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Design And Synthesis of Dual Action Antioxiodant-Inflammatory of Pde4 Agents for Chronic Disease.

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ABSTRACT

Oxidative stress and inflammation are common features of chronic disorders such asthma, chronic obstructive pulmonary disease (COPD), and inflammatory ailments. A viable therapeutic strategy for the treatment of these intricate disorders is the development and manufacturing of dual-action compounds that target the antioxidant and anti-inflammatory pathways at the same time. Because of its anti-inflammatory properties, phosphodiesterase-4 (PDE-4) inhibitors have showed promise in the treatment of conditions like asthma and COPD. However, when paired with antioxidant qualities, which might lessen the oxidative damage linked to certain illnesses, their effectiveness might be increased. The design and production of new PDE-4 inhibitors with dual-action characteristics that target oxidative stress and inflammation are the main goals of this study. We seek to maximize these compounds' potency, selectivity, and safety profile by utilizing molecular docking studies and structure-activity relationship (SAR) analysis. In vitro tests like DPPH and FRAP will be used to assess the antioxidant activity of the produced compounds, in addition to anti-inflammatory tests including leukocyte migration and cytokine production inhibition. Additionally, enzyme inhibition experiments will be used to verify their PDE-4 inhibitory efficacy. The ultimate objective is to create a therapeutic candidate that offers a safer and more effective treatment alternative by addressing the dual pathology of chronic diseases. Contributing to the creation of innovative, multipurpose therapeutic agents that enhance the treatment of chronic inflammatory illnesses is the goal of this effort.

Keywords: Dual-action agents, antioxidant, anti-inflammatory, PDE-4 inhibitors, chronic diseases, oxidative stress, drug design, synthesis, therapeutic development.

INTRODUCTION

1) Chronic Diseases and their Inflammatory-Oxidative Basis

The term "chronic diseases," also known as "non-communicable diseases" (NCDs), refers to a wide range of persistent conditions, including diabetes mellitus, cardiovascular diseases, autoimmune diseases, neurodegenerative diseases, inflammatory bowel diseases, and chronic respiratory diseases (such as COPD and asthma). These disorders have a common underlying pathophysiological mechanism that is focused on oxidative stress and chronic inflammation, despite their varied clinical manifestations. In addition to aiding in the onset of the disease, this dual mechanism is essential for its progression, complications, and resistance to treatment **[1][2].**

Chronic diseases are often characterized by low-grade, persistent inflammation that involves the long-term activation of immune pathways. Chronic inflammation is maladaptive in contrast to acute inflammation, which is self-limiting and protective. It is distinguished by the persistent presence of activated neutrophils, T cells, and macrophages, all of which release pro-inflammatory cytokines like chemokines, interleukins (e.g., IL-1β, IL-6), and tumor necrosis factor-alpha (TNF-α). These mediators encourage a cycle of tissue damage, healing, and remodeling that eventually causes organs to deteriorate structurally and functionally. For example, chronic vascular inflammation in atherosclerosis encourages the formation of plaque and instability, while chronic joint inflammation in rheumatoid arthritis causes bone loss and cartilage erosion. Another key component of chronic diseases is oxidative stress, which results from an imbalance between the body's antioxidant defense systems and the generation of reactive oxygen species (ROS). Although they are byproducts of regular cellular metabolism, ROS—such as superoxide anion, hydrogen peroxide, and hydroxyl radicals—are significantly elevated in pathological settings. They have the ability to harm proteins, lipids, and nucleic acids within cells,

which can result in necrosis, apoptosis, or changed gene expression. In chronic disease states, ROS are primarily produced by inflammatory cell infiltration, mitochondrial dysfunction, and NADPH oxidase activation. **[3]**

Oxidative stress and inflammation are closely related and reinforce each other rather than existing as separate phenomena. Pro-inflammatory genes are expressed more when ROS activate redox-sensitive transcription factors like AP-1 and NF-κB. In the meantime, by increasing the activity of enzymes such as NADPH oxidase and inducible nitric oxide synthase (iNOS), inflammatory cytokines promote the generation of ROS. This leads to a vicious cycle in which oxidative stress and inflammation reinforce and intensify one another. Such a cycle is evident in the pathology of diseases like diabetes, where hyperglycemia-induced ROS exacerbate insulin resistance and beta-cell dysfunction, or in neurodegenerative diseases where microglial activation and oxidative damage contribute to progressive neuronal loss **[4][5].**

In addition to supporting tissue damage and functional decline, this interconnected inflammatory-oxidative axis makes treatment more difficult because conventional monotherapies might not be enough to break this cycle. Comprehending this dual mechanism offers compelling justification for therapeutic approaches that simultaneously address oxidative stress and inflammation with the goal of stopping or even reversing the molecular progression of disease **[6]**.

