dose-dependent surge in luminescence (Figure 4A). This response profile confirmed that obacunone acts as a TGR5 agonist. Limonin also induced cAMP production (Figure 4B), but with lower maximal efficacy and potency compared to obacunone, mirroring the weaker binding affinity observed in CMC. Consistent with previous assays, berberine treatment failed to induce any detectable change in cAMP levels even at high concentrations (Figure 4C), reinforcing that its metabolic effects are TGR5-independent. Treatment with cholic acid stimulated distinct cAMP accumulation,

confirming the sensitivity of the TGR5 signaling assay (Figure 4D). At equimolar concentrations, the cAMP functional assay revealed that obacunone elicited the higher luminescence signal (RLU), compared with limonin and berberine (obacunone > limonin > berberine). This potency rank order mirrors the binding trends observed in the CMC analysis, confirming that stronger receptor affinity correlates with enhanced downstream signaling efficacy.

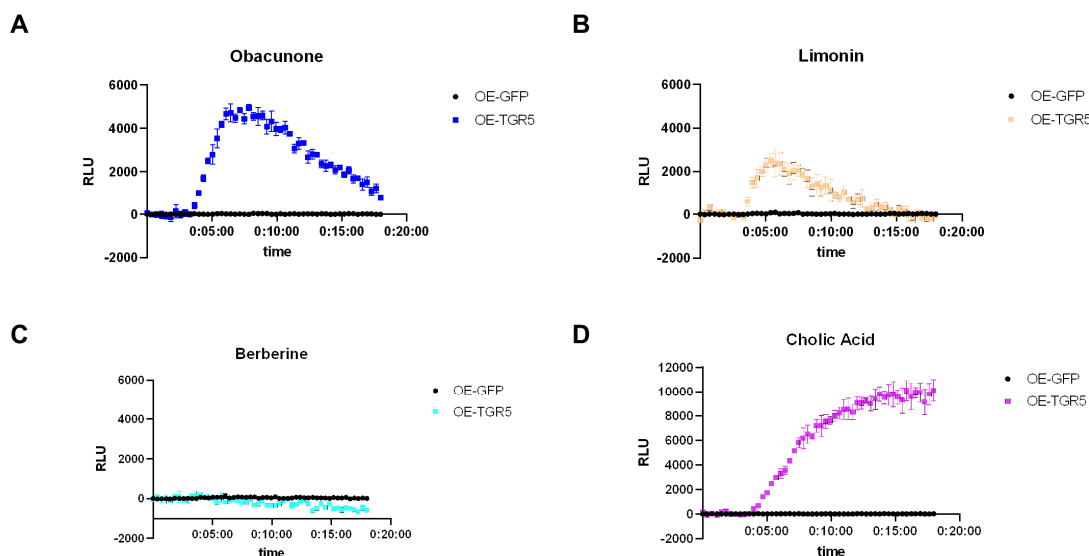


Figure 4. Functional characterization of TGR5 candidates via cAMP assay. (A–D) Comparative analysis of intracellular cAMP levels treated with the indicated compounds, 50 μ M Obacunone (A), 50 μ M Limonin (B), 50 μ M Berberine (C) and 10 μ M Cholic Acid (D). The stable cell lines were designated as OE-TGR5 (TGR5 overexpression) and OE-GFP (vector control). Data represent mean $\pm$ SEM of $n = 3$ independent biological replicates and expressed as relative luminescence units (RLUs).

To quantify the potency of obacunone, we constructed a dose–response curve. Non-linear regression analysis yielded a half-maximal effective concentration (EC₅₀) of 9.6 μ M (9.6×10^{-6} M) (Figure 5A,B). These functional data establish obacunone as a promising agonist of the TGR5.

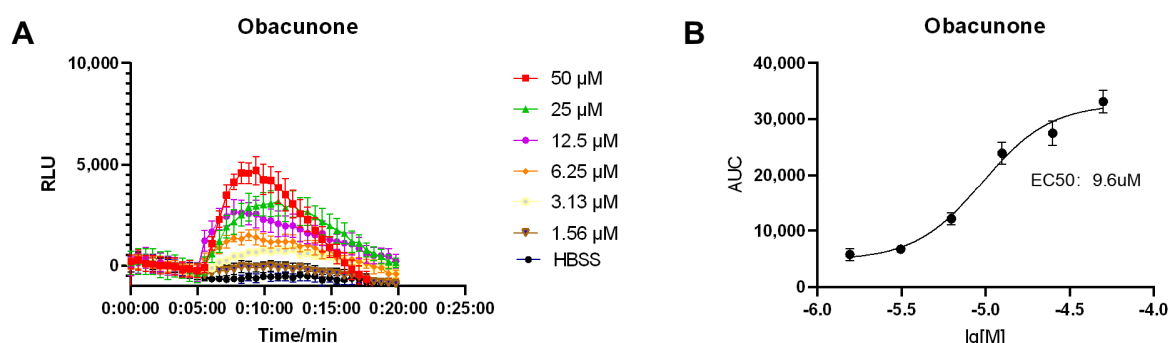


Figure 5. Obacunone acts as a TGR5 agonist to induce cAMP signaling. (A) Treatment with obacunone stimulated cAMP accumulation, confirming its agonist activity. (B) The compound exhibited dose-dependent activation of TGR5 with an EC₅₀ value of 9.6 μ M. Data represent mean $\pm$ SEM of $n = 3$ independent biological replicates and expressed as relative luminescence units (RLUs).

