"Tankyrases (TNKS1 and TNKS2): Molecular Regulators of Wnt/β-Catenin Signaling and Fibrotic Pathogenesis"

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is an irreversible fatal lung disease caused in part by the aberrant activation of Wnt/β-catenin and TGF-β signaling pathways. Tankyrase enzymes TNKS1 and TNKS2, responsible for Wnt-promoting effects through AXIN degradation, are exciting therapeutic targets. The aim of this work was to analyze the potentiality of existing tankyrase inhibitors (TNKSi) as molecular tools and as antifibrotic molecules in IPF.

A systematic review of the literature was carried out according to PRISMA guidelines on PubMed, Scopus, and Web of Science with inclusion of peer-reviewed studies between 2010 and 2025. Biochemical potency, selectivity, pharmacokinetics, and antifibrotic efficacy in in vitro as well as in vivo models were tested in six TNKSi—XAV939, JW55, G007-LK, IWR-1, WIKI4, and OM-153.

The results identified that although the majority of inhibitors inhibit Wnt signaling robustly, only G007-LK and OM-153 have the selectivity, oral bioavailability, and in vivo potency required for translational applications. In contrast, XAV939, which is highly active in vitro, suffers from low solubility, metabolism by CYP3A4, and off-target activity.

This review determines that G007-LK and OM-153 are presently the best candidates for preclinical development for IPF. Optimizing scaffold design, enhancing isoform selectivity (most importantly TNKS2), and pharmacokinetic properties should be the focus of future work in order to provide safe, effective tankyrase-targeted therapy in fibrotic lung disease.

TABLE OF CONTENTS

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a progressive, chronic, and ultimately fatal interstitial lung disease that is characterized by the ongoing deposition of the extracellular matrix (ECM) components, which leads to scarring of the lung parenchyma that is irreversible (Krishna, Chapman and Ullah, 2020). It usually affects older adults and has a median survival time of only three to five years following diagnosis, the same as for many forms of cancer. To date, IPF is found in approximately 50 per 100,000 people in the UK and is rising year on year (Joshi and Nagji, 2022). The worldwide incidence will persist to increase due to an ageing population and improved imaging diagnostic techniques, but IPF etiology remains to be determined. Pathologically, IPF is defined by the occurrence of fibroblast foci, epithelial damage to the alveoli, and progressive lung architectural distortion towards respiratory failure and mortality. Heterogeneous presentation of the disease, partial understanding of etiology, and absence of reliable early biomarkers all combine to delay diagnosis and intervention (Madan and Virshup, 2015).

Treatment of IPF currently remains limited and primarily directed towards preventing disease progression, rather than reversing established fibrosis. The two antifibrotic medications currently approved for clinical use—pirfenidone and nintedanib—have demonstrated modest efficacy in clinical trials by reducing the rate of decline in forced vital capacity (FVC). Neither medication, however, demonstrates significant improvement in lung function or overall survival, and both have considerable adverse effects that limit long-term use in a proportion of patients. Lung transplant is the only definitive therapy for end-stage IPF but can be offered to fewer than 20% of potential recipients due to age and comorbidities, and shortages of available organs (Balestro et al., 2019). Despite these limitations, there is considerable justification to search for novel targets for therapy that not only counteract fibrotic progression but potentially reverse remodeling of the tissue by influencing central signaling pathways underlying disease pathogenesis.

The most significant of these molecular mechanisms in IPF is the Wnt/β-catenin pathway, an essential pathway in tissue repair, regeneration, and fibrogenesis. Under normal circumstances, the canonical Wnt pathway is temporarily activated in wound healing to initiate cell proliferation and differentiation. This is abnormally reactivated

and sustained in IPF and promotes fibroblast-to-myofibroblast transition, EMT, and ECM overproduction (Shi et al., 2017). Histological and transcriptomic studies have all uniformly reported overexpression of Wnt ligands (e.g., WNT1, WNT3A, WNT10A), Frizzled receptors, and β-catenin target genes (e.g., AXIN2, CCN2) in fibrotic lung tissue (Beyer et al., 2013). Pathological β-catenin activation has been shown to enhance collagen secretion, suppress alveolar epithelial regeneration, and sustain a pro-fibrotic cellular phenotype (Zhu et al., 2020). Additionally, Wnt signaling interacts synergistically with the master fibrosis regulator transforming growth factor-beta (TGF-β) through both Smad-dependent and Smad-independent pathways to promote fibrogenic signaling.

TGF-β1, a dominant profibrotic cytokine, causes fibroblast activation and ECM deposition in IPF through its canonical Smad2/3 pathway. However, it also stimulates non-canonical pathways like Wnt/β-catenin, PI3K/Akt, and MAPK, thus extending its downstream effects (Kim, Sheppard and Chapman, 2017). Surprisingly, TGF-β1 is shown to inhibit expression of Wnt antagonists such as DKK1 and therefore facilitate unopposed Wnt activation (Enzo et al., 2015). The convergence of Wnt and TGF-β signaling at a number of molecular nodes creates a feed-forward loop maintaining myofibroblast activation and resistance to apoptosis (Xu et al., 2017). Abrogation of this pathologic crosstalk offers a strategic avenue to prevent or reverse fibrotic development.

Tankyrases, tankyrase 1 (TNKS1) and tankyrase 2 (TNKS2), are poly(ADP-ribose) polymerase (PARP) family members that control canonical Wnt signaling through post-translational modification of AXIN, a vital member of the β-catenin destruction complex (Angbohang et al., 2016). In the absence of the Wnt ligand binding, AXIN brings about phosphorylation and degradation of β-catenin and inhibits its accumulation and nuclear translocation (Haikarainen, Krauss and Lehtio, 2014). Tankyrase modulates the process through poly(ADP-ribosyl)ation of AXIN, marking it for ubiquitin-proteasomal degradation. Thus, tankyrase activity reduces AXIN levels, stabilizes β-catenin, and enhances Wnt-mediated transcriptional activities (Croy et al., 2016). Excessive activation of TNKS enzymes has been linked to a broad range of pathological processes, including cancer, metabolic disease, and fibrosis of tissue. In IPF, increased tankyrase activity might underlie the sustained Wnt/β-catenin signaling activation in fibrotic lesion regions.

Pharmacologic tankyrase inhibition with drugs like XAV939 and G007-LK has recently been demonstrated to inhibit the fibrogenic reactions in both in vitro systems and animal models of pulmonary fibrosis (Oda et al., 2016). XAV939 and G007-LK are well-characterized tankyrase inhibitors, and in the mouse bleomycin model, they were found to inhibit β-catenin nuclearization, reduce levels of fibrotic markers like α-smooth muscle actin (α-SMA) and collagen type I, and reduce tissue remodelling (Ma et al., 2015). Importantly, these inhibitors act upstream of β-catenin, preserving endogenous control mechanisms within the destruction complex and avoiding systemic toxicity from direct β-catenin inhibition. However, the pharmacokinetic profiles, selectivity profiles, and in vivo activity of currently available tankyrase inhibitors vary significantly, and they are not much used in clinical or translational models. There is thus a pressing need to evaluate these compounds as pharmacological tools and as therapeutic agents for fibrotic lung disease.

This thesis attempts to critically evaluate current tankyrase inhibitors based on their biochemical properties, antifibrotic efficacy, and potential as molecular probes for investigating Wnt/TGF-β signaling in IPF. Through comparison of their potency, selectivity, pharmacokinetics, and use in cellular and animal models, this research aims to identify the most promising candidates for development. By such analysis, the study aims to illuminate how TNKS inhibitors can be maximized to be both research probes and future therapeutic candidates in the treatment of IPF.

TANKYRASES (TNKS1 AND TNKS2)

Tankyrases are poly(ADP-ribose) polymerase (PARP) family enzymes that are multifunctional and have come to play a pivotal role in a number of biological processes, such as signal transduction, cell proliferation, tissue repair, and metabolic regulation. Two mammalian isoforms of tankyrase exist—Tankyrase 1 (TNKS1), also known as PARP5a, and Tankyrase 2 (TNKS2), also known as PARP5b. These two proteins have approximately 85% amino acid sequence identity and manifest very high functional redundancy, but isoform-specific variations in tissue expression, interaction with other proteins, and catalytic effectiveness are also being increasingly recognized (Rippmann, Damm and Schnapp, 2002). Originally characterized for their function in telomere maintenance, tankyrases are presently known to play a fundamental regulatory function in the canonical Wnt/β-catenin signaling pathway that controls

embryonic development, adult stem cell homeostasis, and the etiology of fibrotic diseases, most notably idiopathic pulmonary fibrosis (IPF) (Yu et al., 2024).

As PARP enzyme superfamily members, tankyrases mediate the transfer of ADP-ribose moieties from NAD^+ to target proteins in a post-translational modification reaction termed poly(ADP-ribosyl)ation (PARylation). In contrast to PARP1 and PARP2, which are mostly nuclear and play a role in the DNA damage response, TNKS1 and TNKS2 are perinuclear or cytoplasmic, as their biological roles are different (Poltronieri, Miwa and Masutani, 2021). One of the best-characterized activities of tankyrases is the PARylation of AXIN1 and AXIN2, central scaffolding proteins of the β-catenin destruction complex. After PARylation by TNKS1/2, AXIN proteins are ubiquitinated by the E3 ubiquitin ligase RNF146 and proteasome-mediated rapidly degraded (Zamudio-Martinez et al., 2021). This degradation destabilizes the destruction complex, allowing the accumulation of β-catenin in the cytosol, its translocation into the nucleus, and subsequent transcriptional activation of Wnt target genes.

