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Insights into the Design of Anti-inflammatory Agents Against Arthritis Based on Molecular Docking

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ABSTRACT

Introduction. Arthritis encompasses a heterogeneous group of chronic disorders, notably rheumatoid arthritis (RA) and osteoarthritis (OA), which differ mechanistically yet converge on persistent inflammation and progressive cartilage destruction. These states are mediated by overlapping inflammatory cascades, including cyclooxygenases (COX), lipoxygenases (LOX), nuclear factor-κB (NF-κB), TNF- and IL-6 cytokines, and subsequent JAK/STAT signalling. Although numerous therapeutic strategies have been developed, existing therapies are still hampered by toxicity, slower effects, or an inability to control the disease, due to a failure to counter pathway redundancy in molecular design.

Material and methods. A structured literature survey was conducted using PubMed, Scopus, Web of Science, and Google Scholar to identify peer-reviewed studies on molecular docking-guided anti-inflammatory drug design for arthritis-related targets. The relevant studies were assessed for the suitability of the target, docking process, validation method, key ligand-protein interactions, and correlation with experimental anti-inflammatory potential.

Results. The review critically analyses docking-guided studies in natural products, synthetic small molecules, and biologically inspired inhibitors, but especially aims to determine the rich structure-activity relationship (SAR) principles rather than docking scores. The main lessons involve the role of conserved binding poses, regulated molecular flexibility and target selectivity.

Conclusions. The future lies in combining docking, molecular dynamics, ADMET forecasting, and multi-target design techniques to develop sustainable arthritis therapeutics.

Introduction

Rheumatoid Arthritis (RA) is an intrinsically autoimmune-mediated disease characterised by chronic inflammation of the synovium, aberrant activation of immune cells, and dysregulated cytokine release, ultimately resulting in permanent joint destruction [1]. Conversely, Osteoarthritis (OA) has long been considered a degenerative disease, but accumulating molecular data now show that low-grade chronic inflammation and the loss of normal catabolic signalling are actively involved in the breakdown of cartilage and in subchondral bone remodelling [2]. Regardless of such mechanistic variations, the two conditions are united by the prolonged inflammatory signalling and cartilage erosion. More importantly, decades of therapeutic development have largely followed a single-target inhibition paradigm, which has been ineffective at achieving sustained disease control, highlighting a clash between disease complexity and drug design [3].

The inflammatory responses in arthritis are coordinated by closely interrelated and highly redundant networks of signalling processes, rather than by a linear pathway [4]. The overproduction of prostaglandins and leukotrienes, which are mediators of increased pain intensity, vascular permeability, and immune cell recruitment, occurs via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways [5]. At the same time, nuclear factor kappa B (NF-κB) is a central transcriptional regulator that maintains cytokine cascades and strengthens inflammatory feedback [6]. Immune activation is further propagated through the JAK/STAT pathway, which translates extracellular cytokine signals into long-term transcriptional reprogramming [7]. Notably, inhibition of one node of this network is often overcome by compensatory signalling, which is a critical reason why monotherapies have low efficacy and provides a solid mechanistic explanation for multi-target drug design strategies using molecular docking [8].

Non-steroid anti-inflammatory drugs (NSAIDs) provide symptomatic relief but are limited by gastrointestinal and cardiovascular toxicity [9]. Disease-modifying antirheumatic drugs (DMARDs) are associated with delayed onset and variable responses of patients [10]. Although biologic agents are not only mechanistically advanced but also financially unfavourable, they cause systemic immunosuppression. These incompetencies are not to be discussed as clinical inevitabilities but as failures of molecular design that do not effectively address pathway redundancy and network-scale inflammation.

Nor is molecular docking a predictive oracle, but rather a hypothesis-generating method that allows for rational questioning of ligand-target interactions [11]. This is a critical review of docking-guided studies, aimed at extracting transferable design principles based on relationships among structures, activities and interaction patterns, rather than docking scores in isolation.

Methodology

This review was conducted through a structured literature survey to identify and critically evaluate. Studies that employ molecular docking to discover and optimise anti-inflammatory agents for arthritis and related inflammatory disorders. Relevant literature was retrieved from major scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar. The search strategy employed combinations of keywords, including *arthritis*, *rheumatoid arthritis*, *osteoarthritis*, *molecular docking*, *virtual screening*, *anti-inflammatory agents*, *cyclooxygenase inhibitors*, *lipoxygenase inhibitors*, *dual COX/LOX inhibitors*, *JAK inhibitors*, *NF-κB inhibitors*, *matrix metalloproteinase inhibitors*, and *structure–activity relationship*. Additional relevant studies were identified through manual examination of the reference lists of selected articles and review papers.

The review primarily considered peer-reviewed articles published in English between 2015 and 2026. Preference was given to studies investigating molecular targets directly implicated in arthritis pathogenesis, including cyclooxygenases (COX-1 and COX-2), lipoxygenases (particularly 5-LOX), JAK/STAT signalling components, NF-κB pathway regulators, tumour necrosis factor-alpha (TNF-α), and matrix metalloproteinases (MMPs). Studies were selected for their relevance to anti-inflammatory drug discovery and for including molecular docking as a central component of the investigation. Reports lacking sufficient methodological detail on docking protocols, validation procedures, or biological relevance were excluded from the detailed analysis.

For each selected study, particular attention was paid to the biological target investigated, the docking methodology employed, the validation procedures, the key ligand–protein interactions, and the extent to which computational findings were supported by experimental evidence. Emphasis was placed on studies reporting structure–activity relationships, enzyme inhibition data, cellular anti-inflammatory activity, selectivity profiles, or in vivo pharmacological outcomes. Rather than focusing solely on docking scores, the present review critically examined the conservation of binding poses, the mechanistic plausibility of ligand–target interactions, protocol validation strategies, and the correlation between computational predictions and biological activity (see **Table 1**).

The objective of this review was therefore not merely to summarise published docking studies, but to identify recurring design principles, methodological limitations, and best practices that can improve the application of molecular docking in the rational development of anti-inflammatory agents against arthritis.

