

20(S)-Protopanaxatriol Protects Against Angiotensin II-Related Kidney Damage through Regulation of the PTPN1-Dependent AMPK/mTOR Pathway

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ABSTRACT

(20S)-Protopanaxatriol (PPT), a biologically active triterpenoid compound derived from *Panax ginseng*, has traditionally been used in Traditional Chinese Medicine (TCM) for supporting kidney function and maintaining blood pressure balance. This study was designed to investigate the potential protective mechanisms of PPT against renal damage caused by angiotensin II (Ang II). A mouse model of Ang II-induced renal injury was generated using C57BL/6 mice continuously infused with Ang II through osmotic minipumps for four weeks. PPT treatment was started after two weeks of Ang II administration and continued once daily for an additional 14 days. To explore the possible molecular targets of PPT, molecular docking, drug affinity response target stability (DARTS), and cellular thermal shift assay (CETSA) were conducted. Renal function, pathological changes, and alterations in signaling pathways were evaluated through biochemical measurements, histological examination, and Western blot analysis. The results indicated that PPT significantly reduced Ang II-associated renal dysfunction by limiting renal fibrosis and inflammatory responses in mice. Mechanistic investigations revealed that PPT interacted with PTPN1 and suppressed abnormal activation of the AMPK/mTOR signaling pathway, which helped restore autophagy balance in both mouse kidney tissues and Ang II-treated NRK-52E cells. Molecular docking, DARTS, and CETSA analyses further verified PTPN1 as a direct binding target of PPT, supporting its role in PPT-mediated kidney protection. This study provides experimental evidence supporting the traditional application of ginseng in kidney-related disorders and highlights PPT as a promising candidate for managing hypertensive nephropathy through regulation of the PTPN1/AMPK/mTOR signaling axis. Additional clinical studies are required to confirm its therapeutic applicability.

Keywords: Angiotensin II, (20S)-Protopanaxatriol, PTPN1, AMPK, mTOR, Autophagy

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Introduction

The growing prevalence of hypertensive nephropathy, particularly among aging populations in urban environments, has become a major factor contributing to the development of end-stage renal disease [1]. As a serious consequence of long-term hypertension, hypertensive nephropathy is characterized by structural and functional kidney abnormalities, including glomerular sclerosis, tubulointerstitial fibrosis, and infiltration of inflammatory cells [2]. The disease process is closely associated with hypertension-driven activation and damage of glomerular mesangial cells, which promotes increased production of angiotensin II (Ang II) [3]. As a key component of the renin-angiotensin-aldosterone system (RAAS), Ang II plays a central role in kidney injury through multiple pathological mechanisms [4-6].

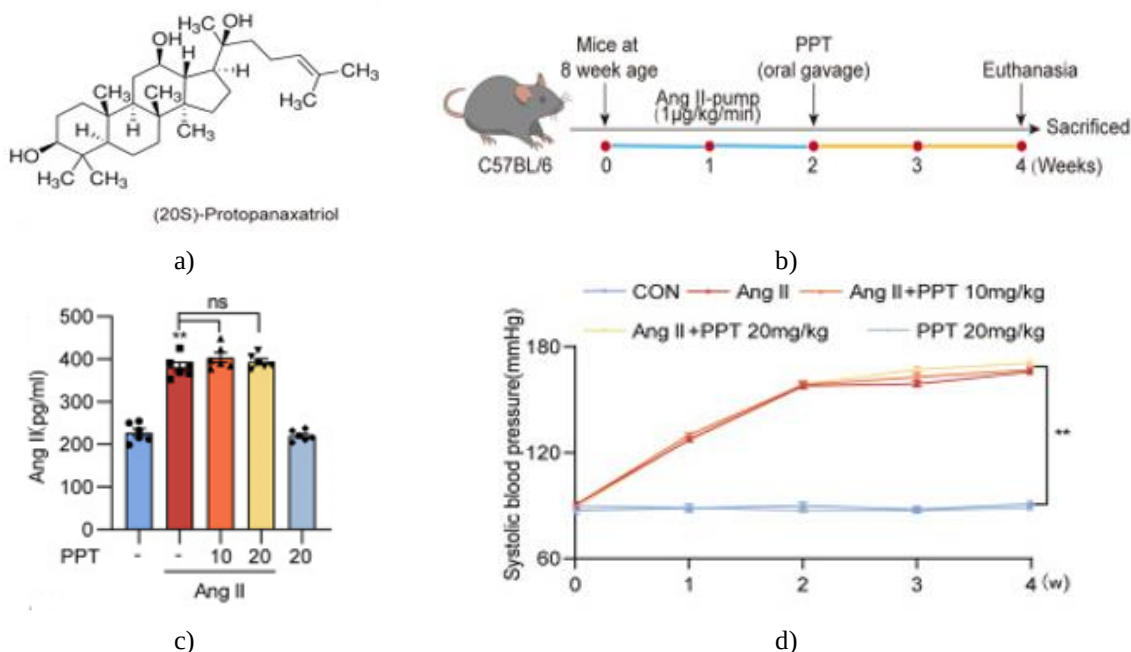
Following renal damage, activation of the local renin-angiotensin system (RAS) occurs through increased expression of several important components, including angiotensinogen, angiotensin-converting enzyme (ACE), renin, and Ang II type 1 receptors [7]. Although Ang II contributes to blood pressure (BP) regulation, excessive Ang II activity can also directly promote kidney injury through blood pressure-independent mechanisms. Previous studies have shown that Ang II binding to AT1 receptors triggers receptor-effector interactions, leading to intracellular calcium accumulation and activation of NF- κ B signaling. This process increases the expression of inflammatory mediators, NHE-3 exchangers, and growth-related factors, ultimately accelerating renal damage [8]. Therefore, identifying new therapeutic compounds capable of controlling Ang II-related oxidative stress and inflammation is important for treating hypertensive nephropathy.