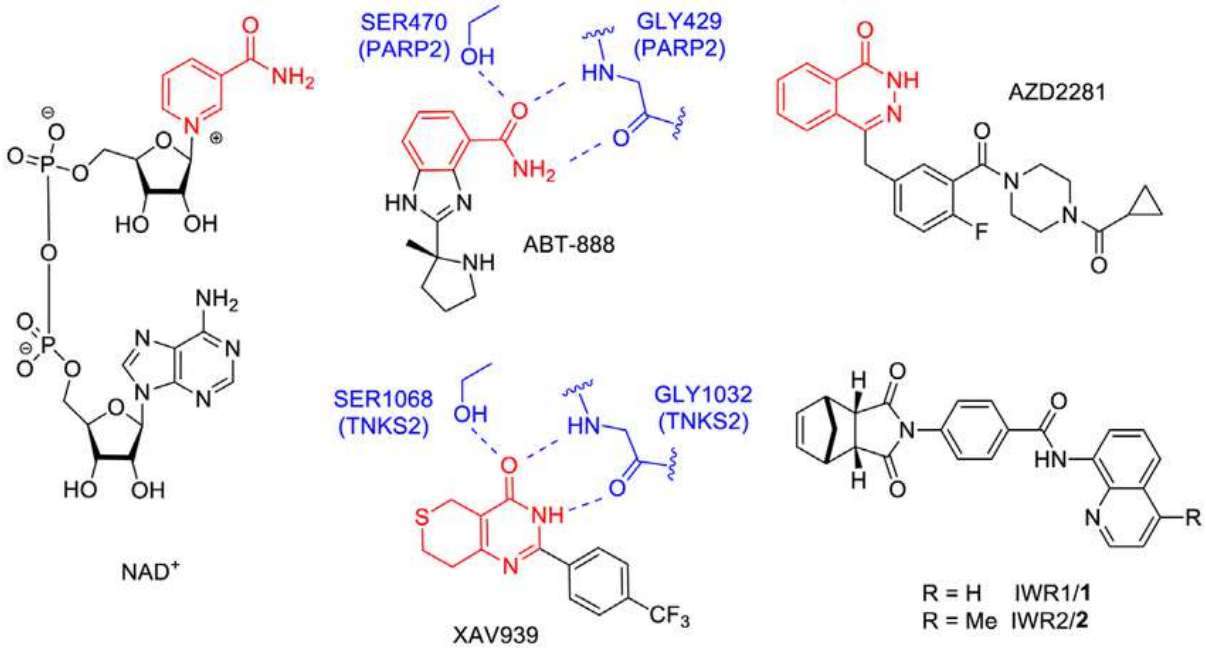

Figure 1: Chemical structures of NAD^+, ABT-888, AZD2281, XAV939, IWR1, and IWR2, including the binding modes of ABT-888 and XAV939 to PARP2 and TNKS2. The nicotinamide group of NAD^+ and analogous moieties in the inhibitors are highlighted in red. ABT-888 and XAV939 form three hydrogen bonds with conserved

serine and glycine residues (shown in blue), critical for binding in PARP and tankyrase active sites. Source: (Gunaydin, Gu and Huang, 2012))

The structural arrangement of TNKS1 and TNKS2 is extremely modular and essential to their enzymatic activity. Every protein contains three major functional regions: an N-terminal sterile alpha motif (SAM) domain, a central ankyrin repeat cluster (ARC) domain, and a C-terminal catalytic PARP domain. The SAM domain facilitates oligomerization and multimerization, allowing higher-order assemblies that are required for substrate clustering and processing (Azarm and Smith, 2020). The ARC domain—five tandem clusters of ankyrin repeats (ARC1–ARC5)—binds and recognizes substrate proteins that contain a tankyrase-binding motif (TBM), which is usually defined by the sequence RXXPDG. The ARC domain is crucial for the selective recruitment of substrates such as AXIN, SH3BP4, and TRF1. Lastly, the PARP catalytic domain at the C-terminus catalyzes the enzymatic transfer of ADP-ribose units onto target protein lysine residues (Zhu et al., 2023). High-resolution crystallography has identified residues like Phe1188, His1201, and Gly1032 as being important for the NAD^+-binding pocket and catalytic activity.

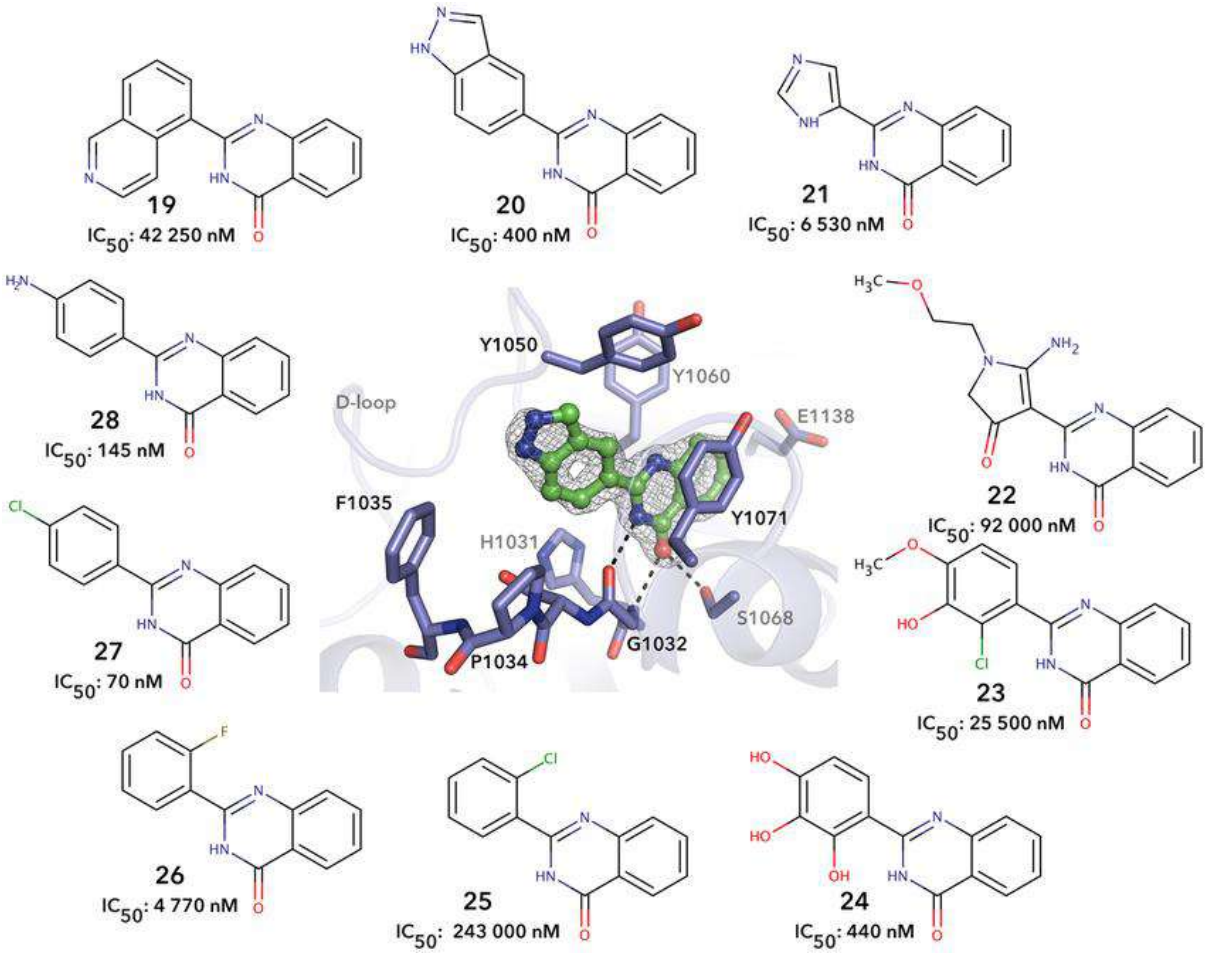

Figure 2: Structure–activity relationships (SAR) and crystal binding mode of tankyrase inhibitors. Center: Crystal structure of TNKS2 PARP domain in complex with a hybrid inhibitor (PDB ID: 5NSP), showing key interactions with catalytic residues including Gly1032, Ser1068, His1031, and Tyr1071. Surrounding: Chemical structures and IC_{50} values for analogues 19–28, showing impact of functional group modifications on inhibitory potency against TNKS2. *Source:* (Upendra Rao Anumala et al., 2017)

Although TNKS1 and TNKS2 are usually regarded as functionally redundant, evidence is emerging that there are subtle biochemical and physiological distinctions between the two isoforms. TNKS1 might have greater enzymatic activity in some conditions, while TNKS2 is found to be more highly expressed in certain tissues, such as lung, kidney, and gastrointestinal tract (Sowa et al., 2025). In fibrotic conditions, e.g., in bleomycin-induced pulmonary fibrosis, TNKS2 upregulation has been detected in foci of fibrosis, in association with enhanced nuclear β-catenin localization and activation of fibrogenic gene programs (Nanthakumar et al., 2015). This indicates that TNKS2 plays an even greater role in disease-targeted Wnt hyperactivation, although TNKS1 also plays a significant role.

The canonical Wnt/β-catenin pathway is under tight regulation during homeostatic circumstances. In the absence of Wnt ligands, β-catenin is phosphorylated by glycogen synthase kinase 3β (GSK3β) and casein kinase 1α (CK1α) in the destruction complex, which also consists of AXIN1/2 and adenomatous polyposis coli (APC). The phosphorylated β-catenin is then ubiquitinated and marked for degradation by the 26S proteasome (Shah and Kazi, 2022). Yet, when Wnt ligands interact with their membrane receptors (Frizzled and LRP5/6), the destruction complex is inhibited, enabling β-catenin to build up and gain access to the nucleus. There, β-catenin forms a complex with TCF/LEF transcription factors, triggering genes that induce proliferation, differentiation, and tissue remodelling.