2) Role of PDE4 in inflammatory and Oxidative Pathways

In many inflammatory and immune cells, such as macrophages, neutrophils, T lymphocytes, and endothelial cells, PDE4 is an essential regulator of intracellular cyclic AMP (cAMP). When PDE4 activity increases, cAMP is hydrolyzed to AMP, lowering cAMP levels and thereby diminishing signaling through PKA and Epac pathways that normally constrain inflammatory responses. PDE4 inhibition raises cAMP, which inhibits NF-κB-mediated transcription of pro-inflammatory genes such as TNF-α and IL-6. At the same time, PKA-CREB activation increases the production of anti-inflammatory IL-10. This dual modulation reduces both innate and adaptive immune activation across a variety of cell types and changes the immune milieu toward an anti-inflammatory state **[7]**.

PDE4 inhibition affects oxidative stress pathways in addition to cytokine regulation. It has been demonstrated that raising cAMP after PDE4 blockade lowers the production of reactive oxygen species (ROS) by preventing iNOS expression and NADPH oxidase activation in activated macrophages. These effects reduce peroxynitrite and associated oxidative damage by limiting the formation of nitric oxide and superoxide. PDE4 inhibitors such as rolipram also cause antioxidant responses in some cellular and animal models, such as the upregulation of heme-oxygenase-1 (HO-1) through the Nrf2 pathway, which provides cytoprotection in inflammatory oxidative conditions **[8][9][10]**.

PDE4 is a therapeutic target for chronic inflammatory diseases that impact the gastrointestinal, pulmonary, vascular, and dermatological systems, according to preclinical and clinical research. In the treatment of dermatitis, psoriasis, psoriatic arthritis, and COPD, approved inhibitors like roflumilast, apremilast, and crisaborole are effective. Their ability to reduce oxidative damage and suppress cytokine networks through cAMP is closely linked to their anti-inflammatory properties. To reduce side effects like gastrointestinal tolerance problems and to optimize benefit-risk profiles in the treatment of chronic diseases, careful design aiming at PDE4 subtype selectivity (e.g., PDE4B) and alternative delivery (topical or inhaled) is crucial when razing pro-inflammatory and oxidative cascades **[11]**.

3) Rationale for Dual – Action Agents

Chronic conditions like chronic obstructive pulmonary disease (COPD), asthma, neurodegenerative diseases, and metabolic syndromes all have a shared pathogenic basis with persistent inflammation and oxidative stress. The two pathological processes are not independent but are rather overlapping, with reactive oxygen species (ROS) inducing pro-inflammatory pathways via redox-sensitive transcription factors such as NF-κB and AP-1. Consequently, the inflammation worsens oxidative damage by attracting and activating immune cells that produce more ROS. This mutual interaction enters into a cycle of self-perpetuating disease worsening. Monotherapies

against either oxidative stress or inflammation alone have proved to be largely ineffective, which is a testament to the requirement for a therapeutic strategy that modulates both mechanisms in tandem **[12][13][14]**.