Arthritis pathophysiology and major molecular targets

COX enzymes mediate prostaglandin synthesis, and overexpression of COX-2 is a major contributor to inflammatory pain and synovial swelling [15] (see **Figure 1**). Selectivity in COX-2 activity should be used to reduce COX-1-mediated gastrointestinal toxicity [16]. Structural examinations indicate that COX-2 has a larger, more flexible side pocket, which accommodates bulky diaryl heterocycles [17]. The difference between COX-1 and COX-2 is largely due to differences in their structural features resulting from amino acid substitutions at position 523, namely, in COX-1, isoleucine has been replaced with valine in COX-2 resulting in an extension of the size and volume of COX-2's secondary side pocket which allows for the accommodation of bulky structures such as sulfonamide and diaryl heterocyclic scaffolds which cannot fit

Table 1. Best Practices for Docking Validation [12–14].

Validation Step	Recommended Practice	Significance
Redocking	RMSD < 2 Å	Confirms protocol reliability
Positive control	Celecoxib, Zileuton, Licofelone	Benchmark comparison
Protonation state analysis	Physiological pH	Realistic interactions
Water treatment	Conserved waters retained	Improved accuracy
Metal parameterization	Essential for 5-LOX	Accurate coordination
Molecular dynamics	Post-docking validation	Pose stability

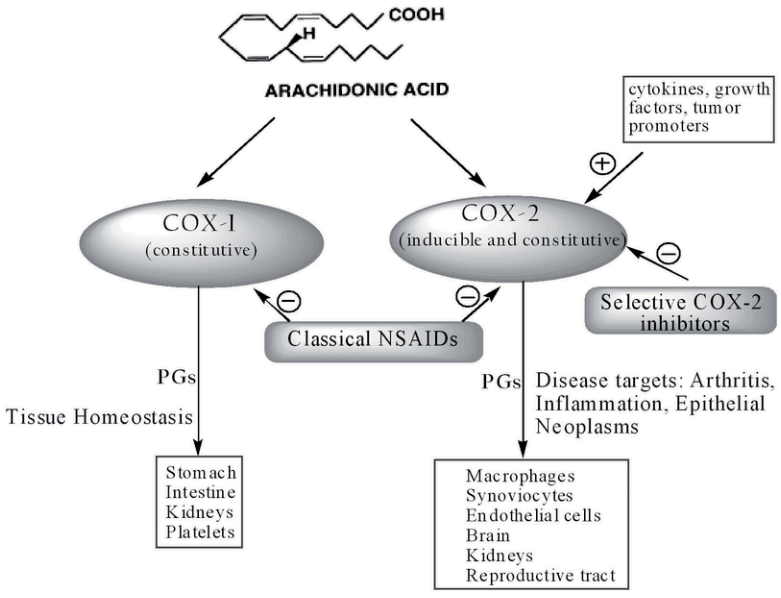

Figure 1. Actions of cyclooxygenases [20].

into the narrower channel of COX-1. Other amino acids, such as Arg513, His90, Ser530, and Tyr385, also influence ligand orientation, hydrogen-bond stability, COX-2 catalytic properties, and COX-2's selective ability to recognise ligands within its active site. For this reason, the optimisation of anti-inflammatory agents through docking-guided approaches requires not only that the agents possess high affinity for the enzyme but also that they maintain geometrically acceptable conformations around these selectivity-determining amino acids [18,19]. Studies on docking consistently indicate that exploiting this pocket is a decisive design property for functional selectivity.

5-LOX facilitates the production of leukotrienes, which play a role in the pathogenesis of chronic inflammation and immune cell infiltration in arthritic joints [21]. Lipooxygenase inhibitors have the potential to reduce leukotriene-mediated inflammation, making them attractive candidates for treating arthritis. In terms of mechanism of action, 5-lipoxygenase (5-LOX) inhibitors can be grouped into four major categories: iron chelators, redox inhibitors, non-redox inhibitors, and 5-lipoxygenase activating protein (FLAP) inhibitors. Iron chelators bind to the non-heme catalytic iron in the active site of 5-LOX, inhibiting leukotriene synthesis by coordinating to the metal. Redox inhibitors convert the active ferric form of 5-LOX to the inactive ferrous form using a redox reaction; however, this class of inhibitor tends to exhibit limited selectivity and is more susceptible to oxidative instability than either iron chelators or non-redox inhibitors. Unlike iron chelators and redox inhibitors, non-redox inhibitors generally occupy hydrophobic regions within the active site without altering the oxidation state of the catalytic iron, making them more specific and pharmacologically stable than either class. FLAP inhibitors act indirectly to inhibit leukotriene synthesis and arachidonic acid transfer by inhibiting FLAP, the membrane-associated protein necessary for efficient FLAP production and FLAP-dependent arachidonic acid transfer. The differences among inhibitor classes are critical considerations when designing anti-inflammatory drugs that employ docking-based screening, because the orientation of the ligands, the coordination of the metal, the hydrophobic occupancy, and the membrane association vary markedly among classes of inhibitors [22,23]. The active site of the enzyme is a catalytic metal core within a hydrophobic channel [24]. Docking-based designs emphasise that successful inhibitors should bind both iron-coordinating motifs and hydrophobic residues, a dual criterion that complicates selectivity and often leads to translation failure [25].

TNF-alpha plays a key role in RA pathogenesis, but blocking it with small molecules is rather complicated [27]. The flat protein-protein interaction interfaces of the cytokine provide few anchoring sites for high-affinity ligands. Docking research tends to overestimate the feasibility of binding, and it reveals the structural incompatibility between conventional small-molecule design and the biophysical properties of cytokine modulation [28] (see **Figure 2**).

IL-6 activates the JAK/STAT pathway to maintain immunity in arthritis. Intervention at ATP-binding sites in JAK kinases is the focus of docking and is primarily potent rather than selective [29]. This bias, however, overwrites allosteric regulatory regions, which can provide safer modulation. This points to a drawback in its design that is more a matter of convenience than a biological consideration for docking (see **Figure 3**).

From a structure-based design standpoint, the dominance of ATP-site focused docking in JAK inhibitors reflects methodological tractability rather than therapeutic optimality [31]. Kinase ATP-binding pockets are highly conserved across the JAK family, which predetermines selectivity and increases the risk of off-target immunosuppression [32]. Regulatory protein protein interfaces and allosteric sites in the JAK/STAT signalling axis have more potential to be selectively modulated. Still, they are structurally dynamic and have not been well represented in fixed docking models. As a result, docking campaigns based on the importance of ATP-site occupancy often yield compounds with high in silico potency but low therapeutic indices [33]. The next generation of docking-based strategies, in turn, will be required to go beyond catalytic-site bias and will need to introduce conformational-state selection or dynamic sampling to match the biological complexity of cytokine signalling with computational design.