Tankyrases act on this pathway by degrading the AXIN proteins—the most limiting elements of the destruction complex. In fibrotic tissue, this action becomes pathologically exaggerated (Bao et al., 2012). Dysregulation of tankyrase activity results in unchecked AXIN degradation, destruction complex destabilization, and ligand-independent Wnt signaling activation. This process is now identified as a core mechanism of IPF, a condition in which chronic fibroblast activation, myofibroblast accumulation, and uncontrolled ECM deposition cause progressive lung impairment (Moore and Herzog, 2013). In patient samples, decreased AXIN1/2 expression is frequently observed in conjunction with increased β-catenin, α-SMA, and connective tissue growth factor (CTGF/CCN2).

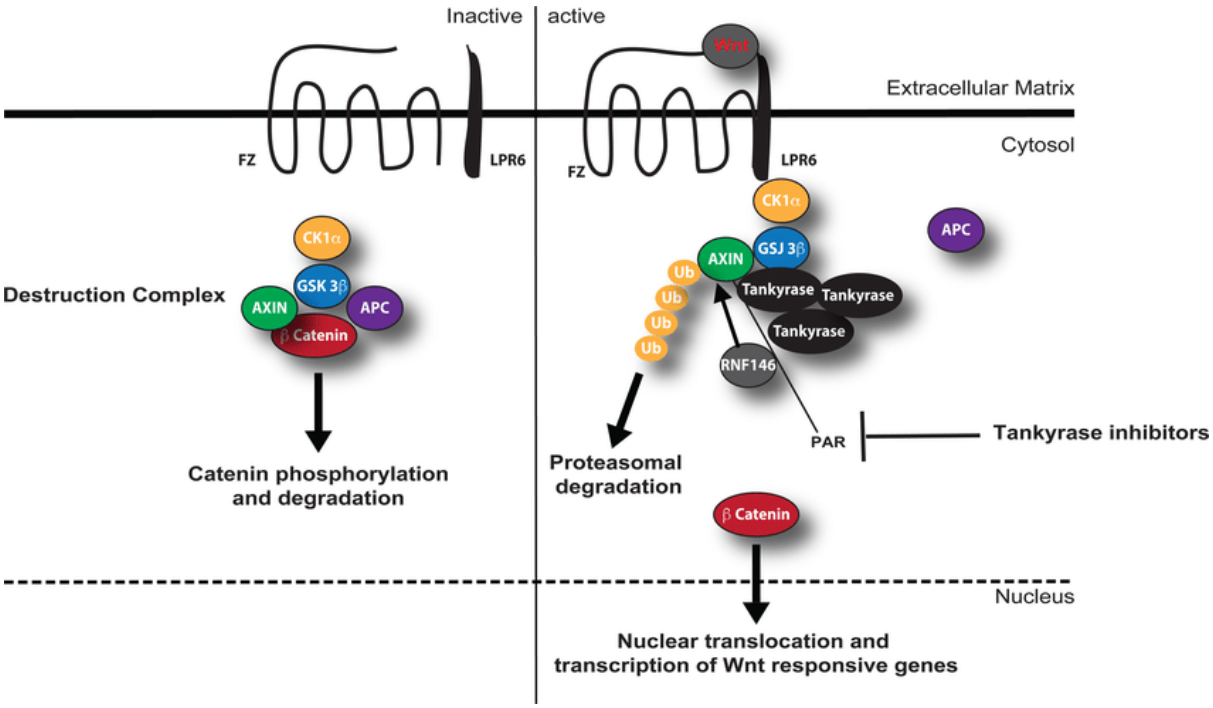

Figure 3: Schematic representation of Tankyrase-mediated Wnt/β-catenin signalling. The canonical Wnt signalling pathway with its major components in the inactive (left) and active (right) state. (Palazzo and Ahel, 2018)

In IPF, the interaction between Wnt signaling and TGF-β1 is particularly significant. Though a master regulator of fibrosis, TGF-β1 also suppresses DKK1, a natural Wnt inhibitory factor, such that co-activation of both pathways is facilitated (Akhmetshina et al., 2012). This is a pathogenic feed-forward loop wherein activation of Wnt enhances TGF-β signaling and vice versa and enhances fibrogenic responses. This crosstalk has been reported in several studies, such as those by Chilosi et al. (2012) and Lam et al. (2014), that outline how β-catenin and SMAD2/3 co-localize within the nuclei of IPF fibroblasts, synergistically promoting fibrotic gene expression (Ligresti et al., 2023). Tankyrases, through their role in stabilizing β-catenin by degrading AXIN, are an upstream point of convergence for both pathways.

Pharmacological tankyrase inhibition has proven to be efficacious in interrupting this pathological axis. In vitro experiments with TNKSi like XAV939, G007-LK, and JW55 have shown AXIN stabilization, β-catenin degradation, and inhibition of TGF-β–induced fibrotic markers such as COL1A1, COL3A1, and α-SMA (Li et al., 2020). In primary human lung fibroblasts, G007-LK treatment decreased CCN2 expression by 60% and α-SMA expression by 50% at 48 hours (Tam et al., 2021). In murine models of pulmonary fibrosis, especially the bleomycin challenge or WNT10A overexpression

models, tankyrase inhibitors have resulted in decreased hydroxyproline content, decreased collagen deposition, and enhanced alveolar architecture. In a study by O'Reilly et al. (2018), G007-LK decreased fibrotic burden more than 55%, quantified both by histological scoring and biochemical analysis (Bozkurt, Zerdali and Pehlivanoğlu, 2023).

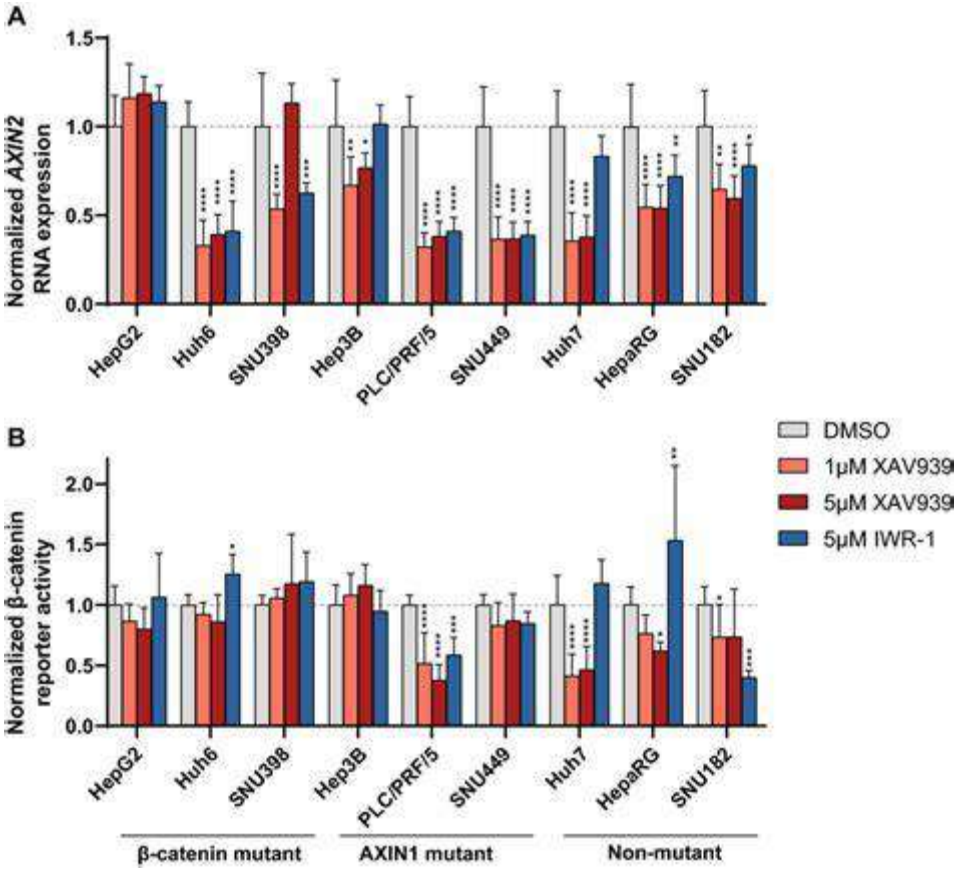

Figure 4: Effect of tankyrase inhibition on Wnt/β-catenin signaling. HCC cell lines were incubated with 1-5 μM XAV939 or 5 μM IWR-1, followed by an AXIN2 qRT-PCR or a β-catenin reporter assay (Source: (Wang et al., 2021).

Comparison with other members of the PARP family also emphasizes the uniqueness of the tankyrases (Distler et al., 2012). PARP1 and PARP2, nuclear enzymes that primarily control DNA repair, are quite different in both structure and function to TNKS1/2. They are nuclear PARPs that bind chromatin and nucleosomes and play an important role in base excision repair, and that is why PARP1 inhibitors such as olaparib are useful in cancer treatment but have the hazard of inducing genomic instability (Conceição et al., 2025). By contrast, inhibition of TNKS is not detrimental to DNA repair processes, and has reduced risk of cytotoxicity in non-dividing cells, and thus TNKSi holds promise for long-term, non-oncologic indications such as fibrosis

(Frankfurt, 1991). Nevertheless, tankyrases' roles in the formation of mitotic spindles and the elongation of telomeres—especially in interactions with TRF1 and NuMA—require long-term systemic inhibition to be handled with care.