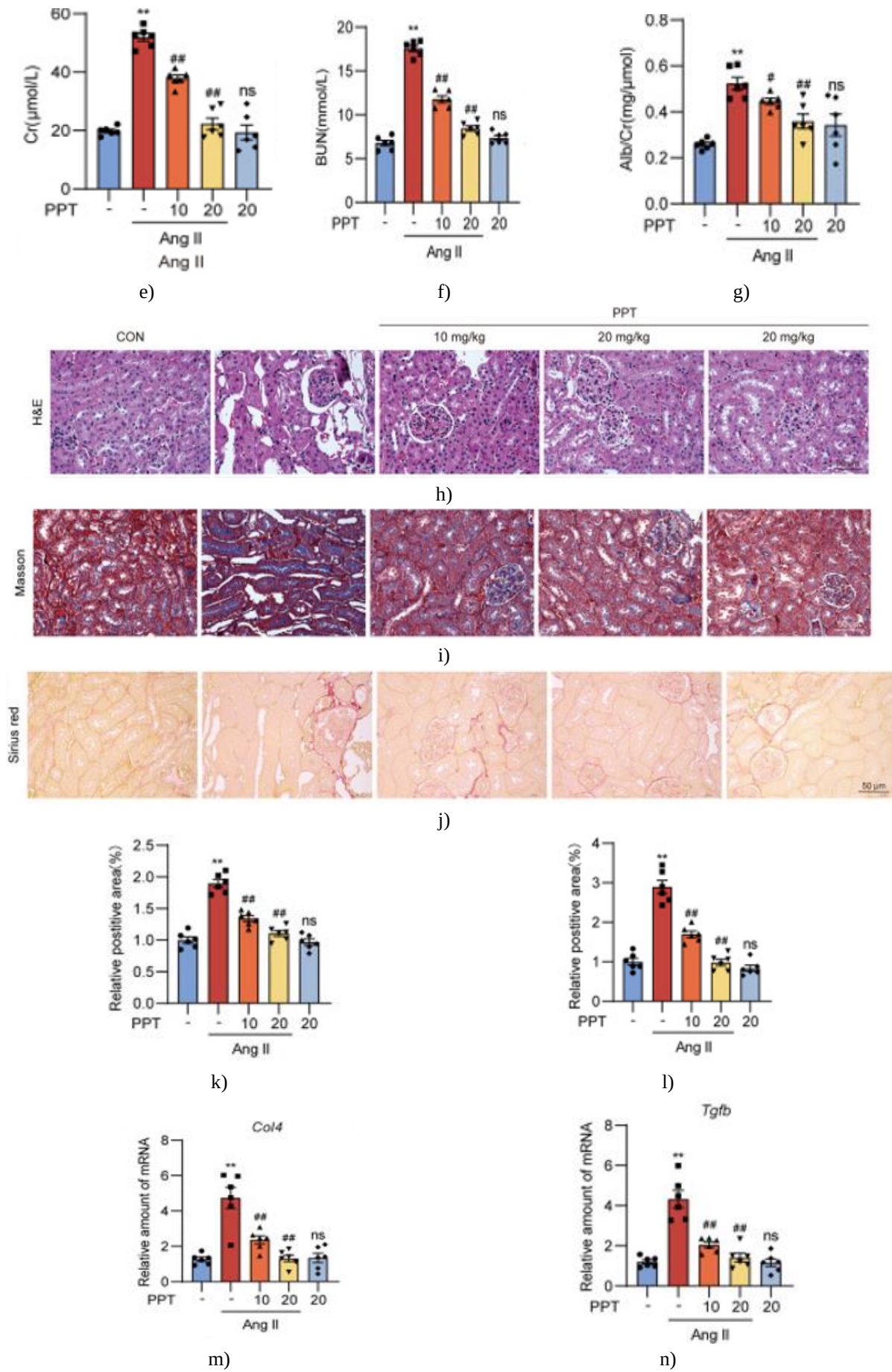
Panax ginseng Meyer is a well-established traditional medicine, and its major pharmacological components are a group of saponins known as ginsenosides [9]. Among these active constituents, (20S)-Protopanaxatriol (PPT), (**Figure 1a**) is considered an important bioactive molecule with antioxidant, anti-apoptotic, and anticancer properties. It has also attracted attention as a potential compound for preventing or improving age-related skeletal muscle decline. PPT is a dammarane-type tetracyclic triterpenoid sapogenin with diverse biological effects [10]. The (20S) stereoisomer, defined by its specific configuration at the C-20 position, demonstrates different pharmacological characteristics compared with the (20R) form [11].

Recent investigations have confirmed the kidney-protective properties of ginseng, mainly due to ginsenosides that regulate oxidative stress, inflammation, and fibrotic processes [12]. Research has demonstrated beneficial effects of ginseng-derived compounds in conditions such as diabetic nephropathy and chronic kidney disease through modulation of pathways including TGF- β /Smad and Nrf2 signaling [13]. These findings connect traditional medicinal applications with modern molecular research and support the potential role of ginseng-derived compounds in renal disease treatment.

Previous studies have shown that PPT improves renal function and reduces kidney damage in different experimental models, including renal injury, nephritis, fibrosis, and diabetic nephropathy [13]. Its strong anti-inflammatory, antioxidant, and antifibrotic activities further suggest that PPT may serve as a valuable therapeutic candidate for kidney disorders [14]. In addition, Han *et al.* reported that PPT-derived saponins suppress uptake activity and proliferation of renal proximal tubular cells by regulating phospholipase A2-related signaling and c-fos/c-jun gene expression [15, 16].

The present study investigates the molecular mechanisms responsible for the renal protective effects of PPT in hypertensive nephropathy, with particular attention to its influence on inflammation, autophagy regulation, and fibrosis development. These findings may contribute to a better understanding of PPT's therapeutic potential and support future development of novel kidney disease treatments.





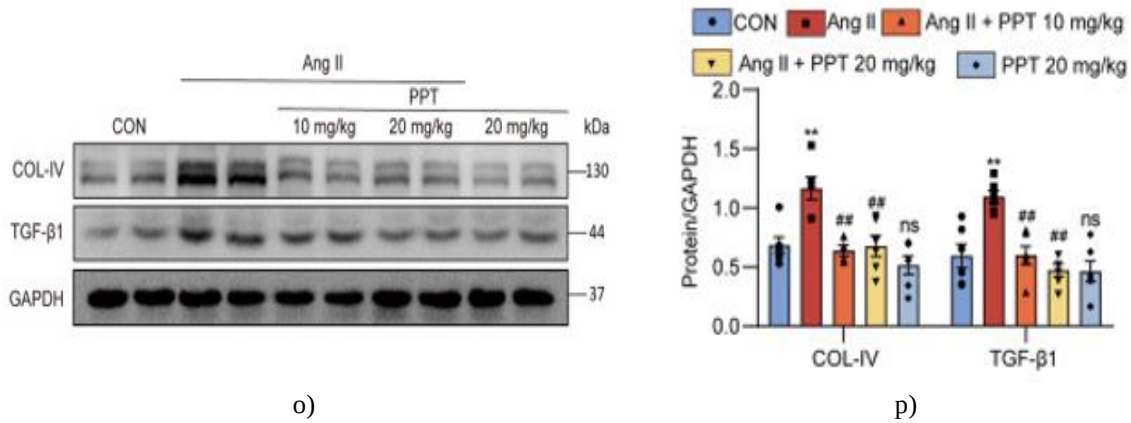


Figure 1. PPT improves Ang II-triggered kidney impairment and fibrosis. The experimental design adhered to standard established protocols. (a) Chemical structure of the PPT molecule. (b) Overview of the study timeline. (c) Serum Ang II concentrations measured by ELISA. (d) Time-course changes in systolic blood pressure. (e) Serum creatinine levels. (f) Blood urea nitrogen (BUN) concentrations. (g) Urinary albumin-to-creatinine ratio assessed at week 4. (h) Histological examination of kidney morphology by hematoxylin-eosin staining (scale bar = 50 μ m). Data are presented as mean $\pm$ SEM (n = 6 per group). (i) Evaluation of collagen accumulation via Masson's trichrome staining (scale bar = 50 μ m). (j) Visualization of interstitial collagen using Picro Sirius Red under polarized light (scale bar = 50 μ m). (k) Quantitative analysis of Masson's trichrome-positive fibrotic regions. (l) Morphometric quantification of Sirius Red-stained collagen area. (m) mRNA expression of the fibrogenic gene Col4, normalized to Actb. (n) Tgfb transcript levels normalized to Actb. (o) Western blot analysis of fibrotic proteins COL-IV and TGF- β 1, with GAPDH as loading control. (p) Densitometric quantification of bands from panel (o). Results shown as mean $\pm$ SEM (n = 6). Statistical comparisons used two-tailed Student's t-test: ns = not significant; *p < 0.05, **p < 0.01 versus control; #p < 0.05, ##p < 0.01 versus Ang II group.

Materials and Methods

Reagents

(20S)-Protopanaxatriol (PPT, CAS No. 30636-90-9) was obtained from Sigma-Aldrich (China). Angiotensin II and Trodusquemine were purchased from Aladdin (USA; Cat. #4474-91-3) and MedChemExpress (USA; Cat. #186139-09-3), respectively. For animal dosing, Ang II and PPT were prepared as suspensions in 0.5% sodium carboxymethyl cellulose. DMSO served as the vehicle for *in vitro* treatments with Ang II, PPT, and Trodusquemine.

Antibodies targeting PTPN1 (Cat. #11334-1-AP), P62/SQSTM1 (Cat. #18420-1-AP), Beclin 1 (Cat. #11306-1-AP), LC3 (Cat. #14600-1-AP), I κ B α (Cat. #10268-1-AP), and p-P65 Ser468 (Cat. #14600-1-AP) were sourced from Proteintech (China). Antibodies for p-AMPK α Thr172 (Cat. #2535), AMPK α (Cat. #2532), p-mTOR Ser2448 (Cat. #5536), and mTOR (Cat. #2983) came from Cell Signaling Technology (USA). Additional antibodies included COL-IV (ab6586), lamin B (ab133741), TGF- β 1 (ab92486), P65 (ab32536), and p-P65 (ab31624) from Abcam (UK), as well as F4/80 (29414-1-AP) and CD86 (13395-1-AP) from Proteintech. GAPDH (5174; Cell Signaling Technology) was used as the internal loading control.