NF-κB is a target of great interest but is evasive because it is a convergence point for various inflammatory stimuli [34]. Direct inhibition is uncommon; most approaches target upstream regulators. Docking experiments often neglect

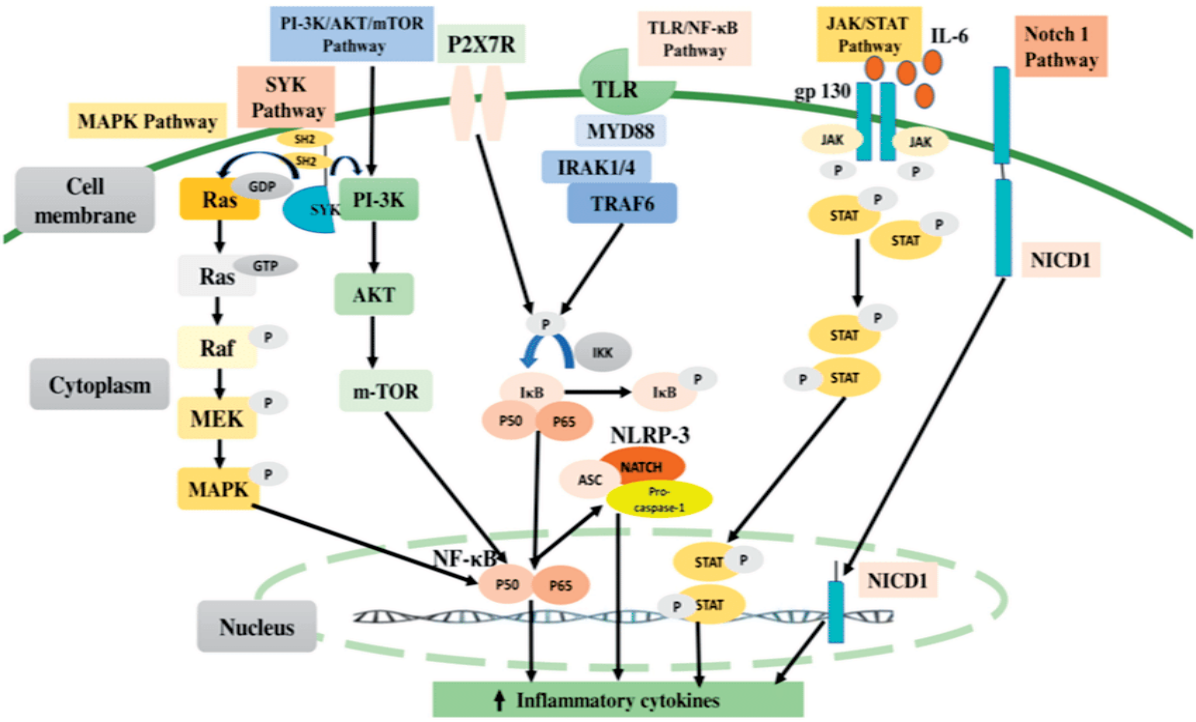

Figure 2. Pathway involved in the pathogenesis of rheumatoid arthritis [26].

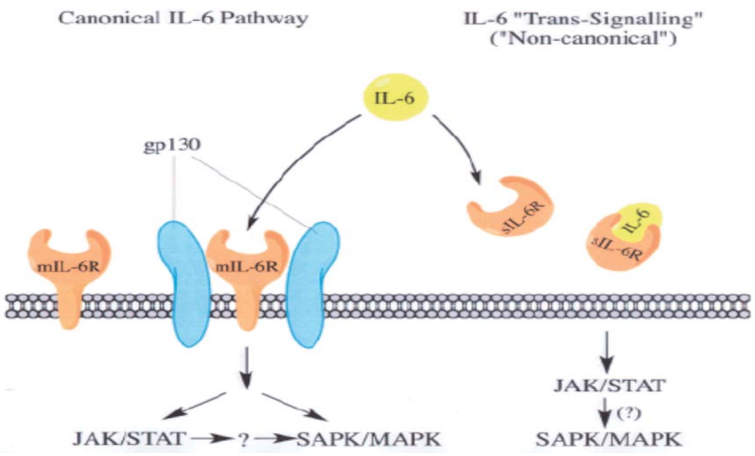

Figure 3. Activation of JAK/STAT by IL-6 [30].

pathway dynamics and temporal activation, reducing intricate signalling behaviour to simple binding events [35]. This oversimplification does not always work into any significant anti-inflammatory activity.

Molecular design of NF-κB has a natural mismatch with a traditional docking paradigm, since its pathology is regulated by signalling kinetics, subcellular localisation, and protein-protein assembly rather than by stable ligand binding to defined pockets [36]. Various docking experiments have been conducted individually on different members of the NF-κB pathway, e.g., IκB kinases or adaptor proteins, without considering the possible activation states of stimuli or transient complexes. Consequently, some compounds predicted to overcome in silico toxicity fail to exhibit significant pathway-inhibitory effects in cells. To achieve effective docking-guided approaches to NF-κB modulation, then, the crucial selection of the target state complementary to dynamic or systems-level modelling is necessary to exclude structurally plausible yet biologically inefficient designs [37].

In RA as well as in OA, MMPs play a significant role in cartilage degradation [38]. Their catalytic zinc ion is an apparent docking site, and as a result, zinc-chelating motifs are commonly used [39]. But this method has yielded general inhibition and intolerable toxicity. The focus of docking-based optimisation is more on interactions between peripheral pockets to enhance selectivity, and on why it is important to move away from highly simplistic approaches based on metal-chelation.

Taken together, these molecular targets fail to act as independent triggers of arthritis but rather as part of a tightly linked inflammatory network [40]. Compensatory signalling occurs through crosstalk between COX/LOX-derived lipid mediators, NF-κB-controlled cytokine cascades, and transcriptional persistence via JAK/STAT Signalling in the event of node inhibition [41]. This reductionist redundancy underlines the relative inefficacy of single-target therapeutic approaches and introduces a fundamental weakness in reductionist drug design approaches [42]. From a molecular design perspective, this network behaviour requires ligands that can achieve multi-target engagement or pathway modulation in a balanced manner. Molecular docking is therefore best used as a systems-informed design strategy rather than a single-target optimisation strategy.