2.5. Transcriptomic Profiling Identifies an Obacunone-Induced Cellular Stress Adaptation Program Mediated by HMOX1

Having established obacunone as a TGR5 agonist, we sought to elucidate the downstream transcriptional landscape remodeled by this activation. We performed high-

throughput RNA sequencing (RNA-seq) on TGR5-overexpressing HEK293T cells treated with 50 μ M obacunone (F1 group) or DMSO vehicle (F0 group) for 24 h. The RNA-seq experiment yielded high-quality sequencing data, with Q30 scores consistently exceeding 97% (Table S3), indicating reliable base-calling accuracy. Furthermore, alignment rates to the human reference genome (GRCh38) surpassed 94% across all samples (Table S4), confirming the integrity of the dataset for downstream analysis. Of the 79,203 genes annotated in the reference genome, 35,157 (44.4%) exhibited detectable expression (defined as having non-zero read counts in at least one sample), underscoring the comprehensive depth of the transcriptome coverage. Principal Component Analysis (PCA) of the gene expression profiles revealed a distinct separation between the control and obacunone-treated groups, with the first principal component (PC1) explaining 27.51% of the total variance (Figure 6A). This clear segregation indicates that obacunone treatment induces a substantial and consistent shift in the cellular transcriptome. The global landscape of differential gene expression was visualized via a volcano plot (Figure 6B). Genes meeting the significance criteria ($p < 0.05$) and fold-change thresholds ($|\log_2\text{FoldChange}| > 0.5$) were highlighted, revealing a balanced distribution of up- and down-regulated transcripts. Differential expression analysis identified a total of 2156 differentially expressed genes (DEGs) based on stringent criteria ($\text{padj} < 0.05$). Of these, 1295 genes were upregulated and 861 were downregulated (Table S5). The top 20 most significant DEGs are summarized in (Table 1). Notably, the list is dominated by stress-response and antioxidant genes. *HMOX1* (Heme Oxygenase 1) emerged as the most significantly up-regulated target ($=1.65$), alongside other key redox regulators such as *GCLM*, and *SQSTM1*. Collectively, these transcriptomic data reveal that obacunone treatment in TGR5-overexpressing cells induces a stress-adaptation program, with *HMOX1* as the most significantly upregulated gene. It is important to note that these findings represent an association with TGR5 activation in an overexpression system; the causal dependence on TGR5 for these transcriptional changes, and their relevance in vivo, remain to be established.

Table 1. Top 20 differentially expressed genes (DEGs).

Gene Name	$\log_2\text{FoldChange}$	padj
HMOX1	1.6529257	1.0×10^{-71}
DDIT4	0.954214291	3.9×10^{-46}
GCLM	0.778998522	5.1×10^{-30}
ODC1	0.551169166	2.2×10^{-25}
TXNRD1	0.562426218	5.2×10^{-24}
FOXO4	−0.589024635	8.1×10^{-23}
HSP90AA1	0.419263699	2.2×10^{-20}
PGGHG	−1.003323896	2.9×10^{-19}
JUN	0.677338005	6.2×10^{-18}
ISYNA1	−0.549767483	7.9×10^{-17}
LAMA5	−0.726324597	8.4×10^{-17}
NOTCH3	−0.843748239	3.7×10^{-16}
PLXNA3	−0.803116405	4.2×10^{-15}
KIFC2	−0.869265067	2.3×10^{-14}
SQSTM1	0.443753513	2.7×10^{-14}
MIR17HG	−0.689930495	2.7×10^{-14}
ITPR3	−0.574121243	3.2×10^{-14}
CHAC1	1.517968596	1.3×10^{-13}
DNAJB1	0.461759766	3.1×10^{-13}
NFATC4	−0.622098998	7.4×10^{-13}