Commercial kits comprised an Ang II ELISA (Shanghai Tongwei Biological Technology, China) and biochemical assay kits for serum creatinine (Cr; C011-2-1), urinary albumin (Alb; E038-1-1), and blood urea nitrogen (BUN; C013-2-1) from Nanjing Jiancheng Bioengineering Institute (China). Histological reagents were obtained as follows: Masson's trichrome (G1340), Picrosirius Red (S8060), and H&E (G1120) from Solarbio Life Sciences (China).

Ang II-induced kidney injury model

All animal experiments complied with National Institutes of Health (USA) guidelines and ARRIVE reporting standards. The protocol was approved by the Beihua University Animal Policy and Welfare Committee (SCXK(JI)2023-0002) [17].

Thirty male C57BL/6 mice (6 weeks old) were obtained from Changchun Yisi Experimental Animal Technology Co., Ltd. and housed under controlled conditions (12-h light/dark cycle, stable temperature, free access to food and water). The oral gavage dose of PPT was selected based on previous reports [18-20]. Following a 2-week adaptation period, mice were randomly divided into five groups ($n = 6/\text{group}$):

1. Control (CON): 0.5% CMC-Na vehicle.
2. Ang II: Ang II infusion + 0.5% CMC-Na.
3. Ang II + 10 mg/kg PPT: Ang II + 10 mg/kg/day PPT.
4. Ang II + 20 mg/kg PPT: Ang II + 20 mg/kg/day PPT.
5. PPT (20 mg/kg): 20 mg/kg/day PPT alone.

Hypertensive kidney injury was induced by continuous subcutaneous infusion of Ang II (1000 ng/kg/min) for 4 weeks using Alzet micro-osmotic pumps (MODEL 1004, USA) [21]. After pump implantation, mice received neomycin (1 g/L) in drinking water and appropriate wound care. Systolic blood pressure was recorded weekly via tail-cuff method (BP-2010A, Softron Biotechnology, Japan) [22]. PPT administration began 2 weeks after Ang II infusion and lasted for the remaining 4 weeks of the study.

At the end of the experiment, mice were euthanized with sodium pentobarbital. Urine, blood, and kidney tissues were harvested. Levels of urinary albumin (Alb), serum creatinine (Cr), and blood urea nitrogen were determined with commercial kits (Nanjing Jiancheng Bioengineering Institute, China). Tissue Ang II content was assessed by ELISA (Shanghai Tongwei Biological Technology, China).

Statistical analysis

All data are reported as mean $\pm$ SEM. Inter-group differences were evaluated by one-way ANOVA followed by LSD post-hoc tests. Longitudinal data were analyzed using two-way repeated-measures ANOVA with Tukey's correction for multiple comparisons, after confirming variance homogeneity. A p -value < 0.05 was considered statistically significant. Analyses were conducted in SPSS 21.0.

Results and Discussion

PPT ameliorates Ang II-induced renal dysfunction and renal fibrosis

To assess the therapeutic effects of PPT in hypertensive kidney disease, we established a model of Ang II-induced nephropathy via 4-week subcutaneous infusion (1000 ng/kg/min) using osmotic minipumps. PPT treatment (10 or 20 mg/kg/day) was initiated on day 14 after Ang II pump implantation (**Figure 1b**). Ang II infusion markedly elevated serum Ang II concentrations and systolic blood pressure; these changes were not altered by PPT (**Figures 1c–1d**). However, PPT treatment significantly reduced Ang II-mediated elevations in serum creatinine, blood urea nitrogen, and urinary albumin-to-creatinine ratio (**Figures 1e–1g**). Histological examination by PAS staining indicated that PPT attenuated glomerular basement membrane thickening, mesangial matrix expansion, and tubular injury. These results demonstrate that PPT exerts renoprotective actions against Ang II-induced functional and structural kidney damage without affecting blood pressure.

Further histopathological analysis confirmed PPT's protective role. H&E staining revealed that PPT substantially preserved glomerular integrity in Ang II-treated mice (**Figure 1h**). Fibrosis, a key feature of hypertensive nephropathy, was markedly decreased in PPT-treated animals as shown by reduced collagen deposition in Masson's trichrome and Picro Sirius Red staining (**Figures 1i–1l**). Consistent with these histological improvements, PPT reversed Ang II-induced upregulation of fibrotic genes Col4 and Tgfb at the mRNA level (**Figures 1m–1n**) and lowered protein expression of COL-IV and TGF- β 1 (**Figures 1o–1p**). Collectively, these findings indicate that PPT effectively counters Ang II-driven renal structural damage and fibrotic progression.

Identification of potential PPT target proteins via network pharmacology and validation of its effect on renal cell autophagy

Potential targets of PPT were predicted using <https://pubchem.ncbi.nlm.nih.gov/> and <http://swisstargetprediction.ch/>. PTPN1 emerged as one of the highest-ranking candidates (**Figure 2a**). Molecular docking analysis suggested that PPT interacts with PTPN1 residues 24ARG, 46TYR, 182PHE, 217ALA, and 219ILE through hydrophobic contacts, and with 24ARG and 262GLN via hydrogen bonds, yielding a binding energy of -6.4 kcal/mol (**Figure 2b**). For comparison, the clinically used kidney disease drug Finerenone

exhibited a binding energy of -6.2 kcal/mol to PTPN1. PPT therefore displayed slightly stronger predicted affinity than Finerenone. Direct interaction was further verified by drug affinity responsive target stability (DARTS) assay, which demonstrated enhanced protease sensitivity of PTPN1 upon PPT exposure (**Figure 2c**). In addition, cellular thermal shift assay (CETSA) showed thermal stabilization of PTPN1 in PPT-treated cells (**Figure 2d**), confirming direct binding in the cellular context.