Anti-inflammatory drug design molecular docking

Principles of molecular docking

Molecular docking is essentially a computational approximation of ligand-target recognition and is based on simplifying assumptions that need to be carefully considered [43]. Modern docking algorithms can be categorised into genetic algorithms, force-field algorithms, and empirical scoring algorithms [44]. Such frameworks vary in search effectiveness and scoring principles, but they share a severe weakness: docking scores are not direct proxies for biological activity [45]. The performance of binding affinity predictions is susceptible to force-field parametrisation, protein rigidity assumptions, and solvent neglect [46]. Therefore, docking results ought to be understood in relative order in a chemically coherent sequence as opposed to absolute score values [47]. The implicit supposition that finding differences in marginal scores indicates greater efficacy is an example of methodological misconception rather than actual insight into structure-activity relationships.

Docking strategy in the design of drugs

The docking utility is dependent. The most appropriate docking method is rigid docking, which treats the protein as a fixed entity; it is most appropriate when virtual screening is used in the early stage, where throughput is valued more than precision [48]. Nevertheless, it cannot be used to interpret mechanisms involving ligand-induced conformational change, which greatly limits its utility for mechanistic interpretation. Flexible docking is one way to partially overcome this, as it allows ligand flexibility and restricted side-chain movement, making it more suitable for structure-activity relationship (SAR) optimisation in well-defined scaffolds [49]. Induced-fit docking is a computationally demanding technique, but it can be relevant during late-stage optimisation, when fine-tuning conformational changes can determine selectivity, particularly when docking with enzymes such as COX-2 or the JAK family of kinases [25]. Notably, rigid docking is abused in many arthritis-oriented studies to perform optimisation tasks that yield overconfident and biologically unrealistic conclusions.

Docking results validation and reliability

Validation is vital to docking credibility, and it is also among the least-attended areas of the literature. Redocking of re-crystalised co-crystallised ligands with root-mean-square deviation (RMSD) values better than 2.0 A must be regarded as a minimum, rather than an option [50]. Control ligands are necessary to put binding scores into perspective [51]. More importantly, automated results need to be supplemented by visualisation of binding poses, which has not yet been fully addressed; the absence of chemically plausible interactions remains a prevailing weakness in published docking-based arthritis studies [52].

Docking as a design filter rather than a predictive tool

Molecular docking, as a predictor of biological potency in anti-inflammatory drug discovery for arthritis, should be conceptualised as a qualita-

tive design filter rather than an objective predictor of biological potency [53]. The most justifiable use of it is to filter out structurally implausible chemotypes, rationalise trends in observed structure-activity relationships (SAR), and select against scaffolds with conserved, mechanistically credible binding poses. Docking is especially useful for determining whether a ligand will meet the required geometric and interaction constraints of a target (e.g., side-pocket binding in COX-2 or iron-proximate hydrophobic binding in 5-LOX) [54]. The interpretation of docking results for COX and LOX inhibitors should not rely primarily on binding energy values. For COX-2, meaningful selectivity requires conservation of the ligand pose within the hydrophobic channel and occupation of the COX-2 secondary pocket, preferably supported by comparison with COX-1 docking and known reference inhibitors. For 5-LOX, docking is more complex because inhibitor activity may depend on iron coordination, redox behaviour, hydrophobic channel occupation, and ligand orientation relative to the catalytic metal centre. Therefore, dual COX/5-LOX inhibitors should be discussed not as simple score-maximising compounds, but rather as ligands that require balanced interaction profiles across two mechanistically distinct enzyme systems. Based on their mode of action, 5-lipoxygenase inhibitors can be classified into several classes. The iron-chelating inhibitors target the non-heme iron in the enzyme's active site and bind it, thereby blocking the oxygenation of arachidonic acid. Redox inhibitors affect enzymatic activity by modifying the oxidation state of the active-site iron. On the other hand, the non-redox competitive inhibitors bind reversibly to the active site of 5-LOX without interacting with the iron atom. Some inhibitors act by inhibiting FLAP (5-lipoxygenase-activating protein). The latter approach indirectly affects leukotriene biosynthesis by inhibiting FLAP, a protein required for proper localisation of 5-LOX at the nuclear membrane [55–57] (see **Table 2**).

Nevertheless, critical determinants of activity, such as solvation effects, entropic penalties, protein conformational dynamics, and kinetic residence time, are systematically ignored when docking scores are used as direct surrogates for efficacy [45]. This misuse is the reason behind the common lack of correlation between high-ranking docked compounds and poor experimental performance. In many studies involving docking of anti-inflammatory compounds with COX-2, NF-kB and LOX targets using curcumin and related curcuminoids, there is often a great disparity between experimental anti-inflammatory effects and computed docking interactions, as curcumin and curcuminoids demonstrate highly favourable docking scores due to a large number of hydrogen bonding and aromatic interactions, but show poor anti-inflammatory effects in vitro due to low bioavailability, fast metabolism and high conformational flexibility, none of which are addressed by docking. Similar findings have also been reported in COX-2 inhibitor studies, where compounds with large predicted binding energies did not yield proportional IC_{50} values experimentally due to unrealistic ligand conformations (i.e., the computed ligand position would not occur in real life) or unstable ligand-receptor interaction geometries. Finally, many dual COX/5-LOX inhibitors also exhibit this limitation, since some ligands exhibit excellent docking affinity for the COX-2 target but do not show comparable interaction stability when evaluated against the structurally distinct 5-LOX target; this results in poor experimental multifunctional activity for these ligands. Collectively, these examples demonstrate that docking studies serve a greater purpose in identifying mechanistic binding patterns than they do in predicting absolute biological activity [58, 59].

Table 2. Major Classification of 5-LOX Inhibitors with docking considerations [22, 58].

5-LOX Inhibitor Class	Mechanism	Key Docking Consideration	Example
Iron-chelating inhibitors	Coordinate catalytic Fe^3	Metal parameterisation is essential	Zileuton
Redox inhibitors	Reduce active iron	Oxidation-state dependence	Nordihydroguaiaretic acid
Non-redox inhibitors	Hydrophobic channel occupation	Pose stability important	Revacept-type compounds
FLAP inhibitors	Indirect inhibition	Membrane-associated target considerations	MK-886

To assess how well molecular docking predictions align with actual biological activity, studies that included both docking analyses and real-world tests against COX-2 and/or 5-LOX were examined.

As shown in **Table 3**, successful dual COX-2/5-LOX inhibitors have good docking scores, appropriate active-site interactions, and coordinate iron when needed. They also pass tests for enzyme inhibition and in vivo anti-inflammatory stuff.