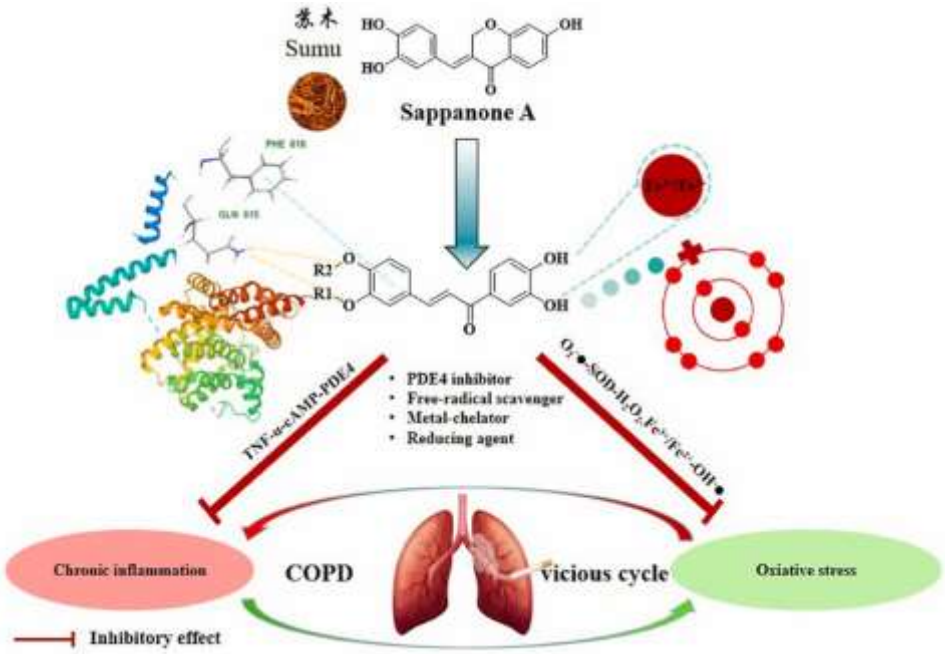
Phosphodiesterase-4 (PDE4) has also drawn major attention because it is involved in the hydrolysis of cyclic AMP (cAMP) in inflammatory and immune cells. An increase in intracellular cAMP by inhibition of PDE4 suppresses the release of pro-inflammatory cytokines like TNF-α, IL-6, and IL-8, and oxidative burst in neutrophils and macrophages. PDE4 inhibitors like roflumilast and apremilast have received clinical approvals to treat COPD and psoriasis, respectively, establishing their anti-inflammatory efficacy. Yet, the therapeutic value of PDE4 inhibitors is generally constrained by dose-limiting side effects such as nausea, vomiting, and weight loss, in addition to the lack of direct antioxidant action. Thus, in spite of their ability to inhibit inflammation, PDE4 inhibitors fail to treat the simultaneous oxidative stress component of chronic diseases**[15]**.

In light of these limitations, the merging of PDE4 inhibition with antioxidant activity into a single molecular species forms a logical approach to addressing the two sides of chronic disease pathology. Antioxidants may scavenge ROS and inhibit pathways driven by oxidative stress, while PDE4 inhibition blunts cytokine-driven inflammation. Dual-action by this strategy can presumably interrupt the feed-back loop between inflammation and oxidative stress more effectively than monofunctional agents. In addition, such bifunctional compounds can decrease the requirement for polypharmacy, thus enhancing patient compliance and lessening side effects of drug interaction**[16][17]**.

Recent medicinal chemistry initiatives have been aimed at developing bifunctional molecules incorporating antioxidant moieties—e.g., polyphenolic structures or catechol groups—with PDE4-inhibitory pharmacophores. A notable example is the construction of sappanone A-derived compounds that were designed by conjugating a catechol-based antioxidant scaffold with a PDE4-inhibitory framework. These compounds showed substantial enhancement of antioxidant activity, reflected in DPPH radical scavenging and reduction of malondialdehyde (MDA), and anti-inflammatory activity, including inhibition of TNF-α secretion and PDE4 inhibition in vitro and in vivo. Dual-functioning such compounds proved more effective in inflammation models than their individual counterparts, either sappanone A or established PDE4 inhibitors, thereby establishing the premise of bifunctional therapeutic design**[18][12]**.

The activity of these dual-action molecules is also consistent with the trends in drug discovery towards multifunctional ligands that can modulate complicated disease networks. Other research has identified dual PDE4/PDE7 inhibitors and hybrid molecules that exhibit improved anti-inflammatory profiles in animal models. Although these do not necessarily have inherent antioxidant activity, they demonstrate the wider scope of multi-targeted molecules in managing chronic diseases. The addition of antioxidant functionality maximizes therapeutic potential, particularly in diseases where oxidative stress is not only an aftermath but an initiator of pathology, as it is in neurodegeneration and pulmonary fibrosis**[19][20]**.

In summary, the synthesis and design of dual-action antioxidant and PDE4-inhibiting agents constitute a promising therapeutic strategy for the treatment of the interlinked functions of oxidative stress and inflammation in chronic diseases. Such agents have the potential to attain greater efficacy by modulating convergent pathogenic processes synergistically. However, challenges do lie ahead, such as pharmacokinetic optimization, off-target toxicity minimization, and selective inhibition of PDE4 without causing emetogenic effects. Ongoing exploration of structure-activity relationships, bioavailability, and tissue-targeting delivery will be essential to the translation of these lead compounds into effective clinical therapies**[21][22]**.



BODY

1) Phosphodiesterase-4 (PDE4): Structure and Biological Role

Phosphodiesterase-4 (PDE4) is a family of phosphodiesterase enzymes that hydrolyses cyclic adenosine monophosphate (cAMP) in a specific manner, an important second messenger in signal transduction pathways. PDE4 is important in maintaining intracellular cAMP levels and hence a range of physiological and pathological processes.