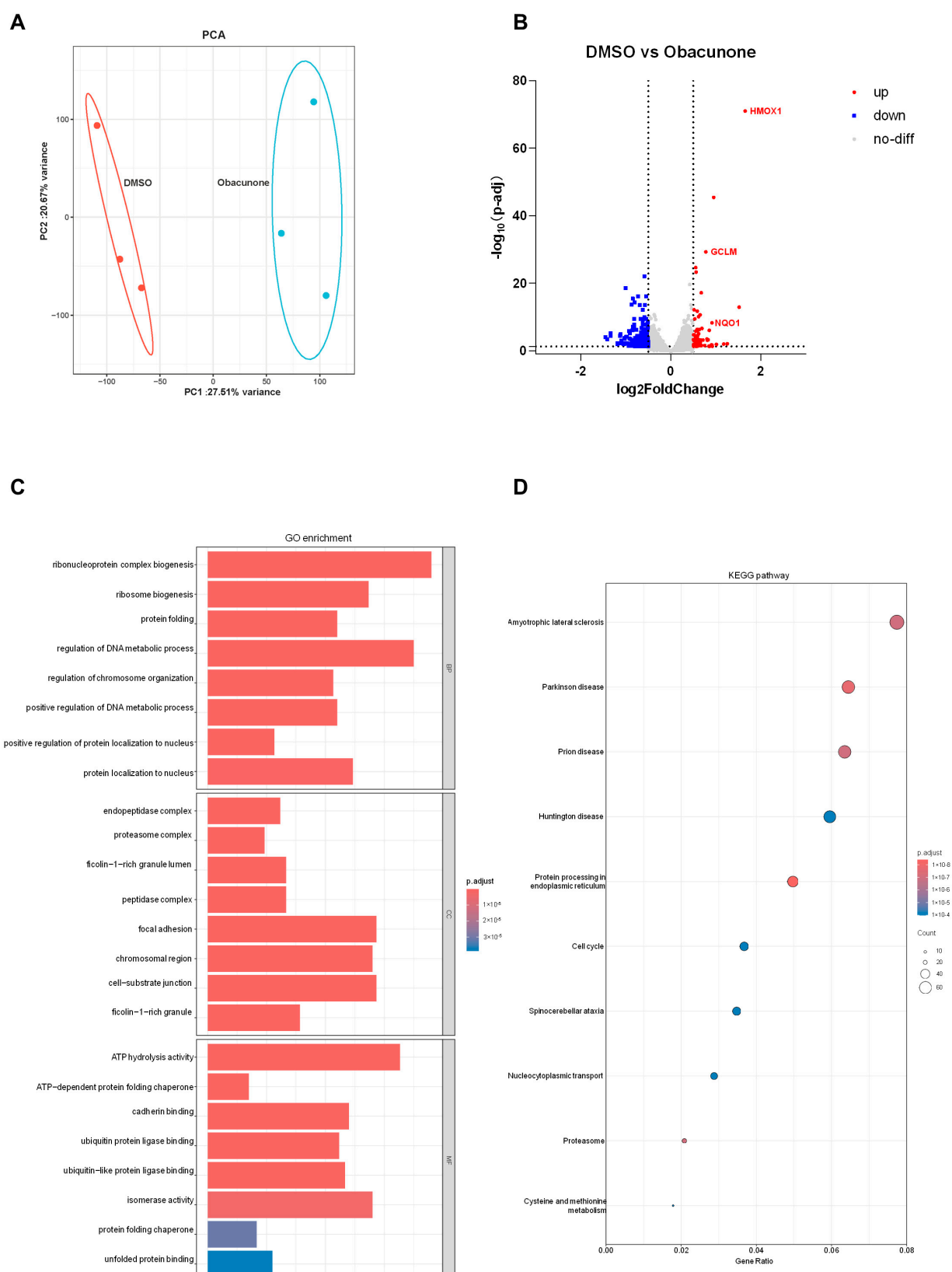


Figure 6. Transcriptomic alterations and functional analysis of DEGs induced by Obacunone. **(A)** Principal Component Analysis (PCA) based on FPKM values from three biological replicates per group. **(B)** Volcano plot visualizing DEGs between Obacunone-treated and DMSO-treated groups. **(C)** Top 8 significantly enriched Gene Ontology (GO) terms across biological process (BP), cellular component (CC), and molecular function (MF) categories. **(D)** Top 10 significantly enriched KEGG pathways. Size of the dots represents the gene count (e.g., 10, 20, 30, 40).

Gene Ontology (GO) enrichment analysis of the DEGs highlighted significant modulation of biological processes related to “ribonucleoprotein complex biogenesis,” “ribosome biogenesis,” and “protein folding” (Figure 6C). Comprehensive results from the GO enrichment analysis of DEGs are provided in (Table S6). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis provided further insight, revealing that the most significantly enriched pathways included “Protein processing in endoplasmic reticulum” and “Proteasome” (Figure 6D). Notably, the KEGG enrichment analysis identified the Non-alcoholic fatty liver disease pathway (hsa04932), which falls under the category of Endocrine and Metabolic Diseases. Comprehensive results from the KEGG enrichment analysis of DEGs are provided in (Table S7). These enrichments suggest that obacunone may trigger a cellular response geared towards maintaining proteostasis and managing protein folding stress.

3. Discussion

Obacunone has been found to potentially regulate the TGR5 signaling transduction and has anti-obesity and anti-hyperglycemic effects in cell and high-fat diet mice models in previous studies [22]. Notably, dietary supplementation with obacunone has been shown to stimulate muscle hypertrophy and suppress hyperglycemia and obesity specifically through the TGR5 and PPAR γ pathways [23]. Although these findings established the functional baseline, the systematic characterization of obacunone’s relative potency, direct binding kinetics, and downstream transcriptional reprogramming in human cells has yet to be fully elucidated. In the present study, we employed a stratified pharmacological approach to systematically elucidate its effect of action. Our data establish obacunone as a promising TGR5 agonist. Obacunone demonstrated a high-affinity interaction with the TGR5 pocket, triggering a distinct Gs-coupled signaling cascade evidenced by rapid, dose-dependent cAMP accumulation. Crucially, our comprehensive structure-activity relationship analysis—integrating molecular docking, CMC, and functional cAMP assays—revealed a striking differential in potency between obacunone and its structural analog, limonin. Despite their high homology, limonin exhibited negligible activity. This contrast underscores the A-ring lactone configuration and the specific orientation of the furan ring as critical pharmacophores for TGR5 activation. These structural features in obacunone appear to favor binding to the TGR5 pocket, whereas the modified A-ring of limonin likely introduces steric hindrance or lacks the necessary binding motifs. Such insights will be essential for guiding rational structural optimization, with the ultimate goal of discovering novel lead compounds that exhibit superior potency and selectivity. In conclusion, we identified obacunone—a TGR5 agonist—from RC and elucidated its key pharmacophore. This workflow offers a scalable and precise pathway for dissecting the possible material basis of TCM and discovering novel GPCR-targeted lead compounds.

Notably, our study determined the physical affinity K_D of obacunone to be 101 μ M via SPR, while its functional potency (EC_{50}) in the cAMP assay was 9.6 μ M. This approximately 10-fold discrepancy—where functional potency exceeds physical affinity—is a hallmark of “signal amplification” in GPCR pharmacology [24]. In systems with receptor reserve (such as TGR5-overexpressing HEK293T cells), occupancy of only a small fraction of receptors is sufficient to fully activate downstream effectors (Adenylyl Cyclase), thereby shifting the functional EC_{50} to a lower concentration than the binding K_D . This suggests that obacunone is an efficient agonist capable of triggering significant signaling even at sub-saturating occupancy levels.

In our study, berberine showed no direct activity in TGR5 binding or functional assays. This negative result is mechanistically significant; it confirms that while berberine exerts potent metabolic regulatory effects primarily through AMPK activation and indirect gut

microbiota modulation, it did not exhibit direct agonism. Therefore, we hypothesize that obacunone may exert an additional effect capable of synergizing with berberine to enhance the overall therapeutic efficacy of RC. This synergistic effect may explain the superior clinical efficacy of the whole herb compared to single agents.

The KEGG pathway analysis revealed a significant enrichment of non-alcoholic fatty liver disease (NAFLD, hsa04932). The NAFLD is complex, having evolved from the classic “two-hit” theory to a more comprehensive “multiple-hit” hypothesis. Despite this shift, oxidative stress remains a central pivot in disease progression. The disruption of redox homeostasis triggers a cascade of lipotoxicity, endoplasmic reticulum (ER) stress, and mitochondrial dysfunction, which collectively drive the transition from simple steatosis to steatohepatitis (MASH) [25]. Central to the cellular defense against this oxidative injury is the Nrf2/HO-1 signaling pathway. Nrf2 functions as a dual-action guardian: it not only scavenges reactive oxygen species (ROS) and electrophiles to preserve mitochondrial integrity but also negatively regulates genes governing hepatic lipid accumulation [26]. Its downstream effector HO-1, further exerts potent anti-inflammatory and anti-apoptotic effects, effectively severing the link between oxidative stress and fibrosis [27]. Our data identify the TGR5-Nrf2 signaling pathway as a potential downstream effector of obacunone, evidenced by the distinct upregulation of *HMOX1*, *NQO1*, *GCLM*, and *SLC7A11*. We propose that obacunone may ameliorate NAFLD specifically by engaging the TGR5-Nrf2-HO-1 signaling pathway, thereby enhancing hepatocyte antioxidant capacity and addressing the core oxidative etiology of the disease.