Comparatively, using docking to identify pose stability in ligand series, maintain interaction networks, and improve predictability against known biological processes, docking significantly downsamples the chemical search space and informs hypothesis-driven optimisation [66]. In the context of arthritis studies, such a restrained application of docking is necessary to prevent score artefacts and to make the computational results informative rather than distorting the medicinal chemistry decision-making process [67].

The comparative evaluation of both successful and unsuccessful docking-guided research projects provides further evidence that biological relevance relies more heavily on consistency among interactions and on experimental verification, rather than on docking score magnitude alone. In general, successful docking studies will show preserved conserved binding orientations, stable interactions with catalytically important residues, and reasonable agreement with either in vitro or in vivo biological activity. On the other hand, there are many examples of failed docking-driven research programs that prioritise numerical score optimisation (with little to no regard for binding-pose conservation, unrealistic ligand conformations, poor reproducibility of interactions, and no experimental validation of the results). Reports of COX-2 and dual COX/5-LOX compounds indicate that compounds with very favourable docking energies did not produce proportionate levels of inhibitory activity due to deficiencies in the representation of key determinants such as protein flexibility, solvation effects, contributions of entropy, and pharmacokinetic behaviour in the program. The above-mentioned observations reaffirm that the primary role of docking should be as a tool to provide mechanistic or structure-activity relationship information rather than as an isolated predictor of therapeutic efficacy [68–70].

Docking-based anti-inflammatory research has a significant limitation: most studies have

Table 3. Characteristic dual COX-2/5-LOX inhibitors representing correlation with key ligand-target interactions, molecular docking predictions, and experimental anti-inflammatory potential.

Compound	Compound class	Key interacting residues	IC50 values	Link with docking	Reference
Thymol-thiazole hybrid	Dual COX-2/5-LOX inhibitor	Favorable occupancy of both active sites; supported by in vivo anti-inflammatory activity	5-LOX IC_{50} = 1.53 ± 0.02 μM. COX-2; IC_{50} = 0.039 ± 0.001 μM	Strong agreement between docking and biological assays.	[60]
Pyridine-thiazole hybrid	Dual COX-2/5-LOX inhibitor	5-LOX: Lys296, His432, Trp599; COX-2: Arg120, Tyr355, Val523	5-LOX IC_{50} = 0.43 ± 0.02 μM; COX-2 IC_{50} = 0.18 ± 0.01 μM	Excellent docking activity correlation.	[61]
Triazine-thiol derivative	Dual COX-2/5-LOX inhibitor	5-LOX: His367, His372, Asn180 and Gln363; COX-2: Arg106, Met508, Val509	5-LOX IC_{50} = 4.90 ± 0.22 μM; COX-2 IC_{50} = 0.33 ± 0.02 μM	Docking predictions supported experimental selectivity and MD stability.	[62]
Hydroxamic acid derivative	Iron-chelating 5-LOX inhibitor	Ile673, Leu368 and Leu414; Coordination with catalytic Fe^{2}	5-LOX IC_{50} = 0.692 μM; COX-2 IC_{50} = 0.794 μM	Docking magnificently explicated dual inhibition by Fe^{2} coordination.	[63]
Thiazolo-pyrimidine hybrid	Dual COX-2/5-LOX inhibitor	5-LOX: π-sulfur interaction with Met145 and hydrophobic contacts. COX-2: His351	5-LOX IC_{50} = 0.38 ± 0.01 μM. COX-2 IC_{50} = 0.09 ± 0.002 μM	Good relationship with docking, enzyme inhibition and reduction of PGE/LTB	[64]
Benzoquinone/ Hydroquinone derivative	Dual COX-2/5-LOX inhibitor	5-LOX:Tyr142 and Arg138; COX-2: H-bonds with Tyr355 and Arg120	5-LOX IC_{50} = 0.28 ± 0.20 μM; COX-2 IC_{50} = 0.55 ± 0.19 μM	Strong link; potent docking interactions related with potent enzyme inhibition and in vivo activity.	[65]

not adequately integrated experimental biological data with computational data. Despite the lack of a strong correlation among computationally determined IC_{50}s, selectivity indices, cellular anti-inflammatory activity, and the in vivo efficacy of the therapeutic, docking scores and predicted binding affinities often receive undue attention. Generally, the best examples of docking-guided investigations yield a correlation between conserved ligand-binding orientations and biological confirmation (as demonstrated by enzyme inhibition assays, cytokine suppression studies, and animal models of inflammation or arthritis). Therefore, the practical value of molecular docking in the discovery of anti-inflammatory agents is based not only on favourable computational predictions but also on rigorous biological testing and confirmation of target specificity and biological activity [68,70,71].

Docking coupled with machine learning

Molecular docking and machine learning (ML) have become increasingly integrated into computational drug development as a result of recent advances in the fields. By combining these techniques, virtual screening can improve reliability through more accurate predictions. The frequent inaccuracy of docking algorithms, insufficient consideration of protein flexibility, and limited correlation between experimental activity and docking scores have motivated the identification and use of ML models trained on large-scale experimental datasets. By identifying complex nonlinear relationships among structural descriptors, ligand interactions, and biological responses, ML models can help overcome some of the challenges that conventional scoring functions cannot adequately address [72,73].

As an example, the combination of docking and ML is extremely useful for designing multi-target anti-inflammatory drugs for arthritic conditions. The challenges encountered in developing dual COX/5-LOX inhibitor drugs are an example of this. Most instances of dual COX/5-LOX inhibitors have had limited ability to develop dual activity due to docking's inability to accurately predict activity, given the distinct structural forms of both COX and 5-LOX. Utilising ML-assisted workflows in conjunction with docking can improve compound ranking accuracy, identify off-target interactions, optimise compound pharmacokinetic properties, and aid in prioritising compounds with optimal multi-target activity. As such, hybrid docking-ML approaches will serve as powerful methodologies to establish the rational discovery of safer and more selective anti-inflammatory drugs [74,75].

Result and discussion

Anti-inflammatory agents classes explored through docking

Natural product–derived anti-inflammatory compounds

Due to their structural diversity and past pharmacological importance, natural products, such as flavonoids, curcuminoids, and terpenoids, dominate the literature on docking-based anti-inflammatory studies [76]. The interaction with several inflammatory targets is consistently reported by docking, which could justify their polypharmacological behaviour. Nevertheless, such seeming versatility is a two-edged sword. Molecular flexibility and abundance of hydrogen-bonding functionalities, which produce high docking scores, in most cases lead to low target specificity and inefficient pharmacokinetic properties [77]. Additionally, docking studies often overestimate binding affinity by ignoring desolvation penalties and entropic costs [45]. Consequently, a significant number of natural compounds may be computationally attractive yet ineffective at demonstrating meaningful in vitro or in vivo activity, a phenomenon that has led to a recurring optimism trap in the form of natural product studies by docking [78]. Docking studies of various curcumin analogues have found that consistently high COX-2 and NF-κB docking scores are not predictive of low IC_{50} values or strong inhibition of inflammatory cytokine production in cell-based assays, revealing that there remains a significant gap between predictions made using computational docking techniques and biological responses [11].