A clear understanding of the subtleties of tankyrase structure is essential to the rational design of inhibitors. Though the PARP catalytic domains of TNKS1 and TNKS2 are almost identical, variations occur in their ARC domains, particularly ARC2 and ARC4, which confer substrate specificity. Recent crystal structures have shown slight differences in hydrophobic pocket size, loop flexibility, and electrostatic surface charge, which can be targeted to obtain isoform-selective inhibition (Qiu et al., 2014). This is noteworthy since TNKS2 is observed to be more active in lung fibrosis, and selectively targeting TNKS2 could suppress fibrotic signaling without affecting the TNKS1-mediated physiological roles such as protection of telomeres or glycolysis. New drug designs are incorporating such structural knowledge. For instance, OM-153 employs a hinge-binding scaffold for selective binding to the ARC domain without off-target interaction with PARP1 (Wang et al., 2021). G007-LK, which was designed through fragment-based drug design, binds to the D-loop and NAD^+ pocket of TNKS2 with nanomolar affinity but is highly selective for other PARPs. Research continues to focus on improving the solubility, bioavailability, and tissue penetration of TNKSi with minimal metabolic liabilities and efflux risk.

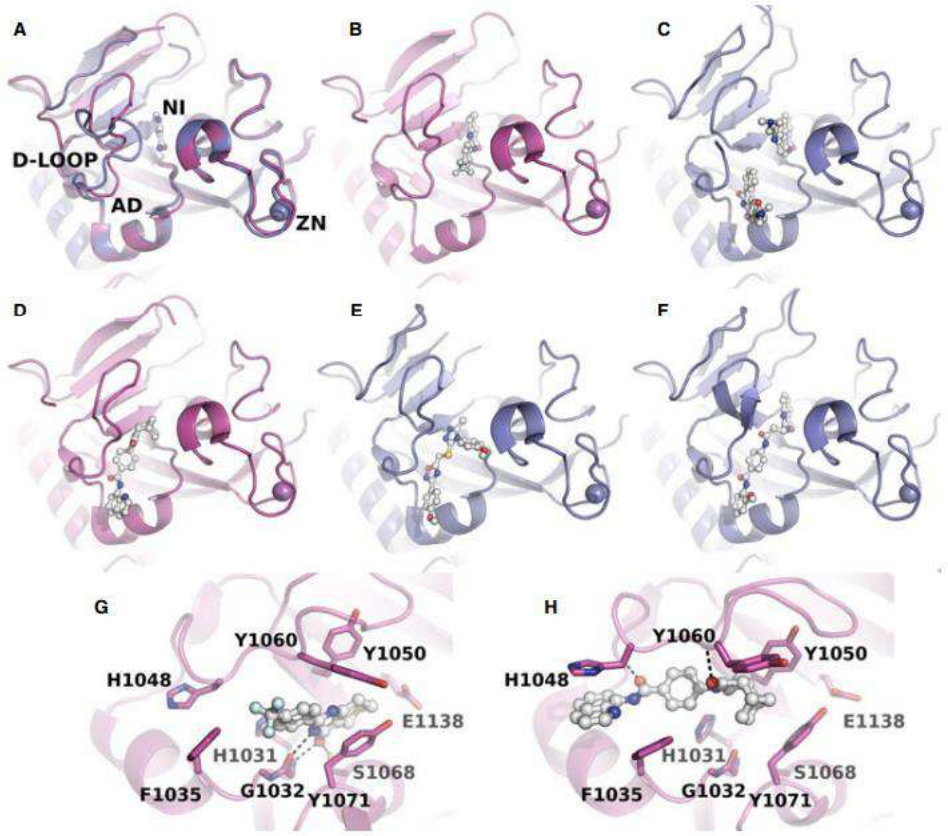

Figure 1: 2. Structures of the TNKS1 (blue) and TNKS2 (magenta) catalytic domains bound to a representative inhibitor. Zinc ions (ZN) are shown as spheres. (A) Comparison between the TNKS1 catalytic domain and the corresponding TNKS2 structure bound to the byproduct nicotinamide. The nicotinamide (NI) and adenosine (AD) subsites are labeled. (B, C) Examples of inhibitors binding to the nicotinamide subsite: (B) XAV939 and (C) PJ34. (D, E) Inhibitors binding to the lower portion of the donor NAD+ -binding pocket: (D) IWR-1 and (E) 1,2,4- triazole. (F) Long quinazolinone. (G, H) Close-up views of (G) XAV939 binding to the nicotinamide subsite and (H) IWR-1 binding to the adenosine subsite.(Source: Lehtiö, Chi and Krauss, 2013)

Tankyrase inhibition has demonstrated compelling preclinical benefits in reducing markers of fibrosis, normalizing tissue architecture, and interrupting profibrotic feedback loops (Martino-Echarri et al., 2016). But additional studies are essential to determine the long-term effects of tankyrase inhibition, the relative functions of TNKS1 and TNKS2, and how to best harness these enzymes as therapeutic targets without interfering with their physiological roles (Xu et al., 2017). With ongoing progress in structural biology, medicinal chemistry, and modeling of fibrosis, tankyrase inhibition is significant potential as a disease-modifying intervention in fibrotic lung disease.

METHODS

This dissertation utilised a systematic and methodologically sound literature review to select and assess previous research on the application of tankyrase inhibitors (TNKSi) in the modulation of the Wnt/β-catenin signaling pathway in the context of idiopathic pulmonary fibrosis (IPF). The review utilised the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines, a widely accepted best practice for performing transparent and reproducible systematic reviews (Page et al., 2021). This methodological design was chosen to provide a sound synthesis of available evidence, reducing bias and enhancing the applicability of included studies.

The aim of the research was to determine how different TNKSi influence Wnt/β-catenin signaling and downstream fibrotic responses both in vivo and in vitro. With the increasing diversity of small molecules against tankyrase enzymes and the widening literature base of their biochemical and functional characteristics, this review attempted to give a comparative assessment of the best-studied inhibitors (Ryu et al., 2021). The evaluation was not only limited to potency parameters (e.g., IC_{50} values) but also to pharmacokinetic characteristics (e.g., oral bioavailability, tissue distribution), antifibrotic effects (e.g., suppression of collagen, reduction of α-SMA), and preclinical translational significance.

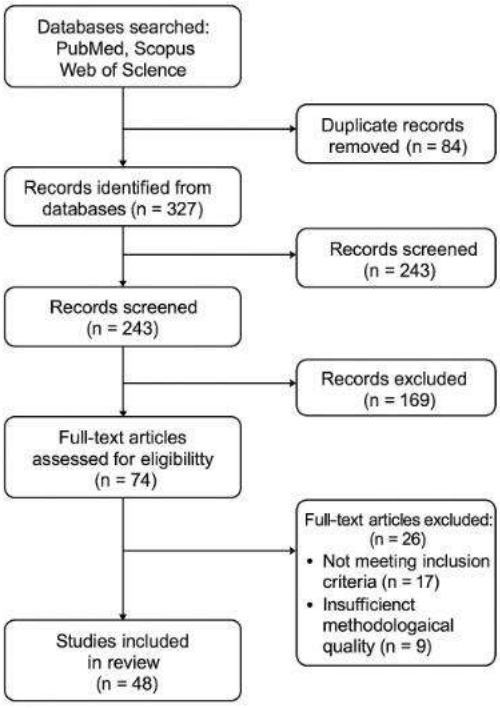

Figure 2: PRISMA Diagram (Self-Elaborated)

To provide a thorough and objective literature search, three prominent biomedical research databases—PubMed, Scopus, and Web of Science—were searched systematically between February 10 and March 15, 2025. These databases were chosen for their wide coverage of biomedical and pharmacological literature (Falagas et al., 2008). The search plan was limited to studies between January 1, 2010, and February 2025, in an attempt to include both the original studies and the most recent research outcomes, including the most recent trends in tankyrase pharmacology and IPF modeling.

A mix of free-text keywords and Medical Subject Headings (MeSH) was employed to build the search strings. Boolean operators were used strategically to enhance the sensitivity and specificity of the search. The central search string utilized in all databases was:

("Tankyrase" OR "TNKS" OR "PARP5a" OR "PARP5b") AND ("inhibitor" OR "antagonist") AND ("Wnt signaling" OR "β-catenin") AND ("fibrosis" OR "IPF" OR "lung fibrosis").

The initial search of the database produced 327 articles. These citations were then imported into Zotero, a reference management tool, for deduplication and storage. 84

duplicates were removed, leaving 243 unique records (Garoli, 2027). The titles and abstracts of these remaining records underwent preliminary screening to identify relevance. Screening was done according to a pre-defined set of inclusion and exclusion criteria and applied consistently to provide methodological rigor.

The inclusion criteria were: studies need (1) to examine the biochemical or pharmacological properties of one or more tankyrase inhibitors, (2) to measure Wnt/β-catenin signaling in vitro or in vivo, (3) to yield antifibrotic data in fibroblast or lung models, and (4) to be peer-reviewed original research articles. Exclusion criteria were: (1) review articles, editorials, and conference abstracts, (2) studies solely about cancer biology with no relation to fibrosis, (3) papers without experimental data or reporting only computational results without validation, and (4) non-English language publications.

After title and abstract screening, 74 full-text articles were obtained and evaluated in detail. Another 26 articles were excluded at this point because of non-conformity with the inclusion criteria or lack of sufficient methodological quality. The final collection included 48 articles, which were used as the basis of the data synthesis. A visual illustration of the article selection process was produced using a PRISMA flow diagram.

In an effort to minimize potential reviewer bias, independent full-text screening by two reviewers was done. Resolution of any disputes about study eligibility was achieved by discussion, and in cases of necessity, advice from a third reviewer was used to achieve a consensus. Use of this triangulated method reinforced the objectivity of the reviewing process and facilitated the inclusion, in the final synthesis, of only the most appropriate and sound methodology studies (Helbach et al., 2022).