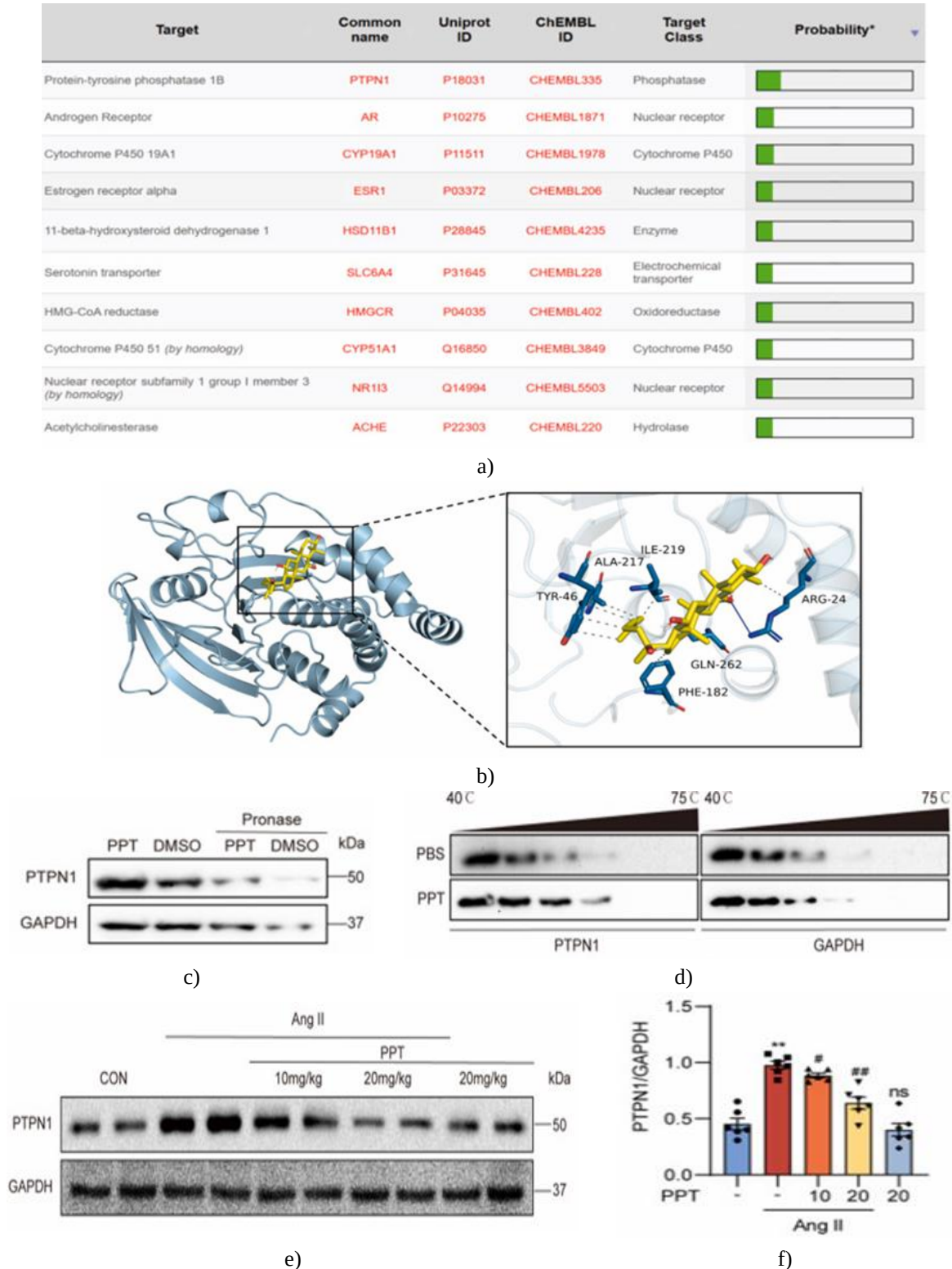
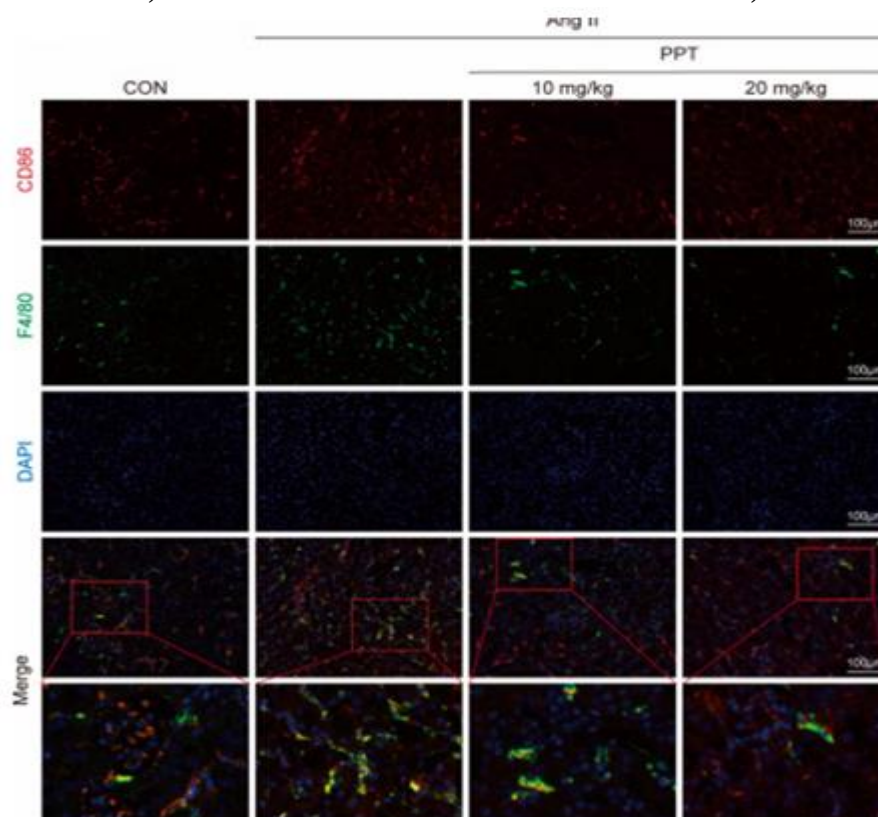
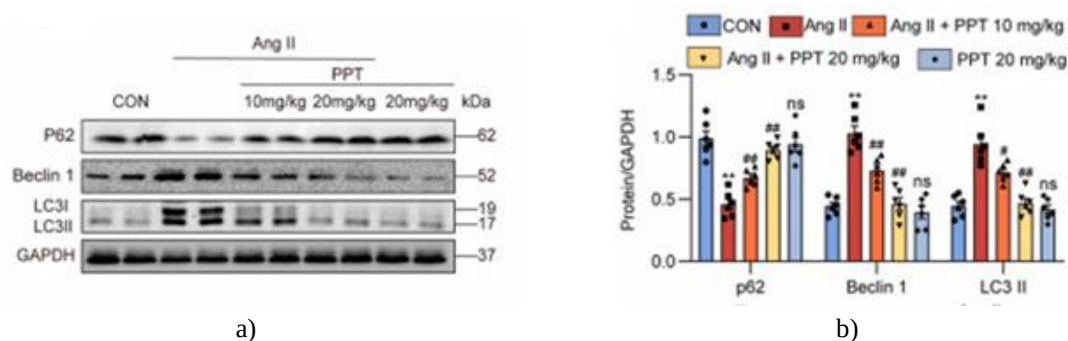


Figure 2. Potential molecular targets of PPT identified by network pharmacology and confirmation of PPT’s impact on renal cell autophagy. (a) Computational prediction of PPT target proteins using online bioinformatics platforms. (b) Molecular docking simulation showing PPT binding to PTPN1. (c) DARTS

assay results indicating that PPT enhances the proteolytic susceptibility of PTPN1 in cell lysates. (d) NRK-52E cells were exposed to 6 μ M PPT for 2 h, then subjected to heat treatment at varying temperatures for 3 min. Following lysis by freeze-thaw cycles, soluble PTPN1 protein abundance was analyzed by Western blot.

(e) Immunoblot analysis of PTPN1 protein expression in kidney tissues. GAPDH served as the loading control. (f) Quantitative densitometry of PTPN1 bands from panel (e) performed with ImageJ software. Data are shown as mean $\pm$ SEM (n = 6 per group). Statistical significance was determined by two-tailed t-test: ns = non-significant; *p < 0.05, **p < 0.01 versus control; #p < 0.05, ##p < 0.01 versus Ang II group.

Earlier research indicated that PTPN1 expression is strongly elevated in podocytes of rats with puromycin aminonucleoside-induced nephrosis [23]. In the present study, Ang II infusion led to increased PTPN1 protein levels in mouse kidney tissue, whereas PPT treatment significantly suppressed this upregulation (**Figures 2e–2f**). Notably, PPT also exerted a beneficial regulatory influence in the Ang II model by normalizing autophagy-related markers. This was reflected in elevated SQSTM1/p62 levels together with reduced Beclin 1 and LC3II expression (**Figures 3a–3b**). Additionally, Ang II enhanced renal mRNA expression of pro-inflammatory cytokines (Il1b, Il6, Tnf), and these increases were markedly reversed by PPT (**Figure 3f**). These observations highlight PPT's ability to simultaneously modulate autophagy pathways and inflammatory responses in the setting of hypertensive kidney disease.