Synthetic small-molecule anti-inflammatory agents

Synthetic small molecules are the best-defined category of docking-directed anti-inflammatory agents. COX-2-selective inhibitors, and espe-

cially diaryl heterocycles, have consistently reappeared as effective scaffolds because of their ability to utilise the enlarged side pocket of the enzyme whilst retaining drug-like characteristics [79]. The role of hydrophobic anchoring and selective hydrogen bonding in discriminating against COX-2 is consistently supported by docking studies [80]. Likewise, 5-LOX inhibitors reveal that dual engagement with the catalytic iron centre and the surrounding hydrophobic residues is required, but selectivity is difficult to achieve [63]. It is interesting to note that dual COX/LOX inhibitors are examples of rational multi-target design, in which docking provides a structural explanation for balanced pathway manipulation [80]. Several experimentally validated dual COX and 5-LOX inhibitors provide strong evidence for conserved docking orientations of these compounds on both targets, leading to balanced inhibition of the two enzymes, decreased production of inflammatory mediators in macrophage-based assays, and enhanced anti-inflammatory action in various animal models of arthritis [81]. In literature, the superiority of scaffolds with rigidity, controlled polarity, and defined pharmacophores over highly flexible designs has been observed, and chemical restraint, rather than complexity, is the basis of effective docking-guided optimisation.

Biologic-inspired small-molecule inhibitors

Biologically inspired small molecules will seek to mimic the activity of cytokine-targeting biologics while addressing the clinical shortfalls associated with global cytokine suppression, including infection risk, immunogenicity, and incomplete patient response [27]. Solutions based on docking to engineer TNF-alpha mimetics are structurally hindered by the fact that cytokine interfaces are typically flat and lack deep binding pockets [82]. As a result, high-affinity docking, as many have reported, is physically implausible. Conversely, IL-6 and JAK inhibitors have been more successful because they are designed to inhibit enzymatic domains rather than protein-protein interfaces [83]. Nevertheless, ATP-site binding is often overemphasised in docking studies that undervalue the possibility of allosteric modulation [84]. This is indicative of a larger surge in which docking convenience determines the choice of target, rather than the subtlety of overall understanding of cytokine signalling biology.

Docking relationships with structure-activity relationships (SAR)

Rigid pharmacophoric characteristics

Docking is truly useful, however, only when it is brought to a point of interaction logic repeated over a series of scaffolds [85]. High-ranking ligands to COX-2-oriented chemotypes are nearly always of a two-anchor shape: (i) polar recognition at the mouth of the channel (typically involving one of the following residues, Arg120/Tyr355), and (ii) deep hydrophobic packing towards the COX-2 side pocket region that facilitates specificity [86]. This binding rationality is recurrently observed in COX docking studies and structural analyses of diaryl heterocycles. In the case of MMPs, docking-derived information about potency is often an artefact of zinc chelation (hydroxamate/carboxylate-like motifs) since the binding of the metal predominates even in cases where the rest of the ligand has not been optimised [87]. The choice of chelator is less selective than S1 pocket engagement, which is poorly modelled or under-modelled in many papers.

As shown in **Table 4**, effective COX-2 inhibitors consistently engage the secondary side pocket of the protein through interactions with amino acid residues such as Arg513, His90, Gln192, and Val523. The selective inhibitors with the highest indices included rofecoxib analogue 17 (SI = 812.4), cyclic imide analogue 6a (SI = 668.3), and cyclic imide analogues 9 and 18 (SI > 333.3). Notably, their biological activity increased not only due to favourable docking energy but also due to the retention of crucial hydrogen-bond formation and occupancy of the side pocket unique to COX-2. These results confirm that molecular docking should be used primarily to identify biologically significant binding modes and to optimise SAR studies, rather than to predict biological activity.

Trends in the substituent and electronics

Substituent electronics are also important when they stabilise certain types of contacts, not just when they raise docking scores [93]. Electron-withdrawing groups tend to increase the strength of H-bond acceptors (stabilised polar anchors), and electron-donating groups can increase the strength of π-density in aromatic stacking within hydrophobic channels, provided the pose geometry allows [86]. The design rule is

Table 4. Representative COX-2 docking-guided anti-inflammatory drugs and experimental evidence.

Compounds	Docking software	Control ligand	PDB ID	Key residues	IC_{50} COX-1	IC_{50} COX-2	Selectivity index	References
Cyclic imide derivative 9	MOE 2008.10	SC-558S	1CX2	His90, Gln192, Arg513	>50	0.15	>333.3	[88]
Cyclic imide derivative 18	MOE 2008.10	SC-558	1CX2	His90, Gln192, Arg513	>50	0.15	>333.3	[88]
Compound 2d (2,5-dimethoxy derivative)	Glide XP	Celecoxib	5KIR, 3KK6	Arg120, Tyr115, Val116, Val523	51.8	74.1	1.43	[89]
Compound 2e (2,4-dimethoxy derivative)	Glide XP	Celecoxib	5KIR, 3KK6	Arg120, Tyr115, Val116, Val523	45.21	54.7	1.21	[89]
Compound 2f (3,4,5-trimethoxy derivative)	Glide XP	Celecoxib	5KIR, 3KK6	Arg120, Tyr115, Val116, Val523	14.7	53.9	3.67	[89]
Compound 2i (Thiophene derivative)	Glide XP	Celecoxib	5KIR, 3KK6	Arg120, Tyr115, Val116, Val523	67	62.1	0.93	[89]
Imidazole derivative 5b	AutoDock 4.0	Celecoxib	6COX	Arg513, Arg120, Leu359	~81.7	0.71	115	[90]
Cyclic imide derivative 6a	MOE	SC-558	1CX2	His90, Arg513, Gln192, Val523, Tyr385	120.3	0.18	668.3	[91]
Cyclic imide derivative 6b	MOE	SC-558	1CX2	His90, Arg513, Gln192	127.2	0.24	530.0	[91]
Cyclic imide derivative 7a	MOE	SC-558	1CX2	Arg513, Gln192, Val523	101.6	0.28	363.0	[91]
Rofecoxib analogue 17 (azidofuranone)	–	Rofecoxib	1CX2	Arg513, Val523 secondary pocket	159.72	0.196	812.4	[92]
Celecoxib analogue 13 (azidopyrazole)	–	Celecoxib	1CX2	Arg513, COX-2 side pocket	>100	1.55	64.5	[92]

critical, which is that electronics only work when the substituent itself is pre-organised to enter a region rich in residues; otherwise, you are maximising noise because scoring functions are not always good quantitative predictors of affinity.