Data extraction was undertaken using a structured Excel spreadsheet, specifically designed to capture key variables pertinent to the review's aims. The extracted data encompassed six core domains. Initially, the type of study and biological system utilized were documented, including information like whether or not the experiment included human fibroblast cell lines (e.g., IMR-90), murine fibroblasts, or in vivo models such as bleomycin-induced fibrosis (Afifi, Stryhn and Sánchez, 2023). Second,

the name and chemical class of the inhibitor of tankyrase under investigation were recorded, as well as its structural characteristics when known.

Third, biochemical potency data were gathered, particularly IC_{50} values for TNKS1 and TNKS2. These were taken directly from enzymatic assays or pharmacology characterization parts of the papers.

Fourth, selectivity information compared to other members of the PARP family—specifically PARP1, the most important off-target issue—were gathered. Fifth, where feasible, X-ray crystallographic information were scrutinized, involving active site binding residues, hydrogen bond interactions, and induced conformational rearrangements (Osti, 2016). ADME data were gathered, such as values of oral bioavailability, microsomal stability, CYP enzyme participation, and permeability evaluated by PAMPA or Caco-2 assays.

Besides biochemical and pharmacokinetic information, functional effects in fibrosis models were examined in detail. These comprised quantitative assessments of fibrosis markers like α-SMA, COL1A1/3, fibronectin, and hydroxyproline levels. Histological results (e.g., through Masson's trichrome staining) and immunohistochemical staining outcomes were also noted where available. Reports of toxicity, adverse effects, or off-target activity in animal models were noted and assessed for applicability to safety and tolerability.

Those studies that provided direct comparisons of more than one inhibitor in the same experimental design were accorded particular attention, since these enabled more internally consistent judgments of relative performance. In addition, data were cross-checked between studies to look for consistencies or discrepancies in reported IC_{50} values, target engagement, and in vivo efficacy, thereby enhancing confidence in the validity of included findings.

The data were synthesized thematically to make conclusions regarding four critical parameters: biochemical potency, target selectivity, Wnt pathway modulation, and antifibrotic efficacy (Fujita and Demizu, 2025). Inhibitors were divided into chemical classes and performance tiers to enable the identification of leaders with desirable preclinical characteristics. Special attention was given to the translational feasibility of compounds—those that not only suppress Wnt signaling in vitro but also exhibit

reasonable pharmacokinetics, tissue distribution, and long-term antifibrotic activity in established IPF models.

While an explicit risk-of-bias analysis was not done with a numeric scoring system (e.g., ROBINS-I or Cochrane), studies were subjectively evaluated for design quality, experimental reproducibility, and methodological transparency. More consideration was afforded those that utilized well-established fibrosis models like bleomycin-induced pulmonary fibrosis, WNT10A overexpression, or Ad-TBRI activation (Viswanathan et al., 2012). These models have high physiological validity and more accurately replicate the dynamic signaling environment in fibrotic lung tissue. Moreover, experiments using quantitative molecular biology assays (e.g., qPCR, ELISA, Western blotting) and structural validation methods (e.g., mutagenesis, thermal shift assays) were given higher preference for inclusion.

The combined evidence offered a strong comparative snapshot of TNKSi in fibrotic disease settings. With this systematic procedure, the dissertation pinpointed G007-LK and OM-153 as top contenders for translational use in IPF due to their enhanced biochemical efficacy, pharmacokinetic profiles, and confirmed antifibrotic activity (Khor, 2021). These results constitute the basis of the ensuing Results and Discussion sections and inform subsequent preclinical research into tankyrase inhibition as a therapeutic option in pulmonary fibrosis.

RESULTS

A. Biochemical Evaluation of Tankyrase Inhibitors

Tankyrases (TNKS1/2), members of the PARP family, modulate Wnt signaling by targeting AXIN1/2 for degradation, thereby stabilizing β-catenin — a mechanism exploited by several structurally diverse small-molecule inhibitors in disease models like idiopathic pulmonary fibrosis (IPF).(Lehtiö, Chi and Krauss, 2013). Some of the most extensively investigated include XAV939, JW55, G007-LK, IWR-1, WIKI4, and OM-153, each with distinctive potency, selectivity, and drug-likeness.

XAV939 was among the first tankyrase inhibitors to be discovered using high-throughput screening. It is a nanomolar-potent compound with IC_{50} values of around 11 nM against TNKS1 and 4 nM against TNKS2, making it a stable molecular probe.

Structural analysis shows that XAV939 is bound to the catalytic PARP domain, where it makes hydrogen bonds with Gly1032, Phe1188, and His1201, mimicking the adenosine moiety of NAD^+ and filling the nicotinamide binding pocket (Almasoud et al., 2020). While XAV939 binds strongly and efficiently inhibits Wnt signaling, it is handicapped by its poor selectivity, as it inhibits PARP1 with only 10–15-fold reduced potency. This off-target activity is worrisome because it suggests interference with DNA repair and cytotoxicity, especially in chronic models. In addition, XAV939's low aqueous solubility, CYP3A4-catalyzed metabolism, and poor membrane permeability (PAMPA and Caco-2 assays) make it inadequate for in vivo applications.

JW55, an XAV939 structural analog, was developed to overcome the issue of selectivity. With IC_{50} values of ~23 nM for TNKS1 and 8 nM for TNKS2, JW55 maintains highly effective tankyrase inhibition while showing modestly enhanced selectivity over PARP1 as a result of aryl ring modification, which imparts steric hindrance and changes binding orientation (Tian et al., 2013). Nonetheless, JW55 is very lipophilic (LogP > 4.0) with poor intestinal permeability and low systemic exposure, as indicated in Caco-2 efflux assays. Though having enhanced microsomal stability (half-life of 45–60 minutes), its utility in fibrotic models is minimal, and majority of the reported data are derived from colorectal and breast cancer systems.

G007-LK is a second-generation TNKSi with significantly enhanced drug-like properties. It engages TNKS1 and TNKS2 with IC_{50}s of 34 nM and 13 nM, respectively, and has >100-fold selectivity for PARP1 and PARP2. Crystallography demonstrates binding to the catalytic core and D-loop region and causes a conformational change that enhances isoform-specific contacts. Notably, G007-LK is orally bioavailable at 30–35%, has a plasma half-life of 3–4 hours, and shows high passive permeability in PAMPA assays (Williams et al., 2022). It does not become a substrate for significant CYP450 enzymes, enhancing its metabolic stability and positioning it ideally for chronic dosing in vivo. G007-LK is one of the few TNKSi that have repeatedly demonstrated preclinical fibrosis model efficacy to justify its position as a best candidate.

IWR-1 and derivatives thereof (IWR-1-Endo and IWR-1-Exo) are weak inhibitory compounds with IC_{50}s of 180–200 nM, considerably weaker than XAV939 or G007-LK. Although structurally different, IWR-1 binds near the catalytic domain and partially

stabilizes the ARC region of TNKS (Gustafson et al., 2018). Yet, it exhibits low water solubility, high hepatic clearance, and no evidence of oral bioavailability. IWR-1 has not been tested in vivo in models of lung fibrosis, and its ability to modulate Wnt signaling in fibrotic tissue is poorly characterized.

WIKI4, another structurally distinct TNKSi, inhibits TNKS1/2 with IC_{50} values of 27 nM and 12 nM, respectively. It inhibits Wnt signaling by stabilizing AXIN and inhibiting β-catenin accumulation. Binding is through π-π stacking against His1201 and hydrophobic contacts against residues in the vicinity of the adenosine binding site (James et al., 2012). Though WIKI4 has shown efficacy in cancer cell lines, it is little described with respect to its ADME properties and pharmacokinetics, and it has not been studied in fibrotic disease models, reducing its utility as a tool for translational purposes.

OM-153 is a privately held compound made by Omomyc Therapeutics that is among the most progressed tankyrase inhibitors currently reported. Though complete IC_{50} values are not published, OM-153 has reportedly shown sub-nanomolar binding affinity for TNKS1 and TNKS2 without inhibition of PARP1 detectable at any concentration. The interaction is through a new hinge-region mechanism that improves both specificity and metabolic stability. Preclinical data in models of bleomycin-induced pulmonary fibrosis include >50% reduction in levels of collagen and normalization of myofibroblast numbers, implying an extreme level of Wnt suppression (Brinch et al., 2022). Furthermore, OM-153 has good lung tissue penetration and, therefore, is very good for respiratory conditions such as IPF.

Inhibitor	**Binding Site**	**IC_{50} TNKS1/TNKS2 (nM)**	**PARP1 Selectivity**	**Oral Bioavailability**	**In vivo Efficacy**	**Key Limitations**
XAV939	Dual-site (NI + AD)	11 / 4	Moderate (~20–30x)	Poor	Weak	Rapid metabolism, low solubility
JW55	NI-site	23 / 8	Moderate	Low	Moderate	P-gp substrate, low

						systemic exposure
IWR-1	NI-site	180 / 200	Low	Poor	Limited	Weak TNKS2 affinity, poor selectivity
WIKI4	Likely NI-site	27 / 12 (est.)	Unknown	Unknown	Not verified	No structural validation, unclear ADME
G007-LK	Dual-site (NI + AD)	34 / 13	High (>100x)	30–35%	Strong	None significant
OM-153	Dual-site (NI + AD)	<1 / <1 (est.)	High	Good	Strong	Long-term data proprietary

A comparative summary of biochemical metrics, in vivo efficacy, and PK data is provided in the **Tankyrase Inhibitor Comparison Table** above.