However, we acknowledge certain limitations in this study. Obacunone has previously been reported to directly activate the Nrf2 signaling cascade, for instance, in protecting retinal pigment epithelium cells from oxidative injury [28]. Previous studies have well established that TGR5 activation triggers the cAMP-PKA signaling cascade, which subsequently promotes the nuclear translocation of Nrf2 and the transcriptional induction of HMOX1 (HO-1). For example, the specific TGR5 agonist INT-777 has been consistently reported to upregulate the Nrf2/HO-1 pathway to mitigate oxidative stress in various cell models [29]. Although our data confirms TGR5 activation (cAMP generation), and literature supports a TGR5-cAMP-Nrf2 signaling axis, we cannot currently exclude the possibility that the observed HMOX1 upregulation arises partly from off-target direct chemical interactions. We can only speculate, based on the coincidence of TGR5 agonism and HMOX1 induction, that the receptor-mediated pathway plays a significant role. To definitively dissect the specific contribution of TGR5 versus off-target effects, future investigations utilizing TGR5-knockout (CRISPR/Cas9) or specific antagonist-treated models are indispensable.

While the use of HEK293T cells effectively demonstrates TGR5 signaling, this model primarily simulates a physiological context and fails to fully recapitulate the complex pathological microenvironments characteristic of diseases such as NAFLD. Consequently, to further validate substantiate the therapeutic potential of obacunone, future inquiries can prioritize the validation of the TGR5-Nrf2-HO-1 signaling pathway using multi-level experimental models, including human organoids and specific animal disease models. Furthermore, employing TGR5-knockout (CRISPR/Cas9) or knockdown (siRNA) strategies is indispensable for verifying target specificity, allowing for the rigorous distinction of TGR5-dependent biological functions from off-target activities. Specially, regarding glucolipid metabolic regulation, a critical objective will be to delineate the impact of obacunone on the incretin axis; specifically, assessing its capacity to stimulate glucagon-like peptide-1 (GLP-1) secretion in enteroendocrine L-cells (e.g., STC-1 or NCI-H716) would provide definitive evidence of its utility in managing metabolic diseases. To definitively establish that the metabolic benefits of obacunone under physiological or pathological conditions

are mediated via TGR5, future in vivo loss-of-function studies utilizing TGR5 knockout animal models or specific antagonists are necessary. In summary, the therapeutic potential of obacunone warrants further comprehensive investigation.

4. Materials and Methods

4.1. Molecular Docking and Virtual Screening

To facilitate virtual screening, a curated ligand library comprising 330 constituents of RC was established. Compound identifiers (PubChem CID and CAS numbers) were retrieved from the GNDC database and verified via an extensive literature search [30]. Three-dimensional Structure Data Files (SDF) were obtained directly from PubChem; for entries lacking 3D coordinates, 2D structures were converted and energy-minimized using the open-source RDKit toolkit (Version 2025.09.1) [31]. Dockey (v1.0.4) with AutoDock-Tools4 was used to prepare ligands and generate PDBQT file.

The cryo-EM structure of the TGR5-Gs complex, complexed with the agonist INT-777, was retrieved from the RCSB Protein Data Bank (PDB ID: 7CFN). Receptor preparation was conducted using Dockey, involving the removal of crystallographic water molecules and native ligands, hydrogenation, and optimization of the hydrogen-bonding network to generate a refined binding interface [32]. AutoDockTools was used to prepare receptor and generate PDBQT file.

Docking simulations were performed using the AutoDock Vina module (v1.2.7) integrated within Dockey. [33] The search space (Grid Box) was defined based on the orthosteric binding site of the INT-777/TGR5 complex, centered at coordinates $x = 96.723$, $y = 122.437$, $z = 115.640$ with dimensions of $14 \times 14 \times 15 \text{ \AA}$ [10]. To ensure stable sampling of the conformational space, the exhaustiveness parameter was set to 8, with all other parameters maintained at default values. Ligands were ranked by binding affinity scores, and critical ligand-receptor interactions—specifically hydrogen bonding and hydrophobic contacts—were characterized using Dockey and visualized with PyMOL (v3.1.3.1) [34].

4.2. Reagents and Stable Cell Line Generation

4.2.1. Chemicals and Reagents

Obacunone (CAS No. 751-03-1), Limonin (CAS No. 1180-71-8), Berberine (CAS No. 2086-83-1), and Cholic acid (CAS No. 81-25-4) were purchased from Taoshu Biotechnology Co., Ltd. (Shanghai, China). Stock solutions were prepared by dissolving compounds in dimethyl sulfoxide (DMSO) or methanol and stored at $-20\text{ }^{\circ}\text{C}$.

4.2.2. Cell Culture

HEK293T cells were obtained from Procell Life Science & Technology (Wuhan, China). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) from Gibco (Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS) from VivaCell (Shanghai, China) and 1% penicillin-streptomycin from Gibco (Waltham, MA, USA) at $37\text{ }^{\circ}\text{C}$ in a humidified atmosphere containing 5% CO_2 . To prevent contamination, cultures were treated with Mycoplasma Removal Agent from Beyotime (Shanghai, China).

4.2.3. Generation of TGR5-Overexpressing Cell Lines

To generate stable TGR5-expressing cells, lentiviral particles were produced by co-transfecting HEK293T cells with the transfer vector (pCDH-TGR5-3 $\times$ Flag-GFP-Puro or the empty control pCDH-3 $\times$ Flag-GFP-Puro), the packaging vector psPAX2, and the envelope vector pMD2.G (all from Youbio, Changsha, China) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol [35]. Viral supernatants were harvested at 48 and 72 h post-transfection and centrifuged at 2000 rpm for 10 min to

remove cellular debris (Figure S1A). For transduction, fresh HEK293T cells were incubated with the viral supernatant [36]. Infection efficiency was assessed 48 h post-transduction by visualizing GFP fluorescence using inverted fluorescence microscopy (Figure S1B). Stable cell lines were established by selecting with 1 µg/mL puromycin. Following initial selection, cells were maintained in medium containing puromycin to ensure sustained transgene retention. TGR5 overexpression was verified via Western blot (Figure S1C). The resulting stable cell lines were designated as OE-TGR5 (TGR5-overexpressing) and OE-GFP (GFP-expressing vector control) for subsequent experiments.