Aromaticity, linkers and flexibility

The repeated rewards for over-flexible ligands are based on the idea that any of the many conformations can fit, as determined by in silico methods; the reality of entropy and desolvation costs punishes them [67]. Rigidity of linkers (or achieving quasi-rigidity through conjugation) is always accompanied by increased pose consistency among docking runs, and more interpretable SAR. Flexible linkers can be argued in a bridging of pockets (e.g., MMP peripheral subsites) that is only justified when you can prove that there are stable binding modes as opposed to more than one pose of incompatibility [94]. If a series is making progress only by adding rotatable bonds, then your SAR is most likely a forgery.

Stereochemistry and binding orientation

A large proportion of docking-SAR papers on arthritis are structurally irresponsible: stereocenters are treated as fungible, and opposite enantiomers are assumed to bind in the same manner [95]. It is a methodological fault, rather than a small omission. Stereochemistry can reverse the direction of the H-bond, alter the ring tilt angles, and entirely change the occupancy of the side-pocket [96]. Unless specific enantiomer docking and stability are reported in the study, consider SAR claims as tentative at best.

High-low affinity comparison

A defensible docking-SAR comparison achieves three things: (1) higher-ranking ligands maintain the same core pose (not a better score), (2) shows that the best score is due to the addition of interactions, and (3) relies on a control ligand to avoid score inflation. This is important since scoring functions are good predictors of binding modes but poor predictors of binding affinity.

Case study analysis

These case studies are selective. They both show in which context docking works best to inform the design of molecules, and in which context it is false when understood naively. It focuses on transferable design logic rather than numerical performance.

Case 1: COX-2 inhibitors – celecoxib-derived diaryl heterocycles

Celecoxib analogues are reported to invariably establish a conserved binding topology at the COX-2 active site, anchoring at the channel entrance (usually involving Arg120 and Tyr355) and occupying the COX-2-specific pocket to a deep extent [20]. High-affinity analogues maintain this posture on docking runs, and the sulfonamide or sulfone group sticks out in the secondary pocket formed by the Val523 toxication, which is not seen in COX-1 [20]. There is great structural similarity between the two proteins, namely, COX-1 and COX-2. The difference between the two lies in the key regions that are important to the active sites of COX-1 and COX-2. One notable feature is that COX-1 has Ile523 at its active site while COX-2 has Val523, which makes it more flexible. COX-2 also has an additional hydrophobic pocket that accommodates larger bulky compounds, while the binding cavity in COX-1 remains smaller. Both COX-1 and COX-2 have similar functional groups, such as His90 and Ser530, that determine the orientation of substrates and inhibitors. Another residue that plays a crucial role in catalysis in COX-2 and COX-1 is Tyr385. In COX-2, there is less steric hindrance due to the larger hydrophobic pocket and altered arrangement of key residues compared to COX-1. Importantly, ligands with high scoring but not oriented in this manner often cannot be selective despite favourable docking energies [86] (see **Figure 4**). In a successful series of celecoxib-derived compounds, retention of this conserved binding orientation was often linked to higher COX-2 selectivity indices and lower experimental IC_{50} values. In contrast, compounds not demonstrating stable side pocket occupation (and non-proportional biological activity) despite good dock scores [97].

Design lesson: COX-2 selectivity is the result of pose conservation and side-pocket exploitation, rather than the result of the improvement of incremental scores [98].

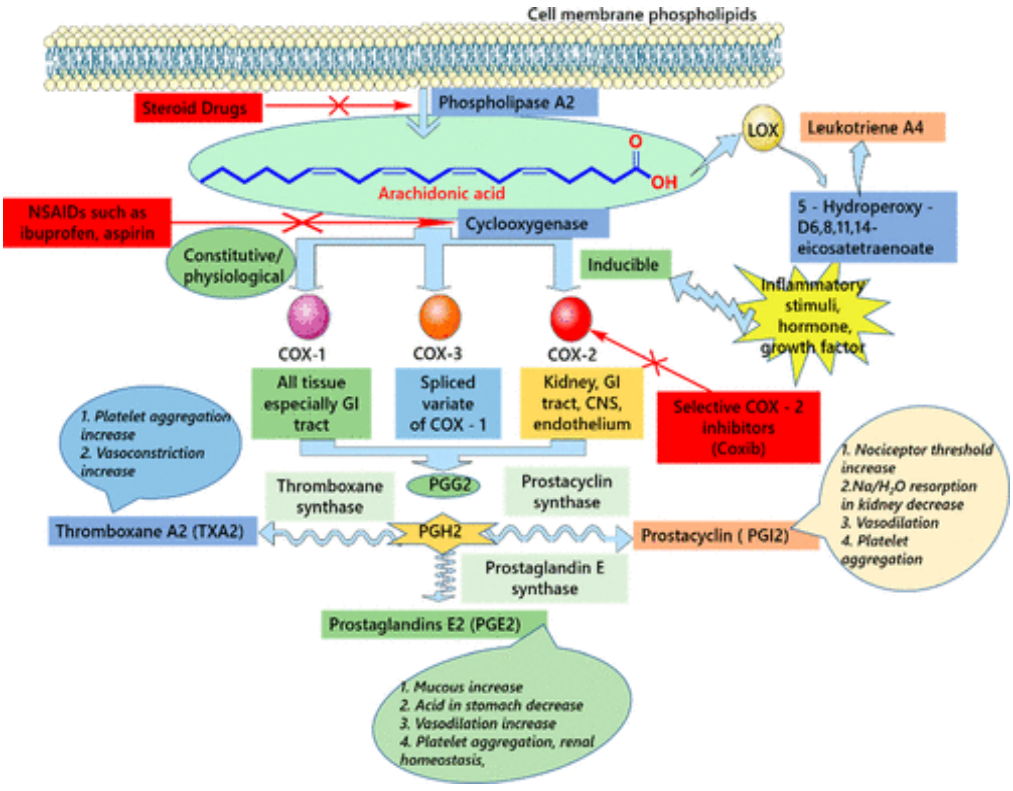

Figure 4. Biochemical pathway of COX inhibitors [99].