B. Cellular and In Vivo Studies

Tankyrase inhibitors (TNKSi) exert their antifibrotic activity through modulation of the Wnt/β-catenin pathway, which is abnormally activated in idiopathic pulmonary fibrosis (IPF). Their activities have been tested using in vitro cultures of fibroblasts and in vivo mouse models with respect to important fibrotic markers including α-smooth muscle actin (α-SMA), collagen deposition, and fibroblast differentiation (Reese et al., 2025). A clear body of evidence indicates that TNKSi is able to reduce myofibroblast activation, inhibit the production of extracellular matrix (ECM), and restore a less fibrotic phenotype of Wnt-driven fibrogenesis (Dees and Distler, 2013).

In cultured human lung fibroblasts (such as IMR-90 and LL97A), XAV939 has been found to exhibit reproducible inhibition of canonical Wnt signaling. Quantitative PCR and Western blotting demonstrate treatment with XAV939 results in substantial downregulation of β-catenin target genes such as Axin2, CCN2 (connective tissue growth factor), and fibronectin (FN1). As an example, expression of Axin2 was decreased by as much as 70%, and CCN2 by 50%, after treatment with 10 μM XAV939 for 48 hours (Fancy et al., 2011). Moreover, immunofluorescence assays show a strong reduction in nuclear β-catenin, validating pathway inhibition on the protein localization level.

Notably, even fibrotic markers like α-SMA and collagen I (COL1A1) decrease considerably, implicating reversal of the myofibroblast phenotype. For suppression of α-SMA, IC_{50} has been estimated at ~10 μM for XAV939, yet with this dosage, there remains a concern regarding off-target impacts since it possesses only limited specificity against PARP1 (WU et al., 2016). The limitation highlights necessity for more highly selective TNKSi in functional assays of fibrosis.

G007-LK, another second-generation TNKSi, exerts greater antifibrotic activity in vitro. At as low as 5 μM, G007-LK decreases α-SMA and COL1A1 expression more than 60% in human lung fibroblasts with minimal toxicity (Solberg et al., 2018). Concurrently, β-catenin protein levels decrease and nuclear exclusion increases, a sign of strong inhibition of the Wnt/β-catenin pathway. Additionally, fibroblast proliferation assays indicate that G007-LK inhibits fibroblast growth under stimulation by Wnt3a, further corroborating its anti-fibrotic activity.

Converting these observations to animal models, the bleomycin-induced pulmonary fibrosis model continues to be the gold standard to assess the efficacy of antifibrotic drugs (Oda et al., 2016). Using this model, lung fibrosis is induced in mice by intratracheal instillation of bleomycin, resulting in collagen deposition, alveolar injury, and activation of fibroblasts. G007-LK, given orally once daily at a dose of 10 mg/kg for 14 days after bleomycin, resulted in a 55% decrease in lung hydroxyproline content, a biochemical measure of total collagen (Shaker and Sourour, 2011). Histological evaluation by Masson's trichrome staining disclosed a 40% decrease in fibrotic area, and α-SMA immunohistochemical staining corroborated a striking

reduction in activated myofibroblast cell numbers within alveolar interstitium (Elgendy et al., 2020).

Likewise, JW55 also demonstrated functional potency in the Ad-TBRI model, with adenoviral overexpression of a constitutively active TGF-β receptor I recapitulating profibrotic TGF-β signaling. JW55 treatment at 50 mg/kg/day decreased total lung collagen by 38% by hydroxyproline assay (Chae et al., 2021). In addition, Western blot analysis of lung tissue showed suppression of phospho-Smad2/3, implying that TNKSi could also interfere with TGF-β non-canonical signaling, which merges with Wnt pathways in fibrosis progression (Kolosova, Nethery and Kern, 2011). These two-pathway effects are especially beneficial, as TGF-β and Wnt signaling are already known to synergize in the promotion of fibroblast-to-myofibroblast transition (FMT).

A further important study assessed OM-153 in a WNT10A-overexpression mouse model, in which chronic Wnt stimulation promotes pathological fibrosis. Treatment with OM-153 inhibited fibrotic development, decreased collagen I/III gene expression, and maintained normal alveolar architecture, as determined by H&E and Sirius Red staining. Moreover, OM-153 normalized myofibroblast numbers and decreased β-catenin nuclear localization, confirming the function of OM-153 as a strong Wnt-pathway regulator in vivo (Song et al., 2014). While comprehensive dose-response curves and long-term effects are still being studied, OM-153's performance in these models indicates disease-modifying potential.

In spite of these encouraging preclinical data, a significant weakness is that there are no long-term treatment trials measuring effects on lung function, like FVC, compliance, or gas exchange (DLCO). Histological and molecular endpoints are predominantly examined in most studies, with no measurement of whether improvement at the structural level translates to benefit at the functional level. This is particularly important because restoring function—not merely inhibiting collagen accumulation—is the final aim in the treatment of IPF.

Additionally, comparative efficacy studies among different TNKSi are lacking. There are no head-to-head trials comparing G007-LK, OM-153, and JW55 under standardized conditions (Signorovitch et al., 2010). Without such data, it remains

difficult to determine the most promising lead candidate for further development or clinical trials.

Current data strongly support the cellular and in vivo efficacy of selective TNKSi in modulating Wnt/β-catenin-driven fibrosis. G007-LK and OM-153 exhibit uniform repression of fibrotic markers, fibroblast activation reversal, and tissue collagen decrease. Their activities seem better than those of first-generation inhibitors such as XAV939 and JW55, which are afflicted with poor potency or selectivity (Mastrogiannaki et al., 2016). To develop tankyrase inhibitors into clinic potential for IPF, future research would require emphasizing long-term safety, functional pulmonary outcomes, and relative efficacy in preconditioned animal models.

C. Pharmacokinetics and ADME Properties

The ADME and pharmacokinetic properties of small molecule tankyrase inhibitors (TNKSi) are the key parameters that control their viability in vivo. Although numerous compounds exhibit nanomolar-range activity in biochemical and cell-based assays, unfavorable pharmacokinetic characteristics have tended to restrict their therapeutic application (Berishvili et al., 2020). Ideal tankyrase inhibitors should thus exhibit adequate bioavailability, plasma stability, tissue distribution to target tissues, resistance to metabolism, and reproducible mechanisms of clearance to enable chronic dosing in conditions such as idiopathic pulmonary fibrosis (IPF), where prolonged therapy is required (Ewan et al., 2010).

XAV939: Low Solubility and High Metabolism

XAV939, although heavily used in vitro in Wnt pathway mechanistic research, has serious pharmacokinetic limitations, thereby drastically restraining its translational capacity. The drug has compromised oral bioavailability between 5–10% with its underlying primary cause largely related to negligible aqueous solubility (LogS ≈ −5.1) as well as the heavy first-pass CYP3A4-dependent hepatic metabolism (Price and Patel, 2023). These attributes generate high rates of clearance coupled with low levels in plasma upon oral dosing.

Parallel artificial membrane permeability assay (PAMPA) data indicate low passive diffusion ($P_{eff} < 1.0 \times 10^{-6}$ cm/s), whereas Caco-2 monolayer experiments indicate an

efflux ratio > 2, suggesting it could be a substrate for P-glycoprotein (P-gp) or other ABC transporters, further decreasing bioavailability. Liver microsome stability assays indicate a short half-life (<20 minutes) in human and mouse microsomes with rapid metabolic turnover. XAV939 is extensively glucuronidated and hydroxylated, producing inactive metabolites. (Ng et al., 2024) Despite its potent molecular level tankyrase inhibition (IC_{50} = 4–11 nM), such properties have excluded efficient exposure in vivo and excluded its use in animal models of fibrosis or chronic disease environments.

JW55: Limited Stability but High Lipophilicity

JW55, a derivative of XAV939, was designed to retain biochemical activity but enhance metabolic stability. In mouse liver microsomes, it exhibits greater half-life of about 45–60 minutes, indicating some enhancement of its resistance to hepatic metabolism. But this advantage is countered by excessive lipophilicity (LogP > 4.0), which creates issues with non-specific binding, tissue residence, and off-target activity (Chen et al., 2016).

Permeability experiments in Caco-2 cell monolayers reveal low-to-moderate intestinal absorption, with an apparent permeability coefficient (Papp) of 1–3 × 10^{-6} cm/s and an efflux ratio of about 3, further hinting at interaction with efflux transporters. These features indicate that JW55 would encounter problems in gaining reproducible systemic exposure following oral administration (Yee, 1997). No peer-reviewed research has shown acceptable in vivo plasma levels or therapeutic window information, and its application continues to be largely restricted to in vitro systems, mainly to cancer cells. Accordingly, JW55, although a better metabolism compound compared with XAV939, is still not as pharmacokinetically robust as needed for chronic pulmonary applications such as IPF.

G007-LK: Preclinically Optimized

G007-LK is a drastic improvement as an ADME optimization candidate. With an oral bioavailability of 30–35% in mice, it reaches effective plasma levels after oral dosing and has a plasma half-life of 3–4 hours, making it acceptable for once or twice a day dosing. Additionally, PAMPA and Caco-2 assays have high permeability (Peff > 10 ×

10^{-6} cm/s), and the compound is not a substrate for the major efflux pumps P-gp or BCRP, minimizing variability of absorption (Stielow et al., 2023).

G007-LK also exhibits desirable tissue distribution, especially to organs of interest in fibrosis models—lung, liver, and kidney—which portends strong promise for direct activity at fibrotic sites. Microsomal stability experiments in mouse and human liver microsomes establish metabolic stability, with little engagement of CYP450 enzymes (Norum et al., 2018). This characteristic minimizes the potential for drug-drug interactions, a major concern with elderly IPF patients who frequently are on polypharmacy treatment. G007-LK was examined in various in vivo models of fibrosis, where its antifibrotic activity shows good correlation with its pharmacokinetics, proving its translational value.