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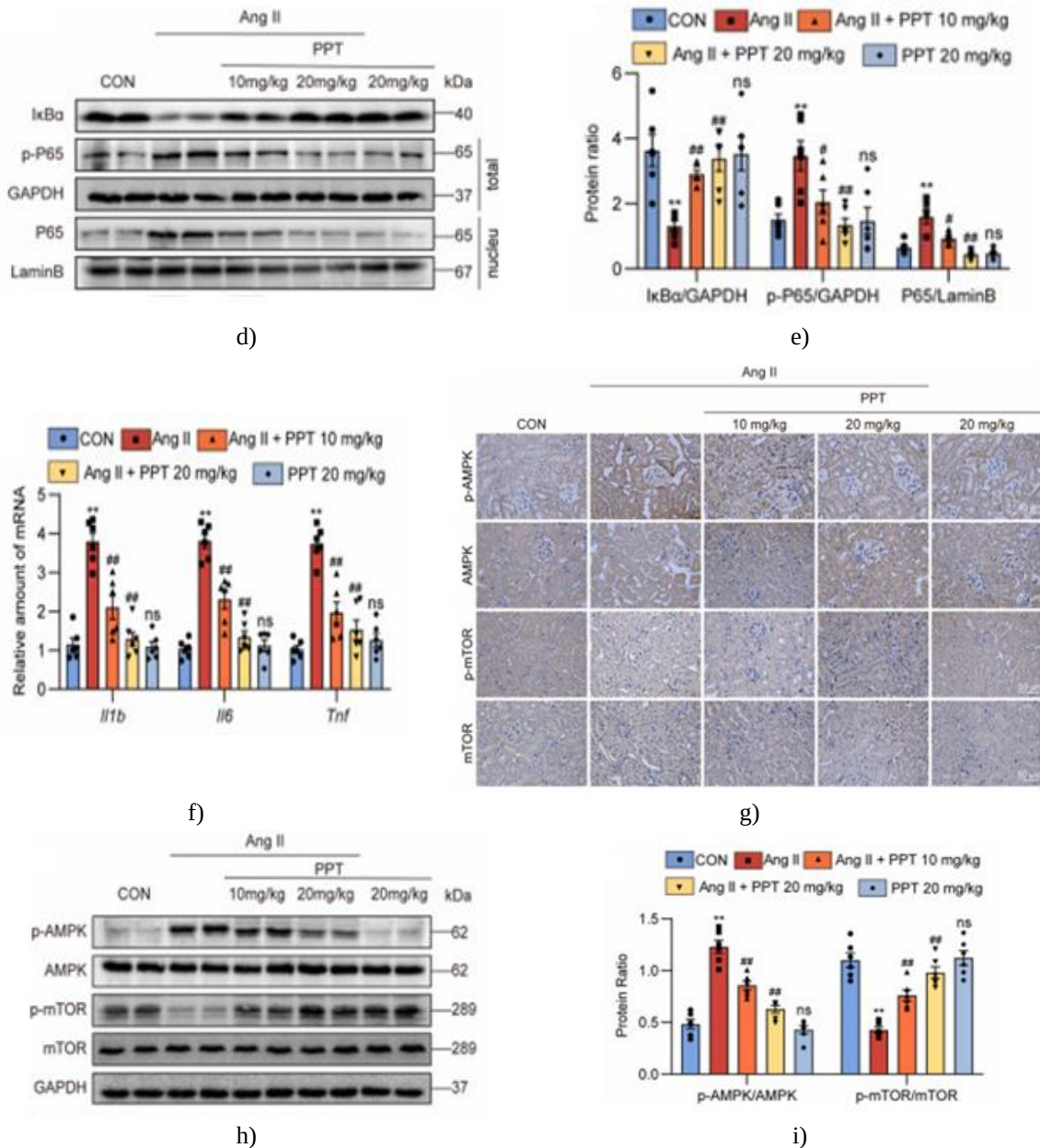


Figure 3. PPT modulates Ang II-mediated kidney damage through the NF-κB and AMPK/mTOR signaling pathways in mice. (a) Western blot analysis of autophagy-related proteins SQSTM1/p62, Beclin 1, and LC3II in kidney tissues, with GAPDH as the loading control. (b) Quantitative densitometry of protein bands from panel (a) using ImageJ software. (c) Multispectral immunofluorescence assessment of CD86+/F4/80+ pro-inflammatory macrophage infiltration in different experimental groups (scale bars = 100 μm). (d) Subcellular fractionation followed by immunoblotting showing cytoplasmic IκBα and phospho-P65 levels versus nuclear P65 in renal samples. GAPDH and Lamin B served as cytoplasmic and nuclear loading controls, respectively. (e) Densitometric quantification of bands from panel (d) with ImageJ. (f) RT-qPCR measurement of renal inflammatory cytokine mRNA levels (Il1b, Il6, Tnf). (g) Representative immunohistochemistry images of p-AMPK, AMPK, p-mTOR, and mTOR expression across groups (original magnification 400×). (h) Western blot detection of p-AMPK, AMPK, p-mTOR, and mTOR proteins in kidney tissue, with GAPDH as loading control. (i) Quantitative analysis of protein bands from panel (h) using ImageJ. All quantitative data are expressed as mean ± SEM (n = 6 biological replicates). Statistical significance was evaluated by two-tailed t-test: ns = non-significant; *p < 0.05, **p < 0.01 versus control; #p < 0.05, ##p < 0.01 versus Ang II group.

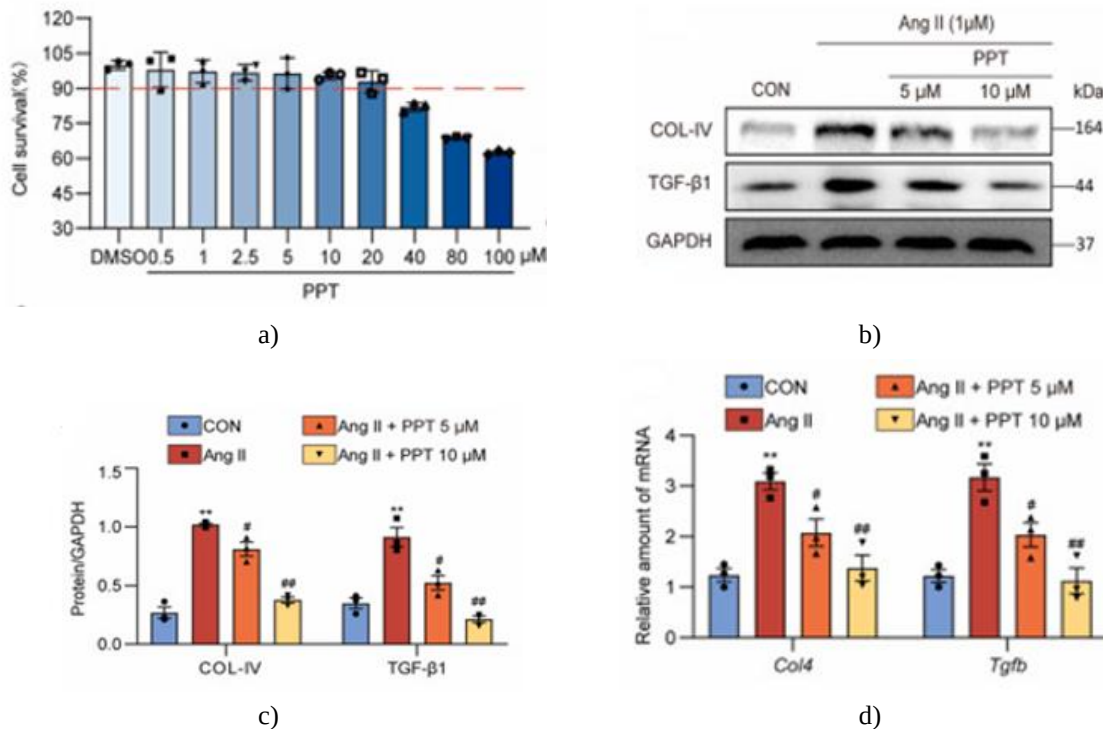
Protective role of NF- κ B in regulating autophagy