1.1) PDE4 Isoforms and Expression

PDE4 consists of four genes—PDE4A, PDE4B, PDE4C, and PDE4D—each coding for several splice variants (e.g. 7 isoforms of PDE4A, 4 of PDE4B, 7 of PDE4C and 12 of PDE4D) by alternative promoters and exons. Isoforms fall into long (with UCR-1 and UCR-2), short (without UCR-1), and super-short (without both).

They all contain a strongly conserved catalytic region (~330 aa, Val357–Ser686 in PDE4A) with the crucial residues (His, Asp, Gln, Phe) and metal-binding HD motif required for cAMP hydrolysis.

Tissue-specific expression patterns: PDE4A, B, and D are highly expressed in inflammatory and immune cells (e.g., monocytes, neutrophils, T-cells, epithelial and endothelial cells), whereas PDE4C is low or undetectable in such cells.

In disease conditions, PDE4A4 is increased in macrophages in the lungs of COPD patients, and in ulcerative colitis models PDE4D > PDE4A, PDE4B, PDE4C in colon tissue**[23]**.

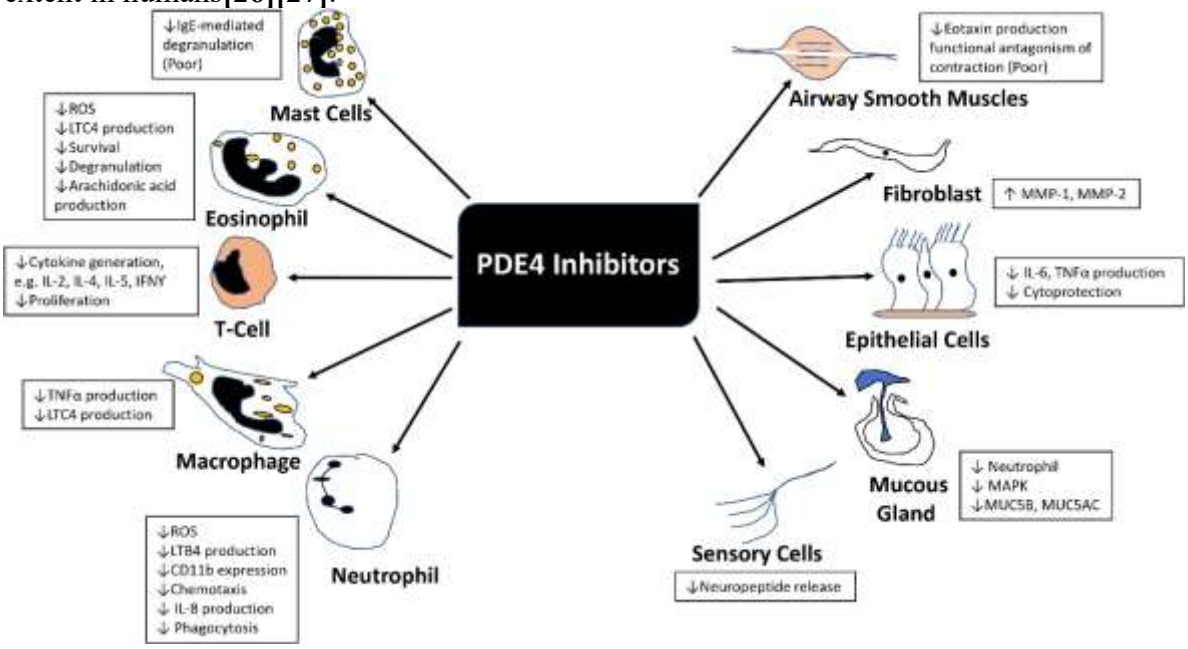
1.2) PDE4 Structure and Catalytic Domain

While more compact in this context, all PDE4 isoforms have a conserved catalytic domain toward the C-terminus that is responsible for hydrolyzing cAMP and must be coordinated by Mg^{2+}/Zn^{2+} through the HD motif. The N-terminal regulatory domain varies by isoform class: UCR1 and UCR2 have regulatory function in long versions, but these can be eliminated in short forms, affecting phosphorylation state, subcellular localization, and protein interactions**[24]**.