4.3. CMC

4.3.1. Preparation of Cell Membrane Homogenates

TGR5-overexpressing HEK293T cells were cultured in 15 cm dishes until they reached confluence. Cells were harvested by trypsin digestion, centrifuged, and the resulting pellets were stored at −80 °C. For membrane extraction, pellets were resuspended in 5 mL of 10 mM Tris-HCl buffer and lysed using a probe-type sonicator (80 W, 9% amplitude; pulse mode: 3 s on, 1 s off) for 30 min on ice [37].

The lysate was centrifuged at 5000 rpm for 10 min at 4 °C to remove nuclei and cellular debris. The supernatant was then ultracentrifuged at 12,000× g for 20 min to precipitate the cell membrane fraction. The resulting pellet was washed repeatedly with physiological saline to remove cytosolic proteins. Finally, the membrane pellet was resuspended and subjected to a second round of micro-sonication (21% amplitude; pulse mode: 3 s on, 1 s off) to generate a homogenous suspension of receptor-bearing membrane fragments [38].

4.3.2. Preparation of the TGR5-CMC Stationary Phase

The TGR5-affinity stationary phase was prepared using an immuno-immobilization strategy. Activated silica gel (Silica-TCT, 50 mg) was dispersed in 1 mL of PBS (pH 9.0). To facilitate directional receptor orientation, 50 µL of anti-Flag antibody from Sigma-Aldrich (St. Louis, MO, USA) was added to the suspension and incubated at 37 °C with horizontal shaking (1500 rpm) for 2 h. After washing to remove unbound antibodies, the prepared TGR5 cell membrane suspension was added and incubated for an additional 1 h at 1500 rpm, allowing the Flag-tagged TGR5 receptors to be captured by the immobilized antibodies. [39] The resulting stationary phase was wet-packed into a chromatographic column using a slurry packing system. The column was packed under a constant flow of 1 mL/min (pressure < 10 MPa) for 30 min to ensure bed stability. This final assembly was designated as the TGR5-CMC column.

4.3.3. Chromatographic Screening

The TGR5-CMC column was equilibrated with pure water at a flow rate of 0.2 mL/min at 37 °C. Test compounds (1 mg/mL in methanol) were injected (10 µL) into the system. Retention times were recorded and compared against a solvent control (methanol). Ligand-receptor interactions were identified by observing marked shifts in retention time on the TGR5 column compared to the control, where a delayed retention indicates binding affinity.

4.4. Purification of Recombinant TGR5 Protein

To obtain high-purity TGR5 protein, HEK293T cells were cultured in 6-well plates until reaching approximately 80% confluence. Cells were transiently transfected with the Flag-tagged TGR5 plasmid (2 µg) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA); the DNA was pre-complexed with 4 µL of P3000 reagent and 4 µL of Lipofectamine 3000 according to the manufacturer's instructions.

After 48 h, cells were harvested and lysed on ice for 40 min in IP lysis buffer supplemented with protease and phosphatase inhibitors. The lysate was clarified by centrifugation

at 12,000 rpm (approx. $13,000\times g$) for 30 min at 4 °C. The supernatant was collected and incubated with 40 µL of anti-Flag magnetic beads (pre-equilibrated with lysis buffer) at 4 °C for 4 h (or overnight) with gentle rotation.

Following incubation, the beads were washed six times with a modified wash buffer (10-fold diluted GPCR lysis buffer) to remove non-specific contaminants; flow-through fractions were retained for validation. Protein elution was performed by incubating the beads with 60 µL of elution buffer (containing $3\times$ Flag peptide and phosphatase inhibitors) for 1 h at 4 °C. The eluate was separated from the beads, and the purified protein was enriched by a secondary centrifugation step (12,000 rpm). Finally, samples were mixed with Loading Buffer and incubated at 37 °C for 10 min (instead of boiling) to prevent heat-induced aggregation of the transmembrane protein [10].

4.5. SPR Analysis

To quantify the binding affinity and kinetics of the Obacunone-TGR5 interaction, SPR experiments were conducted using a Biacore 1K system. Purified recombinant human TGR5 protein was diluted in sodium acetate buffer (pH 4.0) and immobilized onto a CM5 sensor chip via standard amine coupling chemistry (EDC/NHS). A reference flow cell was treated with the same activation and blocking reagents (without protein immobilization) to serve as a control for non-specific binding and matrix effects [40].

The analyte, obacunone, was dissolved in HBS-EP+ running buffer supplemented with 5% DMSO to maintain solubility. A concentration series was generated via 1.5-fold serial dilution (ranging from 52 to 400 µM) and injected over the chip surface at a flow rate of 30 µL/min. The association phase was monitored for 120 s, followed by a dissociation phase of 60 s.

Data analysis was performed using the Biacore evaluation software. Sensorgrams were double-referenced by subtracting the signals from both the reference flow cell and solvent blanks. Due to the fast association and dissociation kinetics typical of low-affinity small molecules (manifesting as square-wave sensorgrams), 1:1 kinetic fitting was not suitable. Therefore, the equilibrium dissociation constant (KD) was derived by fitting the response at equilibrium against concentration to a Steady-State Affinity model. The immobilization density of the TGR5 protein was carefully controlled at approximately 4000–5000 Response Units (RU) to ensure a sufficient signal-to-noise ratio for small molecule detection while minimizing steric hindrance.

4.6. cAMP Functional Assay (GloSensor™)

To evaluate the functional activation of the TGR5-Gs signaling cascade, intracellular cAMP dynamics were monitored using the GloSensor™ cAMP biosensor assay (Promega) [41]. HEK293T cells stably expressing TGR5 (or the pCDH vector control) were seeded into white-walled, clear-bottom 96-well plates (NEST,701701) at a density of 40,000 cells/well. Upon reaching 70–80% confluence, cells were transiently transfected with 100 ng of the pGloSensor™-22F plasmid per well using Lipofectamine 3000 according to the manufacturer's protocol.

Twenty-four hours post-transfection, the culture medium was replaced with an equilibration medium (CO₂-independent balanced salt solution) supplemented with 0.5 mM D-luciferin. The plates were incubated for 2 h at room temperature to establish a stable basal luminescence signal. Following equilibration, cells were treated with serial dilutions of the candidate compounds (Obacunone, Limonin, and Berberine). Real-time luminescence intensity was recorded continuously for 30 min using a Molecular Devices iD3 microplate reader (San Jose, CA, USA). Functional potency was quantified by plotting the Area Under the Curve (AUC) against the logarithm of the compound concentration. The half-maximal

effective concentration (EC50) was calculated via non-linear regression (four-parameter logistic fit) using GraphPad Prism 9.5.