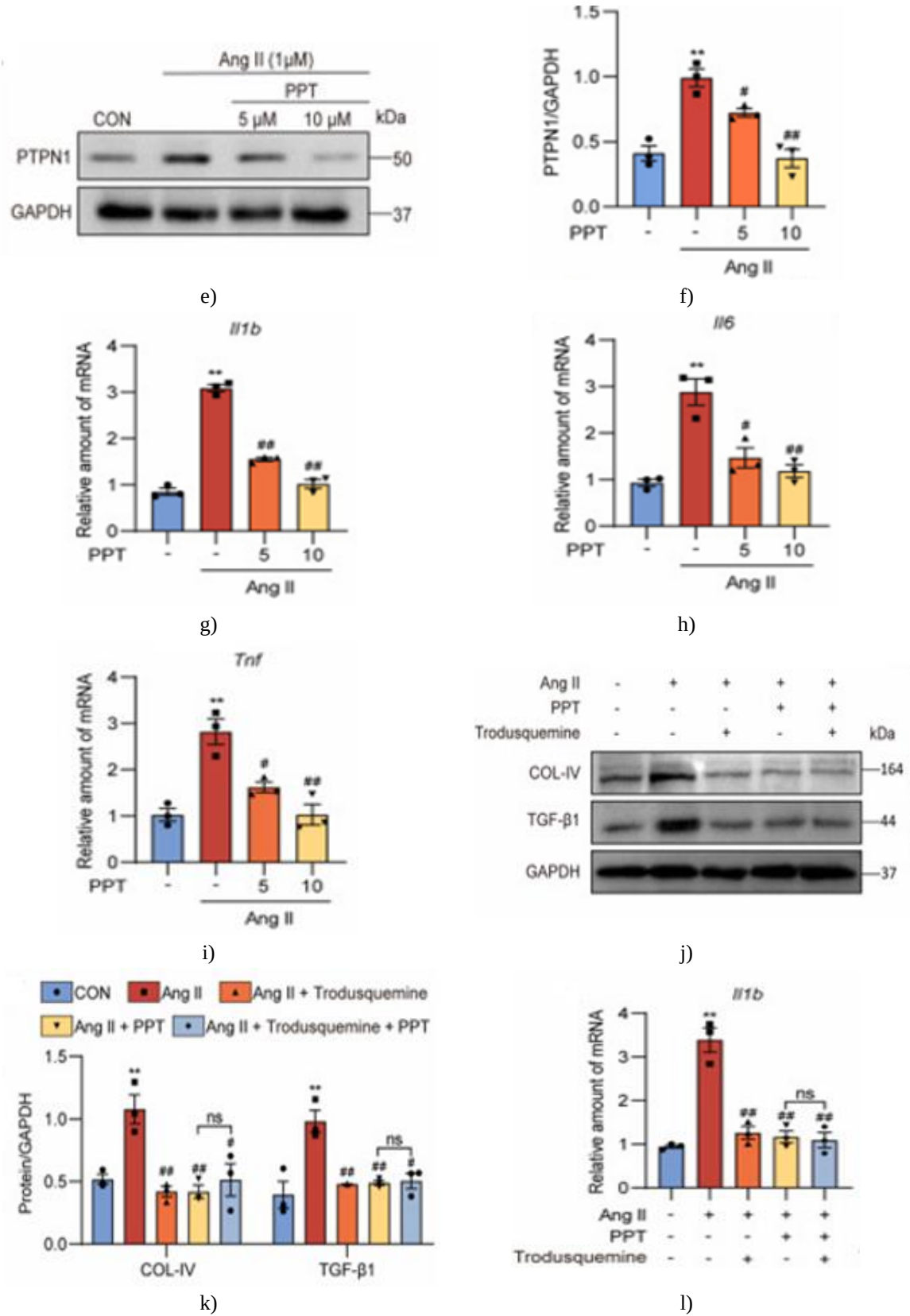
Autophagy and NF- κ B signaling exhibit a close interconnection during inflammatory processes [24]. Ang II acts as a major driver of pathology by activating the NF- κ B pathway, which serves as a primary mediator of its pro-inflammatory actions [25]. Treatment with PPT substantially reduced the infiltration of pro-inflammatory CD86+ F4/80+ macrophages into the kidneys of Ang II-exposed mice (**Figure 3c**), supporting its immunomodulatory properties. At the mechanistic level, Ang II promoted I κ B α degradation and p65 phosphorylation—key indicators of NF- κ B activation—in renal tissues, and these effects were strongly blocked by PPT. Furthermore, PPT markedly inhibited the nuclear translocation of p65, a critical step in NF- κ B pathway activation [26], as demonstrated by decreased nuclear p65 levels (**Figures 3d–3e**). Together, these data illustrate that PPT interferes with Ang II-induced NF- κ B signaling by suppressing both cytoplasmic activation and subsequent nuclear translocation.

Immunohistochemical staining showed that Ang II infusion lowered the p-mTOR/mTOR ratio while elevating the p-AMPK/AMPK ratio relative to controls; both changes were reversed by PPT treatment (**Figure 3g**). These *in vivo* observations were corroborated by Western blotting, which confirmed that PPT increased the p-mTOR/mTOR ratio and decreased the p-AMPK/AMPK ratio in Ang II-stimulated kidney tissues (**Figures 3h–3i**). The findings indicate that PPT inhibits the AMPK/mTOR signaling pathway under these conditions.

PPT attenuates Ang II-induced fibrosis in NRK-52E cells

To extend the *in vivo* antifibrotic results, we examined PPT's direct actions on renal tubular epithelial cells in culture. Initial dose-response experiments demonstrated concentration-dependent suppression of NRK-52E cell proliferation, with evident cytotoxicity at 20 μ M (**Figure 4a**). Therefore, subsequent studies employed non-cytotoxic doses (5–10 μ M). Stimulation with Ang II significantly increased the expression of profibrotic proteins COL-IV and TGF- β 1, along with their corresponding mRNA transcripts (Col4 and Tgfb) in NRK-52E cells, recapitulating the fibrotic pattern observed *in vivo*. PPT administration reduced these Ang II-evoked elevations in both protein and mRNA levels in a dose-dependent manner (**Figures 4b–4d**), confirming preservation of its antifibrotic activity across model systems. In line with the animal data, Ang II elevated PTPN1 protein abundance in cells, an effect that was attenuated by PPT (**Figures 4e–4f**). RT-qPCR analysis further showed that PPT dose-dependently counteracted Ang II-induced upregulation of inflammatory cytokine transcripts (Il1b, Il6, Tnf), with the 10 μ M concentration restoring expression close to baseline levels (**Figures 4g–4i**).





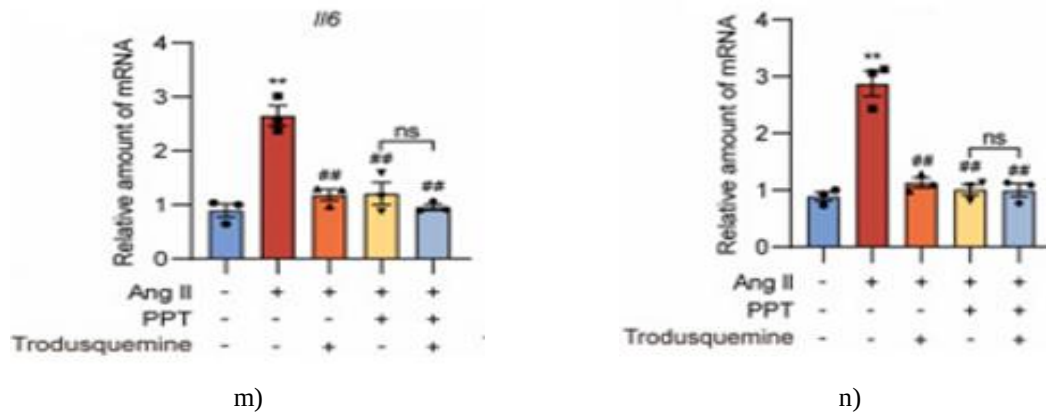
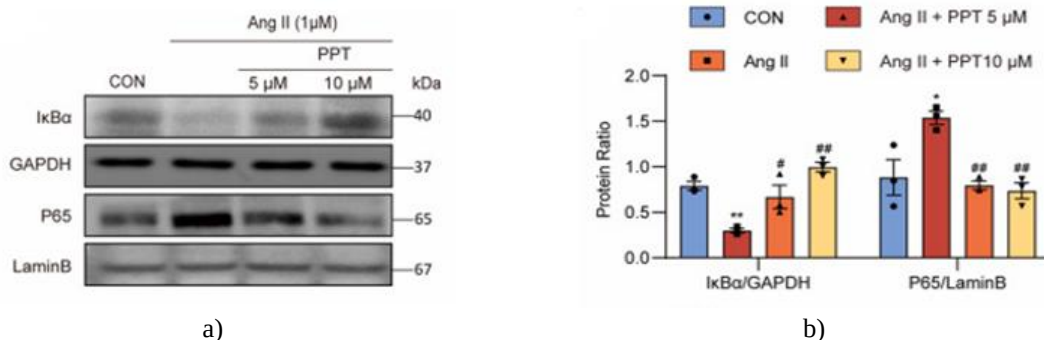