1.3) PDE4 in Inflammation and Oxidative Stress

PDE4 regulates intracellular cAMP critically, which goes on to affect inflammatory signaling. PDE4 activity decreases cAMP, which facilitates release of pro-inflammatory cytokines like TNF-α, IL-1β, IL-6 in macrophages, dendritic cells, T-cells, etc.PDE4B expression is LPS/TLR4 stimulus inducible, particularly in microglia, monocytes and neutrophils, and hence plays a crucial role in innate immune reactions and neuro-inflammation; PDE4D expression is relatively unresponsive to TLR activation**[25]**.

Experimental models (e.g., A549 lung epithelial cells, LPS-stimulated macrophages) exhibit upregulation of PDE4A and PDE4B promotes inflammatory signaling through mediators such as p38-MAPK and NF-κB, whereas PDE4 inhibitors recover cAMP levels and inhibit these mediators. Breakdown of cAMP also increases oxidative stress; PDE4 inhibition diminishes the generation of reactive oxygen species and ER stress, ensuring cytoprotection through cAMP-AMPK pathways, as evident in murine NAFLD but to a lesser extent in humans**[26][27]**.

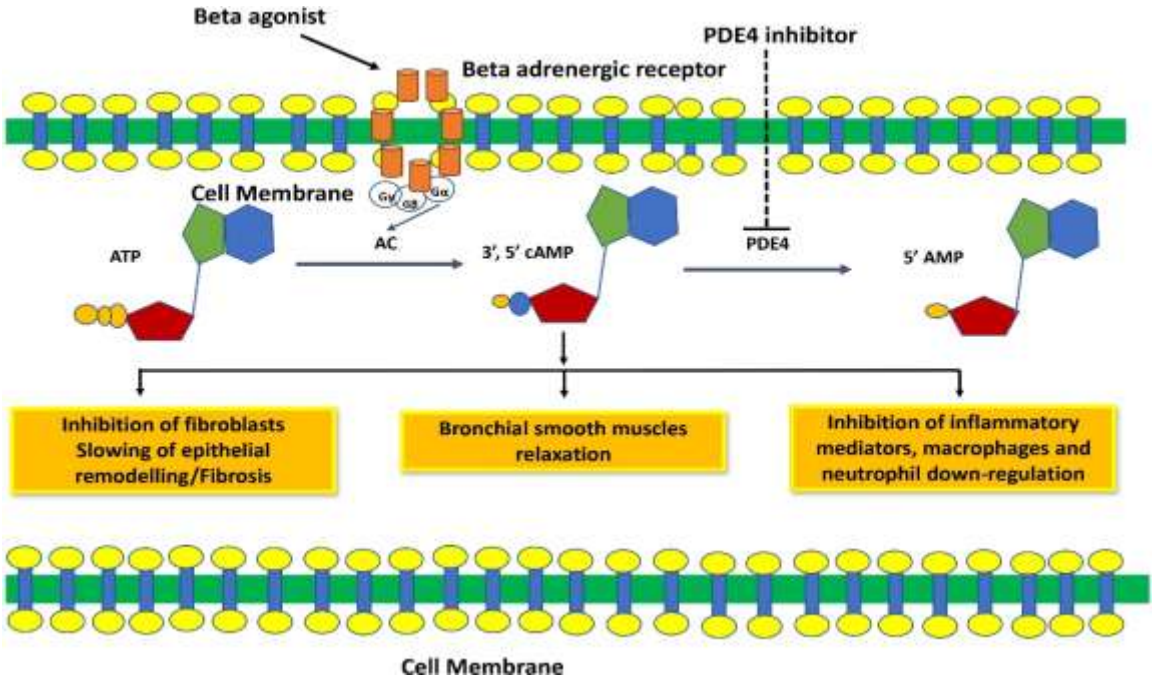


1.4) Therapeutic Potential of PDE4 Inhibitors

PDE4 inhibitors inhibit cAMP degradation, increase intracellular cAMP, and stimulate downstream targets like PKA and EPAC1/2. This results in inhibition of NF-κB, JAK-STAT, MAPK and other inflammatory cascades, decreases cytokine release, and enhances barrier function through stimulation of CREB and Rap1/SOCS3 in models such as ulcerative colitis and synovial inflammation.

Clinically available agents include roflumilast for treatment of exacerbations of COPD, apremilast for psoriatic disease and psoriasis, and crisaborole (PDE4B-targeted) for atopic dermatitis.First-generation rolipram, although efficient in research, has limited therapeutic window owing to emetic and GI side effects**[28][29][30]**.