4.7. Transcriptome Sequencing (RNA-Seq) Strategy

To delineate the signaling network activated by obacunone, a comparative transcriptomic analysis was performed using TGR5-overexpressing cells.

4.7.1. Experimental Design and Sample Preparation

HEK293T-TGR5 cells were seeded in 6-well plates at a density of 4.5×10^5 cells/well. Cells were treated with either 50 μ M obacunone (F1 group, $n = 3$) or 0.1% DMSO vehicle control (F0 group, $n = 3$) for 24 h prior to harvest.

4.7.2. RNA Extraction

Total RNA was extracted using RNAiso Plus (Takara, Tokyo, Japan) following the manufacturer's instructions. RNA concentration and purity were quantified using a NanoDrop™ One Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and RNA integrity was verified by 1% agarose gel electrophoresis (200 V for 10 min).

4.7.3. Library Construction and Sequencing

RNA-seq libraries were constructed using the VAHTS® Universal V6 RNA-seq Library Prep Kit for MGI (Cat#: NRM605, Vazyme Biotech Co., Ltd., Nanjing, China). Briefly, eukaryotic mRNA was enriched from total RNA using oligo(dT) magnetic beads and randomly fragmented. First-strand cDNA was synthesized using random hexamers, followed by second-strand cDNA synthesis. The double-stranded cDNA underwent end repair, A-tailing, and ligation with sequencing adapters. The resulting library was amplified and circularized to form DNA Nanoballs (DNBs) via rolling circle amplification (RCA). The DNBs were loaded onto a high-density DNA nanochip and sequenced on the DNBSEQ-T7 platform (MGI Tech Co., Ltd., Shenzhen, China) using Cooperative Probe-Anchor Synthesis (cPAS) technology.

4.7.4. Bioinformatics Analysis

Raw sequencing reads were processed using fastp (v0.23.2) to remove adapters, poly-N sequences, and low-quality reads ($Q < 20$). High-quality clean reads were aligned to the Homo sapiens reference genome (GRCh38, Ensembl release 115) using STAR (v2.7.10a). Gene expression was quantified using RSEM (v1.3.1) and normalized to Fragments Per Kilobase of transcript per Million mapped reads (FPKM).

Differential expression analysis between the obacunone-treated (F1) and control (F0) groups was performed using the DESeq2 R package. Differentially Expressed Genes (DEGs) were identified based on a threshold of $|\log_2\text{FoldChange}| > 0.5$ and an adjusted p -value (Padj) < 0.05 . Functional enrichment analysis (GO and KEGG) was conducted using the clusterProfiler R package, with a corrected p -value < 0.05 considered statistically significant. Alternative splicing events and novel transcripts were predicted using rMATS and StringTie, respectively.

4.7.5. Statistical Analysis

Statistical differences between groups were evaluated using one-way analysis of variance (ANOVA). All data are presented as mean $\pm$ standard deviation (SD) of at least three independent replicates. Statistical analyses were performed using GraphPad Prism 9.5 software (San Diego, CA, USA), with $p < 0.05$ considered statistically significant.

5. Conclusions

In summary, this study's *in vitro* evidence presented here identifies obacunone as a novel, natural TGR5 agonist derived from RC, providing a molecular basis for its potential TGR5-mediated metabolic regulatory effects. Furthermore, transcriptomic analysis revealed that obacunone treatment induces a specific Nrf2/HO-1 antioxidant gene expression signature, suggesting a potential link between TGR5 activation and redox regulation that warrants further investigation. These findings not only expand the pharmacological material basis of RC but also establish a screening paradigm for identifying GPCR-active components in traditional medicines. Future studies should focus on validating these effects through *in vivo* disease models and exploring the structural optimization of obacunone to enhance its potency for clinical translation.

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Abbreviations

The following abbreviations are used in this manuscript:

HEK293T	Human Embryonic Kidney 293T
GFP	Green fluorescent protein
TGR5	Takeda G protein-coupled receptor 5
DMEM	Dulbecco's modified eagle medium
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
DEGs	Differentially expressed genes
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
PCA	Principal component analysis
FPKM	Fragments Per Kilobase of exon per Million mapped reads
SPR	Surface Plasmon Resonance
CMC	Cell membrane chromatography
cAMP	Cyclic Adenosine Monophosphate
Padj	adjusted <i>p</i> values
GNDC	Gene-encoded Natural Diverse Components Repository

CA	Cholic Acid
RC	Coptis chinensis
TCM	Traditional Chinese medicine
AMPK	Adenosine Monophosphate-Activated Protein Kinase
DMSO	Dimethyl Sulfoxide
FBS	Fetal Bovine Serum
GLP-1	Glucagon-Like Peptide-1
HMOX1	Heme Oxygenase 1