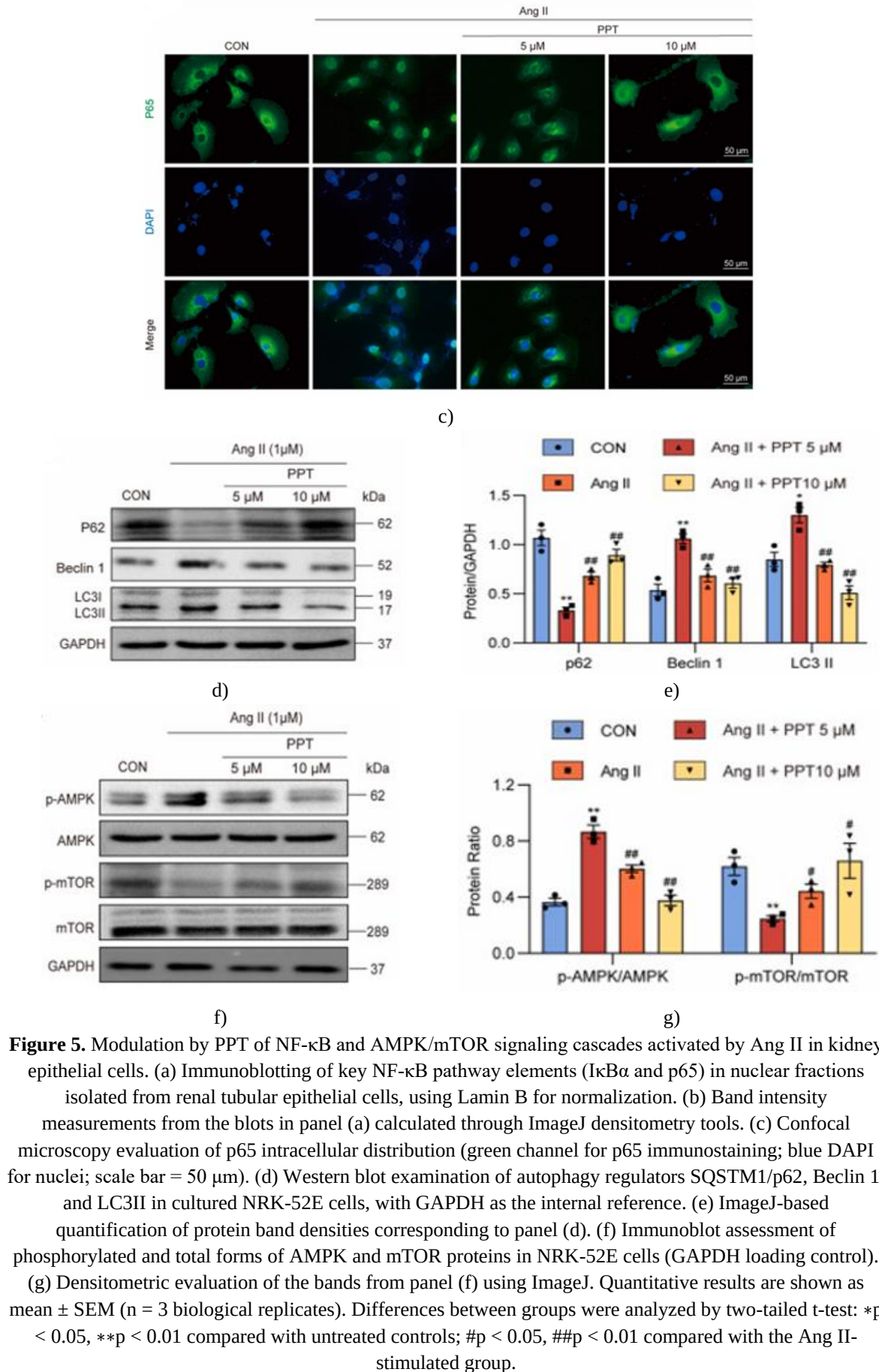
Figure 4. PPT suppresses fibrosis triggered by Ang II in NRK-52E renal tubular epithelial cells. Cells were pretreated with 5 or 10 μ M PPT for one hour, then stimulated with 1 μ M Ang II for 8 or 24 hours. (a) Assessment of cell viability using the MTT assay ($n = 3$ technical replicates). (b) Western blot showing levels of fibrosis-associated proteins COL-IV and TGF- β 1, normalized against GAPDH. (c) Band intensity analysis of the blots in (b) performed with ImageJ. (d) RT-qPCR-based measurement of Col4 and Tgfb gene expression. (e) Immunoblotting for PTPN1 protein expression in NRK-52E cells (GAPDH as loading control). (f) Densitometric evaluation of PTPN1 bands from panel (e) via ImageJ. (g–i) Quantitative RT-qPCR analysis of pro-inflammatory cytokine mRNAs (Il1b, Il6, Tnf). (j) Inhibitor validation experiment: NRK-52E cells received a 1-hour pretreatment with PPT (5 or 10 μ M), either alone or combined with 5 μ M Trodusquemine (PTPN1 inhibitor), prior to 2-hour Ang II (1 μ M) challenge. Protein and RNA were then extracted for immunoblotting and gene expression studies. Shown are representative Western blots for COL-IV and TGF- β 1 (GAPDH loading control). (k) Densitometry quantification of the blots in panel (j). (l–n) RT-qPCR data for Il1b, Il6, and Tnf transcript levels. Results are mean $\pm$ SEM from 3 independent biological experiments. Statistical analysis by two-tailed t-test: * $p < 0.05$, ** $p < 0.01$ vs. untreated control cells; # $p < 0.05$, ## $p < 0.01$ vs. Ang II-stimulated group.

Trodusquemine represents one of the best-characterized small-molecule allosteric inhibitors of PTP1B [27]. Using this PTPN1 inhibitor pharmacologically reproduced the protective actions of PPT, leading to strong suppression of Ang II-induced elevations in fibrotic proteins (COL-IV and TGF- β 1) as well as inflammatory cytokines (Il1b, Il6, Tnf) (**Figures 4j–4n**). These consistent outcomes from independent pharmacological strategies demonstrate that PPT's beneficial effects on renal epithelial cells depend on PTPN1 inhibition, which in turn blocks downstream fibrotic and inflammatory pathways activated by Ang II.

Influence of PPT on Ang II-activated AMPK/mTOR and inflammatory signaling pathways in renal cells

PPT effectively prevented the degradation of I κ B α and blocked the nuclear import of NF- κ B p65 subunit in Ang II-treated NRK-52E cells (**Figures 5a and 5b**). Supporting immunofluorescence data showed prominent cytoplasmic retention of p65 when cells were exposed to PPT, further confirming inhibition of the NF- κ B cascade (**Figure 5c**). Overall, these results indicate that PPT acts via a coordinated dual mechanism: stabilizing I κ B α to keep NF- κ B sequestered in the cytoplasm and consequently reducing the expression of downstream inflammatory genes.