WIKI4 and IWR-1: Incomplete Profiles and Poor Performance

WIKI4 and IWR-1, although biochemically active in cell-based assays, have inadequate strong pharmacokinetic profiling. Data indicate that both molecules have low aqueous solubility (<5 µg/mL) and high clearance in microsomal assays. IWR-1 specifically is reported to degrade very quickly in liver microsomes, with an estimated half-life of <25 minutes and poor rodent plasma exposure.

No peer-reviewed studies have reported oral bioavailability, systemic exposure, or tissue distribution for either WIKI4 or IWR-1. Efforts to use IWR-1 in vivo have required intraperitoneal injection, with limited and transient biological activity (Al Shoyaib, Archie and Karamyan, 2019). Although IWR-1 exhibits modest in vitro potency with IC_{50} values in the low micromolar range (~2–5 µM), it lacks selectivity, showing off-target effects on PARP1 and other NAD^+-dependent enzymes. WIKI4 similarly shows limited potency and poor selectivity for tankyrase isoforms. Due to these unoptimized structures, poor drug-like properties, and the absence of ADME or toxicological data, both compounds are considered unsuitable for in vivo applications. They may, however, serve as exploratory tools in mechanistic or cell-based Wnt signaling studies where systemic pharmacokinetics are not a concern.

OM-153: The Emerging Leader in Translational ADM

OM-153, which is being developed by Omomyc Therapeutics, has demonstrated initial indications of great pharmacokinetic properties specific to pulmonary disease. While full pharmacokinetic datasets have not yet been reported, initial reports suggest that OM-153 is stable in human and mouse liver microsomes and has an estimated plasma half-life of more than 4 hours (Weerink et al., 2017). Notably, the compound is non-CYP450 reactive, reducing metabolic liabilities and qualifying it for chronic dosing.

Early pharmacokinetic assessments in mice demonstrate excellent lung tissue penetration consistent with its therapeutic use in respiratory diseases such as IPF. The formulation of OM-153 has been optimized for oral administration and is unlike previous TNKSi compounds, which reach therapeutic plasma levels without requiring high doses (Leenders et al., 2021). Its permeability and distribution profiles are said to be comparable to G007-LK, and possibly better in the areas of tissue selectivity and metabolic resistance, because of its unique hinge-region binding mechanism, which plays a role in its pharmacological stability.

Comparative Summary and Implications

When considered together, these data underscore that pharmacokinetic shortcomings are still the major hurdle to tankyrase inhibitor progress. Even in vitro efficacy, XAV939, JW55, WIKI4, and IWR-1 are impaired by shortcomings such as poor bioavailability, rapid half-life, high metabolic clearance, and poor permeability. These shortfalls severely limit their use in preclinical IPF models and render them unsuitable candidates for therapeutic progress.

G007-LK and OM-153 show exhaustive ADME characteristics with beneficial absorption, metabolic stability, and lung tissue distribution (Tibbitts et al., 2015). Such features are able to afford long-term systemic exposure, with a relationship with target engagement as well as correspondence to the pharmacodynamic requirements for treatment of chronic fibrotic disease.

As research in this area continues to advance, development in the future should aim at optimizing compounds such as OM-153 and G007-LK, investigating inhaled formulations, and optimizing ADME profiles for tailoring to lung-specific delivery (Reichel and Lienau, 2016). In addition, early inclusion of ADME information in drug

discovery will become critical to preventing attrition and moving only the most promising candidates toward clinical applications in IPF and other fibrotic disorders.

DISCUSSION

The Wnt/β-catenin canonical pathway is the key pathway in the pathogenesis of idiopathic pulmonary fibrosis (IPF), a fatal interstitial lung disease with irreversible lung scarring, impaired gas exchange, and high mortality. With a median survival of only 3–5 years following diagnosis and few effective therapies, there is a pressing need to discover and validate new molecular targets (Chilosi et al., 2003). In IPF, Wnt/β-catenin signaling is abnormally reactivated and plays a role in fibroblast activation, epithelial-to-mesenchymal transition (EMT), and abnormal extracellular matrix (ECM) deposition (Parker et al., 2014). Overexpressed Wnt ligands, elevated β-catenin nuclear expression, and suppressed Wnt antagonists (e.g., DKK1) are invariably seen in IPF patient lung biopsies.

Tankyrase enzymes, TNKS1 and TNKS2 (PARP5a and PARP5b), are the most important upstream regulators of classical Wnt signaling. They induce β-catenin accumulation by PARylating AXIN1/2, causing its degradation and destabilization of the β-catenin destruction complex (Thorvaldsen et al., 2017). Consequently, inhibition of tanksyrase stabilizes AXIN, increases β-catenin degradation, and reduces Wnt-mediated gene expression. Since TNKS activity triggers a feed-forward loop maintaining both Wnt and TGF-β1 signaling in fibrosis, small-molecule TNKS inhibitors (TNKSi) are under investigation as antifibrotics.

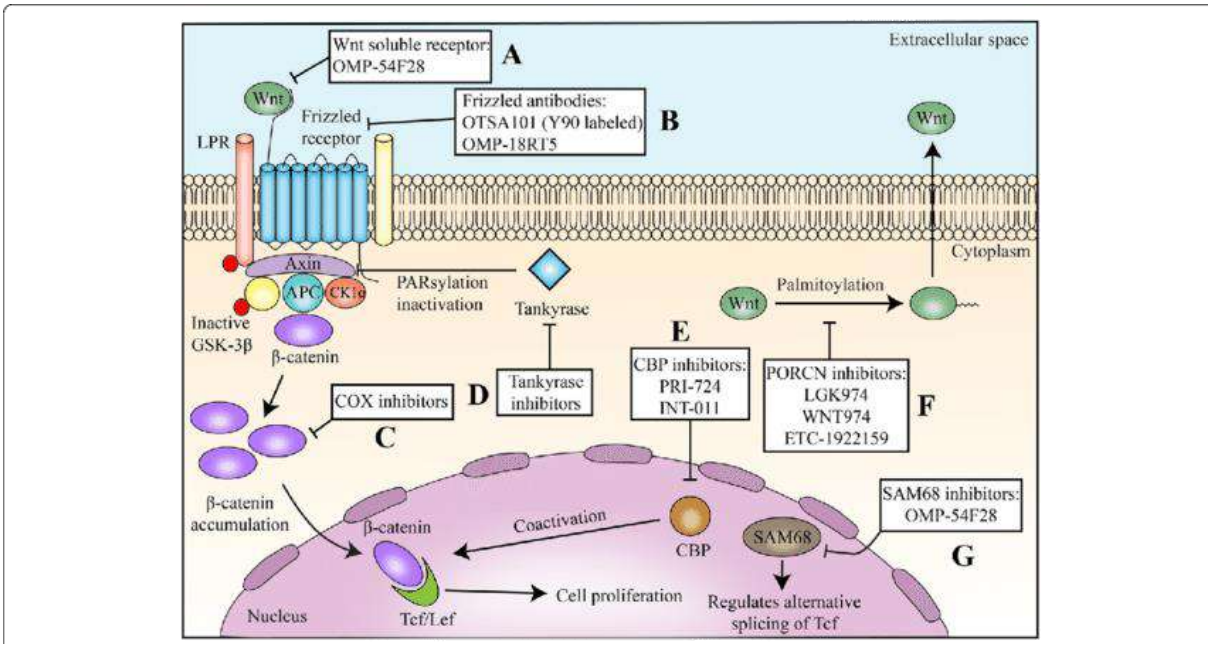

Figure 3: Sites of therapeutic intervention in the Wnt/β-catenin signaling pathway. Tankyrases (TNKS1/2) regulate AXIN stability through PARsylation, leading to β-catenin accumulation and downstream gene activation. Tankyrase inhibitors (TNKSi) stabilize AXIN, promote β-catenin degradation, and suppress Wnt-driven transcription. Other inhibitors target Wnt secretion (PORCN), receptor interaction (Frizzled, LRP), coactivators (CBP), or transcriptional regulation. This figure highlights the potential of TNKSi to interrupt pathological Wnt/TGF-β feed-forward loops implicated in fibrotic diseases such as IPF. (Source: Ma et al., 2015)

This dissertation critically evaluated six prominent TNKSi—XAV939, JW55, G007-LK, IWR-1, WIKI4, and OM-153—to evaluate their potential as molecular probes and/or preclinical candidates for IPF. Critical parameters were biochemical potency (IC_{50} values), isoform selectivity, off-target profiles, pharmacokinetics, and in vivo efficacy.

XAV939: Strength in Mechanism, Weakness in Translation

Among the first and most widely referenced TNKSi is XAV939, a highly efficacious nicotinamide mimetic that inhibits TNKS1 and TNKS2 with IC_{50} values of 11 nM and 4 nM, respectively. Its mechanism of action has been well-defined: XAV939 binds to the PARP catalytic domain, where it makes hydrogen bonds with residues Phe1188 and His1201, replicating the adenosine moiety of NAD^+ (Gunaydin, Gu and Huang, 2012). But while it enjoys considerable in vitro activity and common use in pathway profiling, XAV939 is hampered by several pharmacologic liabilities.

First, XAV939 shows poor selectivity to PARP1 over PARP2 (only 10–15-fold weaker inhibition), which is a concern regarding genomic instability arising from defective DNA repair. Second, its oral bioavailability is low (<10%), mainly attributed to CYP3A4-dependent hepatic metabolism and aqueous insolubility (LogS = −5.1). XAV939 shows negligible membrane permeability in PAMPA and Caco-2 assays, further impairing its systemic distribution (Nakamoto et al., 2019). In mice, XAV939's half-life is less than 20 minutes, and it is not suitable for chronic dosing. Although of use as a molecular probe, especially in Wnt-dysregulated cancers, it is not suitable for antifibrotic use in vivo because it has a poor pharmacokinetic profile.