References

1. Pharmacopoeia of the People's Republic of China. In 2020—National Library of Medicine Institution; Zhongguo Yi Yao Ke Ji Chu Nan She: Beijing, China, 2020. Available online: https://catalog.nlm.nih.gov/discovery/fulldisplay/alma9918821387706676/01NLM_INST:01NLM_INST (accessed on 18 January 2026).
2. Kang, X.; Jin, D.; Ji, H.; An, X.; Zhang, Y.; Duan, L.; Yang, C.; Zhou, R.; Duan, Y.; Zhang, Y.; et al. The Clinical Efficacy of Gegen Qinlian Decoction in Treating Type 2 Diabetes Is Positively Correlated with the Dose of Coptidis Rhizoma: Three Randomized, Doubleblind, Dose-Parallel Controlled Clinical Trials. *Drug Des. Dev. Ther.* **2024**, *18*, 5573–5582. [\[CrossRef\]](#)
3. Ran, Q.; Wang, J.; Wang, L.; Zeng, H.; Yang, X.; Huang, Q. Rhizoma Coptidis as a Potential Treatment Agent for Type 2 Diabetes Mellitus and the Underlying Mechanisms: A Review. *Front. Pharmacol.* **2019**, *10*, 805. [\[CrossRef\]](#)
4. Wang, H.; Guo, L.; Shang, H.; Ren, M.; Wang, X.; Wang, D.; Chen, J.; Li, S.; Chen, L.; Wang, Y.; et al. Jinqijiangtang Tablets for Pre-Diabetes: A Randomized, Double-Blind and Placebo-Controlled Clinical Trial. *Sci. Rep.* **2017**, *7*, 11190. [\[CrossRef\]](#)
5. Pang, B.; Yu, X.-T.; Zhou, Q.; Zhao, T.-Y.; Wang, H.; Gu, C.-J.; Tong, X.-L. Effect of Rhizoma coptidis (Huang Lian) on Treating Diabetes Mellitus. *Evid.-Based Complement. Altern. Med. ECAM* **2015**, *2015*, 921416. [\[CrossRef\]](#)
6. Friedemann, T.; Otto, B.; Klätschke, K.; Schumacher, U.; Tao, Y.; Leung, A.K.-M.; Efferth, T.; Schröder, S. *Coptis chinensis* Franch. Exhibits Neuroprotective Properties against Oxidative Stress in Human Neuroblastoma Cells. *J. Ethnopharmacol.* **2014**, *155*, 607–615. [\[CrossRef\]](#)
7. Cheng, Z.; Kang, C.; Che, S.; Su, J.; Sun, Q.; Ge, T.; Guo, Y.; Lv, J.; Sun, Z.; Yang, W.; et al. Berberine: A Promising Treatment for Neurodegenerative Diseases. *Front. Pharmacol.* **2022**, *13*, 845591. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Xu, X.; Yi, H.; Wu, J.; Kuang, T.; Zhang, J.; Li, Q.; Du, H.; Xu, T.; Jiang, G.; Fan, G. Therapeutic Effect of Berberine on Metabolic Diseases: Both Pharmacological Data and Clinical Evidence. *Biomed. Pharmacother.* **2021**, *133*, 110984. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Li, D.; Feng, G.; Li, Y.; Pan, H.; Luo, P.; Liu, B.; Ding, T.; Wang, X.; Xu, H.; Zhao, Y.; et al. Benefits of Huang Lian Mediated by Gut Microbiota on HFD/STZ-Induced Type 2 Diabetes Mellitus in Mice. *Front. Endocrinol.* **2023**, *14*, 1120221. [\[CrossRef\]](#)
10. Yang, F.; Mao, C.; Guo, L.; Lin, J.; Ming, Q.; Xiao, P.; Wu, X.; Shen, Q.; Guo, S.; Shen, D.-D.; et al. Structural Basis of GPBAR Activation and Bile Acid Recognition. *Nature* **2020**, *587*, 499–504. [\[CrossRef\]](#)
11. Guo, C.; Chen, W.-D.; Wang, Y.-D. TGR5, Not Only a Metabolic Regulator. *Front. Physiol.* **2016**, *7*, 646. [\[CrossRef\]](#)
12. Duboc, H.; Taché, Y.; Hofmann, A.F. The Bile Acid TGR5 Membrane Receptor: From Basic Research to Clinical Application. *Dig. Liver Dis.* **2014**, *46*, 302–312. [\[CrossRef\]](#)
13. Katsuma, S.; Hirasawa, A.; Tsujimoto, G. Bile Acids Promote Glucagon-like Peptide-1 Secretion through TGR5 in a Murine Enteroendocrine Cell Line STC-1. *Biochem. Biophys. Res. Commun.* **2005**, *329*, 386–390. [\[CrossRef\]](#)
14. Drucker, D.J. The Biology of Incretin Hormones. *Cell Metab.* **2006**, *3*, 153–165. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Thomas, C.; Gioiello, A.; Noriega, L.; Strehle, A.; Oury, J.; Rizzo, G.; Macchiarulo, A.; Yamamoto, H.; Matak, C.; Pruzanski, M.; et al. TGR5-Mediated Bile Acid Sensing Controls Glucose Homeostasis. *Cell Metab.* **2009**, *10*, 167–177. [\[CrossRef\]](#)
16. Watanabe, M.; Houten, S.M.; Matak, C.; Christoffolete, M.A.; Kim, B.W.; Sato, H.; Messaddeq, N.; Harney, J.W.; Ezaki, O.; Kodama, T.; et al. Bile Acids Induce Energy Expenditure by Promoting Intracellular Thyroid Hormone Activation. *Nature* **2006**, *439*, 484–489. [\[CrossRef\]](#)
17. Chen, W.; Yu, Z.; Leng, L.; Sun, D.; Liu, H.; Gong, R.-Z.; Cong, Z.; Xiao, W.; Zhang, G.; Yang, L.; et al. Artificial Intelligence-Curated Repository of Gene-Encoded Natural Diverse Components from Herbal Medicines. *Innovation* **2025**, *6*, 101011. [\[CrossRef\]](#)
18. Zhang, C.-C.; Chen, G.; Liu, J.-L.; Li, Y.; Xu, X.-Y. Cell membrane chromatography and its application progress in studies of traditional Chinese medicines. *Zhongguo Zhong Yao Za Zhi Zhongguo Zhongyao Zazhi/China J. Chin. Mater. Medica* **2013**, *38*, 1881–1886.
19. Ma, W.; Wang, C.; Liu, R.; Wang, N.; Lv, Y.; Dai, B.; He, L. Advances in Cell Membrane Chromatography. *J. Chromatogr. A* **2021**, *1639*, 461916. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Cooper, M.A. Optical Biosensors in Drug Discovery. *Nat. Rev. Drug Discov.* **2002**, *1*, 515–528. [\[CrossRef\]](#)
21. Li, X.; Wang, C.-Y. From Bulk, Single-Cell to Spatial RNA Sequencing. *Int. J. Oral Sci.* **2021**, *13*, 36. [\[CrossRef\]](#)