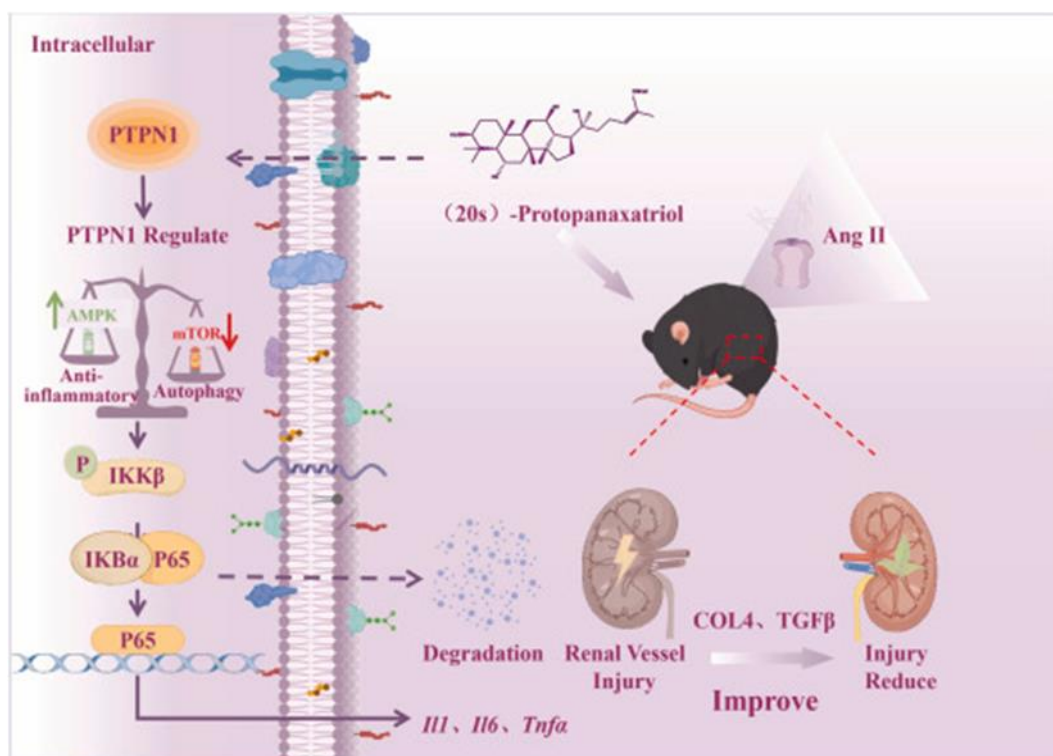


Figure 6. Schematic summary illustrating how (20S)-Protopanaxatriol relieves hypertensive inflammatory renal damage by acting on the PTPN1-regulated AMPK/mTOR/autophagy signaling cascade.

In agreement with the animal experiments, PPT restored normal autophagy function in cultured cells by elevating p62 protein levels while lowering Beclin1 and LC3II expression (**Figures 5d–5e**). Similarly, PPT raised the p-mTOR/mTOR ratio and lowered the p-AMPK/AMPK ratio in Ang II-exposed NRK-52E cells (**Figures 5f–5g**). These observations further verify that PPT suppresses both autophagy and the AMPK/mTOR pathway under in vitro conditions.

This investigation reveals a previously unreported protective pathway in which PPT counters hypertensive inflammatory kidney damage by modulating the PTPN1-dependent AMPK/mTOR/autophagy axis. The results expand knowledge regarding the renoprotective actions of ginsenoside metabolites and identify a potential targeted approach for managing hypertension-related nephropathy (**Figure 6**).

Existing pharmacological treatments for hypertensive nephropathy, such as thiazide diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers [28], primarily control blood pressure but carry side effects and require careful dose adjustment. These shortcomings underscore the demand for innovative therapeutic agents.

(20S)-Protopanaxatriol (PPT), an active metabolite derived from ginsenosides, functions as a versatile regulator of autophagy and inflammation in multiple pathological contexts. For instance, PPT counters D-galactose-induced brain aging in mice by enhancing mitochondrial autophagy through upregulation of TFEB and LAMP2 [18]. Molecular docking studies have also predicted PPT's interaction with Akt, leading to inhibition of excessive autophagy and apoptosis in triple-negative breast cancer cells via the Akt/mTOR route [20]. Additionally, PPT influences AMPK family members, mTOR, and IGF-1 transcription factors, thereby targeting IGF-1/mTOR and PI3K/AKT pathways to protect against age-related kidney disorders [14]. This coordinated control of upstream signals and lysosomal biogenesis sets PPT apart from rapamycin-like compounds. In models of inflammatory bowel disease, ginseng-derived PPT potently blocked NLRP3 inflammasome activity in various primary cells, reduced systemic inflammation, improved liver function, and lessened hepatic inflammation and fibrosis in MCD diet-induced MAFLD mice [29]. In ulcerative colitis, PPT modulated circulating pro-inflammatory cytokines (TNF- α , IL-6, IL-1) and colonic MPO and NO levels, effectively alleviating metabolic disturbances [30]. Furthermore, in T2DM mice, PPT decreased serum TNF- α and IL-6, downregulated hepatic inflammatory gene expression, boosted antioxidant defenses via elevated superoxide dismutase, and lowered malondialdehyde

concentrations [31]. Such broad-spectrum activities establish PPT as a distinctive modulator of autophagy and inflammation with adaptable tissue-specific benefits. Our data add to this body of evidence by confirming PPT's ability to combat oxidative stress and structural injury in hypertensive nephropathy. PPT lowered blood urea nitrogen and serum creatinine, indicating improved renal function. Histological examinations (H&E, Picro Sirius Red, and Masson's trichrome) further demonstrated substantial attenuation of renal fibrosis in treated mice.

Substantial literature highlights the importance of autophagy indicators such as p62, LC3-II/LC3-I ratio, and Beclin1 across various disorders including neurodegeneration, malignancies, and metabolic conditions [32]. In Alzheimer's disease, p62 buildup signals defective autophagic clearance of protein aggregates, while diminished LC3-II/LC3-I and Beclin1 levels are associated with autophagy failure and worsened neuronal loss [33]. In contrast, heightened Beclin1 and LC3-II/LC3-I ratios in hepatocellular carcinoma support excessive autophagy that aids tumor cell survival during metabolic challenge [34]. In the current work, PPT increased p62 levels, reduced the LC3-II/LC3-I ratio, and modulated Beclin1 in a manner consistent with autophagy restoration. These changes correlated with PPT's influence on the AMPK/mTOR pathway, where it inhibited AMPK and activated mTOR to restrain autophagy, ultimately decreasing inflammatory kidney injury. This mechanism is consistent with reports showing that AMPK/mTOR-mediated autophagy restrains NLRP3 inflammasome activity and limits pro-inflammatory cytokine production [35]. Prior research has also linked autophagy enhancement to reduced renal fibrosis and inflammation in hypertensive nephropathy [36], supporting the therapeutic potential of PPT's targeting of this pathway in autophagy-related inflammatory conditions.

Robust evidence establishes that overactive NF- κ B signaling drives renal fibrosis progression. Excessive NF- κ B activity promotes immune cell recruitment and activation, amplifying inflammation. This leads to oxidative stress through generation of reactive oxygen and nitrogen species from leukocytes and tissue-resident cells, ultimately causing cell death, tissue injury, and fibrosis [37]. NF- κ B activation involves IKK-mediated phosphorylation and degradation of I κ B α , freeing p50/p65 dimers for phosphorylation and nuclear entry to drive target gene transcription [38]. In both Ang II-infused mouse kidneys and NRK-52E cells, we detected increased p-P65, decreased I κ B α , and elevated nuclear p65. PPT administration reversed these alterations, demonstrating its protective role through NF- κ B suppression. These observations are in line with findings by Wang *et al.* showing Cofilin1 contributes to hypertensive renal injury via NF- κ B regulation in tubular epithelial cells [39]. Collectively, our results indicate that PPT mitigates Ang II-induced kidney damage by dampening the interconnected autophagy/NF- κ B inflammatory cascade. This underscores PPT's strong anti-inflammatory capacity and its promise as a candidate therapy for hypertensive nephropathy. However, detailed pharmacokinetic characteristics of PPT are still not fully characterized, representing a current limitation. Additional PK/PD investigations will be essential to refine dosing strategies and advance its clinical development.

Conclusion

In conclusion, this study identifies a novel mechanism through which PPT protects against hypertensive inflammatory kidney injury by inhibiting PTPN1 and thereby regulating the AMPK/mTOR/autophagy pathway. These insights enrich our knowledge of ginsenoside metabolites' renoprotective properties and position PPT as a viable therapeutic option for hypertension-associated nephropathy. Selective modulation of the PTPN1/AMPK/mTOR/autophagy axis may provide an innovative approach to reduce renal inflammation and damage in hypertensive states, opening avenues for future translational studies.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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