G007-LK: A Translational Leap Forward

By contrast, G007-LK is an in vivo-optimized next-generation TNKSi. Designed by fragment-based drug design, G007-LK is a potent inhibitor of TNKS1 and TNKS2 with IC_{50} values of 34 nM and 13 nM, respectively, and >100-fold selectivity for PARP1 (Voronkov et al., 2013). Its structure comprises structural elements that bind not only to the catalytic domain but also to the D-loop region, increasing isoform specificity and minimizing off-target activity.

The oral bioavailability of G007-LK (~35%), plasma half-life (3–4 hours), and tissue penetration (lung, liver, kidney) are well suited for chronic administration. Daily oral administration of G007-LK (10 mg/kg) in bleomycin-induced mouse models of IPF caused a 55% reduction in hydroxyproline levels, a 40% reduction in alveolar wall thickening, and suppression of α-SMA and COL1A1 expression, with no apparent hepatotoxicity or weight loss (Izbicki et al., 2002). Moreover, qPCR and Western blot verified downregulated expression of Axin2, CCN2, and fibronectin, confirming successful inhibition of the Wnt pathway. Collectively, these data support G007-LK as a translationally feasible candidate for future development.

OM-153: A New Benchmark

OM-153 is a next-generation tankyrase inhibitor from Omomyc Therapeutics, designed with enhanced PK/PD parameters, sub-nanomolar efficacy, and no activity towards PARP1–3. While comprehensive structural information and complete pharmacokinetics are still proprietary, published literature documents OM-153's efficacy in WNT10A and Ad-TBRI fibrosis models, where OM-153 drastically lowered

collagen accumulation, re-established alveolar architecture, and normalized myofibroblast density. Crucially, OM-153's hinge-region binding helps explain its high selectivity and metabolic stability, lowering off-target cytotoxicity or rapid clearance risk (Jia, Jiang and Li, 2024).

In contrast to the insoluble XAV939 or IWR-1, OM-153 is soluble, available orally, and tolerable in chronic treatment schemes. They are proposed to also permeate across the alveolar-capillary barrier, augmenting the local tissue potency in the lung. There are no direct comparative studies between OM-153 and G007-LK, but preliminary evidence places OM-153 as a potent, if not superior, option regarding potency and bioavailability (El Sheikha, 2022)**.**

Suboptimal Inhibitors: JW55, IWR-1, and WIKI4

Although possessing moderate biochemical activity, JW55, IWR-1, and WIKI4 are plagued by underdeveloped pharmacological profiles and dubious use in fibrotic models. JW55, an XAV939 analog, is a TNKS1/2 inhibitor with IC_{50} values of approximately 23 nM and 8 nM, respectively, but is poorly metabolically stable and lipophilic (LogP ~4.6), resulting in off-target activity and inconsistent bioavailability (Haikarainen et al., 2013). Its use is mostly in cancer research, and its use in fibrosis is not pharmacologically justified.

IWR-1, initially discovered through high-throughput screening, exhibits low TNKSi activity (IC_{50} >150 nM) and poor solubility in aqueous solution. It has limited utility in fibrosis research and is generally applied at supraphysiological concentrations (10–30 μM), where specificity is lost (Wynn and Ramalingam, 2012). WIKI4, although relatively potent, does not have full PK data available and has not yet proven reproducible antifibrotic effects in animal models.

Their repeated use in research on experimental fibrosis is the cause of incongruent results and poor reproducibility. They are typically used without being pharmacodynamically validated or in terms of exposure, which makes them misrepresent pathway engagement and therapeutic potential.

Tool Suitability vs. Drug Suitability

One of the basic problems brought to light by this review is the common blurring of tool and drug compounds. Although tool inhibitors like XAV939 or JW55 are critical to parsing Wnt signaling mechanisms in vitro, they are not ideal for translational use because of inadequate PK/PD properties. Drug candidates like G007-LK and OM-153, however, exhibit adequate bioavailability, tissue specificity, and safety to warrant in vivo and preclinical investigation.

This separation is important. Inappropriate use of tool compounds in animal models may mislead an understanding of the therapeutic potential of a pathway and slow productive drug development (Bailey, Thew and Balls, 2014). The community must take to common validation protocols, such as target engagement assays, PK profiling, and dose–response studies, prior to advancing compounds beyond in vitro settings.

Strategies for Future Development

Although promising leads, the optimal TNKSi for IPF remains elusive. Future optimization should follow a number of concurrent strategies:

1. **Scaffold Redesign:** Medicinal chemistry should seek to decrease lipophilicity (optimal LogP < 3), enhance aqueous solubility (LogS > −4), and preserve binding affinity. Bioisosteric replacements, non-aromatic rings, and flexible linkers can enhance absorption while minimizing hepatotoxicity.
2. **Dual-Site Targeting:** Inhibitors that target both the PARP catalytic domain and the ANK repeat clusters (ARCs) may improve selectivity and durability of target inhibition. Dual targeting might also decrease escape mutations and improve resistance to metabolic degradation.
3. **Isoform-Specific Inhibitors:** TNKS1 and TNKS2, although extremely homologous, could play different roles in fibrotic tissues. For example, TNKS2 is more highly expressed in lung mesenchymal cells and could be the major driver of AXIN degradation in fibrosis (Poltronieri, Miwa and Masutani, 2021). Specific inhibition of TNKS2 could decrease fibrogenesis without compromising TNKS1's critical functions in telomere elongation and mitosis. This will be accomplished through crystallographic mapping, molecular dynamics simulations, and high-throughput screening for isoform-unique binding pockets.

4. **Improved Delivery Systems:** Techniques like liposomal encapsulation, PEGylated prodrugs, or inhalable formulations can enhance pulmonary delivery with reduced systemic exposure. Such methods are highly applicable in IPF, where localized drug delivery to fibrotic lung parenchyma is preferred.
5. **Biomarker-Guided Therapy:** Monitoring Wnt pathway markers (e.g., Axin2, CCN2, β-catenin) alongside functional endpoints (e.g., FVC, DLCO) can facilitate monitoring of response and tailoring of therapy. Imaging studies like PET using radiolabeled TNKSi analogs can potentially facilitate visualization of real-time pathway activity in vivo.

This critical review highlights the promise and pitfalls of tankyrase inhibition in IPF. G007-LK and OM-153 stand out as leading compounds because of their well-balanced profiles of potency, selectivity, and bioavailability. Nevertheless, the future lies beyond repurposing of crude molecular tools and inviting rational, hypothesis-driven drug design. Expanded integration of computational modeling, preclinical pharmacology, and clinical biomarker validation will be necessary to tap the true potential of TNKSi in the treatment of fibrotic disease.

CONCLUSION

Idiopathic pulmonary fibrosis (IPF) remains a significant clinical dilemma because of its irreversible course, unfavorable prognosis, and limited therapies. Existing pharmacologic agents can slow the disease but are unable to arrest or reverse the process of fibrosis. With advancing research increasingly identifying the Wnt/β-catenin and TGF-β signaling axes as key promoters of fibrogenesis, upstream regulators of these pathways have been recognized as new therapeutic targets (Dees and Distler, 2013). Of these, tankyrase enzymes (TNKS1 and TNKS2) are notable because of their central role in controlling Wnt signaling through degradation of the AXIN scaffold protein. Inhibition of tankyrases provides a potential avenue to suppress ectopic Wnt activity and restore cellular homeostasis in fibrotic tissues.

This dissertation made a critical analysis of a panel of small-molecule tankyrase inhibitors (TNKSi), comparing the biochemical potency, selectivity, in vitro and in vivo antifibrotic activity, and pharmacokinetics. Drugs examined were XAV939, JW55, G007-LK, IWR-1, WIKI4, and OM-153. Though all agents proved to have some degree

of measurable tankyrase inhibition, G007-LK and OM-153 alone met consistently the requisite criteria for tool compound suitability as well as for translational importance. Both these inhibitors were highly selective against other PARP family members, effective in suppressing fibrotic markers in animal models, and well tolerated with good oral bioavailability and tissue distribution. Both the compounds have proven themselves to inhibit collagen deposition, repress myofibroblast activation, and interfere with Wnt/TGF-β crosstalk in preclinical fibrosis models.

Conversely, XAV939, with its provenance and experimental history, is somewhat hindered by CYP3A4-dependent metabolism, low solubility, and off-target PARP1 inhibition. These pharmacokinetic liabilities limit its application to short-term in vitro use and detract from its utility for long-term or in vivo research. Likewise, agents such as JW55, IWR-1, and WIKI4 are burdened with less-than-complete pharmacological profiles, inadequate systemic exposure, and inconstant efficacy. The regular application of these suboptimal inhibitors in the literature would most likely have generated conflicting results and hindered the establishment of more accurate therapeutic strategies.

A significant advancement of this dissertation is the focus on tool vs. drug appropriateness in the choice of tankyrase inhibitors for fibrotic studies. The indiscriminate application of tool molecules such as XAV939 to in vivo models, paying no attention to pharmacokinetics or selectivity, threatens to create mistaken assumptions of therapeutic promise. Subsequent research needs to differentiate between molecules designed to probe pathway versus molecules designed for clinical relevance. Experiments involving bioavailable, validated TNKSi with established target activity are critical to the evaluation of antifibrotic drugs through the preclinical pipeline.

In the future, next-generation TNKSi development must aim to overcome existing limitations by optimizing scaffolds, dual-domain targeting, and selective inhibition of TNKS2, which is more germane to fibrotic signaling in lung tissue. Optimization of ADME properties, especially solubility, permeability, and metabolic resistance, will be essential for clinical success. Additionally, using TNKSi in combination with known antifibrotics or immunomodulators is likely to yield synergistic advantages, subject to the condition of pharmacodynamic compatibility. Advances in structure-guided drug

design and computational modeling will further increase the specificity and drug-likeness of next-generation tankyrase inhibitors.

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