Case 2: natural compounds – curcumin and curcuminoid analogues

Curcumin analogues exhibit consistently high docking scores on COX-2, NF-& 0x, and LOX targets, in large part owing to numerous hydrogen-bond donor/acceptor groups and long 0x-systems [100]. The pose analysis, however, frequently reveals several incompatible binding orientations, indicating low conformational commitment [81]. Docking becomes much more predictable, and target-specific interactions can be observed when structural constraints are added (e.g. mono-carbonyl or cyclic curcuminoids). This proves that the main confounder affecting curcumin docking studies is the flexibility rather than affinity [100]. Additional experimental investigations confirmed that most curcumin analogues with good docking results in computer modelling studies have either poor or limited anti-inflammatory activity in cell culture assays and exhibit poor pharmacokinetic properties in vivo. Therefore, the strength of binding alone does not indicate therapeutic benefit when developing new compounds [68].

Design lesson: one should use docking to punish over-flexibility, rather than to reward promiscuous binding.

Case 3: Multi-target dual COX/5-LOX inhibitors

Dual COX/5-LOX inhibitors are among the most obvious achievements of docking-guided anti-inflammatory design [71]. The best scaffolds usually contain a hydrophobic core capable of forming enzyme channels, a polar motif that can interact with COX-2, and the ability to withstand an iron-containing LOX active site [101]. Comparison of docks demonstrates that effective dual inhibitors exhibit the same relative orientations in both targets, rather than maximising their interactions with one target at the expense of the other. Ligands optimised for COX-2 tend to lose engagement with LOX [71]. Studies using enzyme inhibition assays demonstrated that ligands with stable interactions with both targets tended to provide better dual inhibition and more balanced selectivity profiles than compounds optimised solely for COX-2 affinity [81].

Design implication: the efficacy of multi-target systems requires a compromise in interactions at equilibrium, rather than pharmacophore additivity.

Case 4: TNF-a small-molecule mimetics – docking limitations at protein-protein interfaces

The inhibition of TNF-a highlights the limitations of classical docking [102]. Most experiments report high docking scores for small molecules when docked onto TNF-alpha monomers, but do not account for the trimeric interface-based biology of TNF signalling [103]. The small molecule SPD-304 provides an instructive example: the activity of this agent does not arise from tight binding to one of the pockets, but from disruption of the TNF trimer via perturbation of an interface [104]. Systematic docking studies that do not model oligomeric states overestimate feasibility.

Design implication: docking to PPIs should be interface- and assembly-conscious; otherwise, it is structurally deceptive.

Case 5: matrix metalloproteinase (MMP) inhibitors – zinc chelation to selectivity

Initial experiments with MMP docking focused on zinc chelation, yielding high-scoring, non-selective inhibitors [105]. Recent case studies have shown that selective inhibition does not correlate well with metal-binding strength but rather with high interactions in the S1 antibacterial specificity pocket, which differs significantly among [106] MMP isoforms. Comparison of docking models between selective and non-selective inhibitors demonstrates that both chelate zinc successfully; however, only selective compounds may preserve constant peripheral pocket interactions [107].

Design lesson: zinc binding must be made, but not too much; selectivity must be designed at the periphery, not at the catalytic core [108].

In each of them, the same trend can be observed: effective docking-guided design maintains pose identity, manages flexibility, and matches high scores with effectiveness, and failed programs equate high scores with efficacy. The most useful docking is when it discriminates why a ligand is active rather than just first.

Critical limitations

The most common form of failure in docking-based arthritis discovery is score worship – using a numerical result as a proxy of potency [53]. Another commonly noted limitation of docking studies in the literature on anti-inflammatory agents is the lack of proper biological validation

following computational screening. Many of the studies describe computational docking scores and/or interaction maps. Still, they do not relate either to IC_{50} measurements, cytokine suppression assays, selectivity indices, or animal studies of anti-arthritic activity. As a result, the usefulness of the computational predictions is limited in terms of their potential for clinical practice [11]. That is scientifically unacceptable, since docking scoring functions are not predictive of binding energies and may depend on test sets [70]. There is an additional systematic distortion due to protein rigidity: numerous inflammatory targets (COX channels, kinase loops, MMP subsites) are similarly conformationally plastic and cannot be captured by rigid docking, yet the SAR conclusions in papers are still drawn based on a single static pose [109]. Reproducibility is also not good: many engines, grid settings, protonation states, and water treatment can change rank order – frequently unreported [70]. Lastly, such correlations with in vitro activity are often weak due to the lack of explicit solvent, entropy, induced fit, and kinetic effects from docking; thus, top hits can be either permeability-limited or unstable under physiological conditions. Unless redocking, RMSD, controls, and pose sanity checks are reported, one should interpret a study's docking conclusions as a hypothesis rather than evidence [70].

Prospects

The second step is not more docking, and it is superior integration. Machine-learning and deep learning scoring systems can be better at ranking, but only when they are trained on the correct structural/affinity data and tested fairly across targets [110]. The docking must be used together with molecular dynamics (MD) to ensure pose stability and with water-mediated interaction testing to remove non-viable chemotypes in the initial stages, particularly natural-product-like scaffolds, which are vulnerable to false optimism [111]. Design strategies, including multi-target design, will be more rational when pipelines are explicitly designed to optimise the targeted occupancy rather than maximising a single target score [112]. Lastly, the growth of structural biology, particularly Cryo-EM and novel high-quality structures, will reduce reliance on homology models and enhance target-state relevance (active/inactive conformations), which is already a silent but significant source of docking error.

Conclusions

Molecular docking accelerates anti-inflammatory discovery for arthritis only when used as a design instrument, not as a validation shortcut. Its real value is the extraction of interaction rules—which residues anchor binding, which pockets confer selectivity, and which chemical modifications preserve pose stability across a series. The field's persistent weaknesses are also clear: over-interpretation of scores, neglect of protein dynamics, and SAR built on pose drift rather than conserved binding logic. Arthritis biology is networked and redundant; therefore, future therapeutics will need multi-target or pathway-balanced designs, supported by docking validated through controls (RMSD, pose inspection) and integrated with MD and ADMET screening. In short: docking should guide mechanistically honest medicinal chemistry, not replace it.

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