22. Ono, E.; Inoue, J.; Hashidume, T.; Shimizu, M.; Sato, R. Anti-Obesity and Anti-Hyperglycemic Effects of the Dietary Citrus Limonoid Nomilin in Mice Fed a High-Fat Diet. *Biochem. Biophys. Res. Commun.* **2011**, *410*, 677–681. [\[CrossRef\]](#)
23. Horiba, T.; Katsukawa, M.; Mita, M.; Sato, R. Dietary Obacunone Supplementation Stimulates Muscle Hypertrophy, and Suppresses Hyperglycemia and Obesity through the TGR5 and PPAR γ Pathway. *Biochem. Biophys. Res. Commun.* **2015**, *463*, 846–852. [\[CrossRef\]](#)
24. Buchwald, P. Quantification of Signal Amplification for Receptors: The K_d/EC₅₀ Ratio of Full Agonists as a Gain Parameter. *Front. Pharmacol.* **2025**, *16*, 1541872. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Buzzetti, E.; Pinzani, M.; Tsochatzis, E.A. The Multiple-Hit Pathogenesis of Non-Alcoholic Fatty Liver Disease (NAFLD). *Metabolism* **2016**, *65*, 1038–1048. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Gugliandolo, A.; Bramanti, P.; Mazzon, E. Activation of Nrf2 by Natural Bioactive Compounds: A Promising Approach for Stroke? *Int. J. Mol. Sci.* **2020**, *21*, 4875. [\[CrossRef\]](#)
27. Li, N.; Hao, L.; Li, S.; Deng, J.; Yu, F.; Zhang, J.; Nie, A.; Hu, X. The NRF-2/HO-1 Signaling Pathway: A Promising Therapeutic Target for Metabolic Dysfunction-Associated Steatotic Liver Disease. *J. Inflamm. Res.* **2024**, *17*, 8061–8083. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Huang, D.-R.; Dai, C.-M.; Li, S.-Y.; Li, X.-F. Obacunone Protects Retinal Pigment Epithelium Cells from Ultra-Violet Radiation-Induced Oxidative Injury. *Aging* **2021**, *13*, 11010–11025. [\[CrossRef\]](#)
29. Zuo, G.; Zhang, T.; Huang, L.; Araujo, C.; Peng, J.; Travis, Z.; Okada, T.; Ocak, U.; Zhang, G.; Tang, J.; et al. Activation of TGR5 with INT-777 Attenuates Oxidative Stress and Neuronal Apoptosis via cAMP/PKC ϵ /ALDH2 Pathway after Subarachnoid Hemorrhage in Rats. *Free Radic. Biol. Med.* **2019**, *143*, 441–453; Erratum in *Free Radic. Biol. Med.* **2024**, *216*, 78–79. <https://doi.org/10.1016/j.freeradbiomed.2024.03.004>. [\[CrossRef\]](#)
30. Yang, Y.; Hua, Y.; Chen, W.; Zheng, H.; Wu, H.; Qin, S.; Huang, S. Therapeutic Targets and Pharmacological Mechanisms of Coptidis Rhizoma against Ulcerative Colitis: Findings of System Pharmacology and Bioinformatics Analysis. *Front. Pharmacol.* **2022**, *13*, 1037856. [\[CrossRef\]](#)
31. Landrum, G. RDKit: Open-Source Cheminformatics 2006. Available online: https://github.com/rdkit/rdkit/releases/tag/Release_2016_09_4 (accessed on 31 January 2026).
32. Du, L.; Geng, C.; Zeng, Q.; Huang, T.; Tang, J.; Chu, Y.; Zhao, K. Dockey: A Modern Integrated Tool for Large-Scale Molecular Docking and Virtual Screening. *Brief. Bioinform.* **2023**, *24*, bbad047. [\[CrossRef\]](#)
33. Eberhardt, J.; Santos-Martins, D.; Tillack, A.F.; Forli, S. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *J. Chem. Inf. Model.* **2021**, *61*, 3891–3898. [\[CrossRef\]](#)
34. Schrödinger, LLC. *The PyMOL Molecular Graphics System*; Schrödinger, LLC: New York, NY, USA, 2026.
35. Zhang, N.; Lo, C.-W.; Utsunomiya, T.; Maruyama, M.; Huang, E.; Rhee, C.; Gao, Q.; Yao, Z.; Goodman, S.B. PDGF-BB and IL-4 Co-Overexpression Is a Potential Strategy to Enhance Mesenchymal Stem Cell-Based Bone Regeneration. *Stem Cell Res. Ther.* **2021**, *12*, 40. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Elegheert, J.; Behiels, E.; Bishop, B.; Scott, S.; Woolley, R.E.; Griffiths, S.C.; Byrne, E.F.X.; Chang, V.T.; Stuart, D.I.; Jones, E.Y.; et al. Lentiviral Transduction of Mammalian Cells for Fast, Scalable and High-Level Production of Soluble and Membrane Proteins. *Nat. Protoc.* **2018**, *13*, 2991–3017. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Xia, Z.; Zhu, Q.; Shan, Y.; Lu, J.; An, M.; Mo, X.; Wang, S.; Yang, W.; Qian, H.; He, H.; et al. MrgX2-Targeting Ligand Screen for Antipseudoallergic Agents by Immobilized His-Tag-Fused Protein Technology. *J. Med. Chem.* **2025**, *68*, 5942–5953. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Zhang, P.; Shi, Y.; He, X.; Sun, W.; Lv, Y.; Hou, X. Study on Screening Potential Allergenic Proteins from Infant Milk Powders Based on Human Mast Cell Membrane Chromatography and Histamine Release Assays. *J. Pharm. Anal.* **2019**, *9*, 55–61. [\[CrossRef\]](#)
39. Shan, Y.; Lu, J.; Qian, H.; Xia, Z.; Mo, X.; An, M.; Yang, W.; Wang, S.; Che, D.; Wang, C.; et al. Immobilized Protein Strategies Based on Cell Membrane Chromatography and Its Application in Discovering Active and Toxic Substances in Traditional Chinese Medicine. *Pharmacol. Res.* **2024**, *210*, 107492. [\[CrossRef\]](#)
40. Zhang, G.; Liu, B.; Qiao, D.; Gao, S.; Zhu, X.; Huang, J.; He, W.; Gao, R.; Zhang, L.; Wang, J.; et al. PROTAC-Mediated Structure-Function Disruption of CD26: A Therapeutic Strategy for Driver-Negative Non-Small Cell Lung Cancer. *eBioMedicine* **2025**, *122*, 106026. [\[CrossRef\]](#)
41. Wang, F.I.; Ding, G.; Ng, G.S.; Dixon, S.J.; Chidiac, P. Luciferase-Based GloSensorTM cAMP Assay: Temperature Optimization and Application to Cell-Based Kinetic Studies. *Methods* **2022**, *203*, 249–258. [\[CrossRef\]](#) [\[PubMed\]](#)

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