To enhance tolerability and efficacy, second-generation inhibitors with subtype selectivity (e.g. PDE4B-selective nerandomilast; PDE4D modulators such as zatolmilast; PDE4B/D inhibitors like orismilast and PF-07038124) are undergoing late-stage clinical trials for disorders such as idiopathic pulmonary fibrosis, fragile X syndrome cognitive impairment, psoriasis, hidradenitis suppurativa and atopic dermatitis**[31]**.



2) Antioxidant and Anti-inflammatory agents: Mechanism of action

2.1) Reactive Oxygen Species (ROS) and Cellular Damage

Reactive oxygen species (ROS)—superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), singlet oxygen ($^1O_2$), and others—are produced as byproducts of cellular metabolism (e.g., mitochondrial electron transport) or activation of inflammatory cells. Endogenous protective mechanisms like superoxide dismutase (SOD), catalase, glutathione, and peroxiredoxins detoxify ROS efficiently under physiological conditions. But when ROS formation exceeds antioxidant capacity, oxidative damage results: DNA and RNA strand breaks, modification of bases; lipid peroxidation of cellular membranes; protein oxidation and inhibition of enzymes are typical consequences. These molecular damages may directly induce inflammation and cell death, leading to such chronic diseases as atherosclerosis, neurodegeneration, diabetes, cancer, and arthritis**[32]**.

2.2) Anti-inflammatory Pathways (COX, NF-κB, Cytokines)

Cyclooxygenase (COX)

COX enzymes (constitutive COX-1 and inducible COX-2) catalyze arachidonic acid conversion to prostaglandins. Inflammation leads to upregulation of COX-2 expression, which makes a contribution to the production of prostaglandin E_2 and inflammatory swelling/pain.

NSAIDs block the activity of COX, thus inhibiting prostaglandin-induced inflammation but potentially with side effects**[33]**.

NF-κB Signaling

NF-κB is a quick-acting transcription factor induced by stimuli like TNF-α, IL-1β, LPS, and ROS. When activated through IκB kinase (IKK), NF-κB migrates to the nucleus and promotes pro-inflammatory gene expression—such as TNF-α, IL-1β, IL-6, chemokines, COX-2 and iNOS. Most antioxidant phytochemicals suppress NF-κB signaling, thus reducing inflammatory cytokines and mediators**[34]**.

Cytokines (TNF-α, IL-1β, IL-6)

Critical cytokines TNF-α, IL-1β and IL-6 induce inflammation through leukocyte recruitment, expression of adhesion molecules and metalloproteases, and the activation of downstream effectors such as NF-κB and AP-1. Failure of regulation of these cytokines is responsible for chronic inflammatory and autoimmune conditions (e.g. RA, SLE, MS)**[35]**.

2.3) Mechanisms of Antioxidant & Anti-inflammatory Agents

Antioxidants directly scavenge ROS (e.g. vitamins C/E, glutathione), increase endogenous enzyme defenses (SOD, catalase, HO-1, NQO1) through Nrf2 activation, and normalize redox balance to avert ROS-mediated amplification of inflammatory signals. The Keap1-Nrf2 pathway is essential: stress favors dissociation of Nrf2, which translocates to the nucleus and induces transcription of antioxidant response element (ARE) genes (e.g. HO-1, GCLC/M, NQO1, SRXN1). At the same time, numerous phytochemicals (e.g. baicalein, quercetin, celastrol, myricetin) suppress NF-κB and MAPKs, lower COX-2, iNOS, cytokine levels and inflammasome activation**[34][36]**.

2.4) Synergistic Effects in Dual-Targeted (Antioxidant + Anti-inflammatory) Therapies

Synergistic effectiveness is observed in combination therapies that act on both oxidative stress and inflammation. Ceria nanoparticle + TLR-4 inhibitor resatorvid co-decorated nanocarriers (Ox-CS/CeRT), for example, had a marked decrease in ROS and pro-inflammatory cytokine secretion in acute lung injury models and performed better than single agents. Similarly, CeO_2 with quercetin in mesoporous silica nano-platforms (CeO_2/Que@$mSiO_2$) exhibited strong ROS scavenging and inhibited IL-1β, IL-6, TNF-α better than quercetin alone. Phytochemical mixtures—like cereal-derived extracts or polyphenol blends—have greater antioxidant and anti-inflammatory activities than their component parts, possibly because they have superior bioavailability, combined signaling modulation, and interaction with gut microbiome. Medicinal chemistry research is also giving rise to compounds engineered to stimulate Nrf2 concurrently with inhibiting NF-κB/COX pathways, providing multi-targeted therapeutics for multifaceted disease**[37].**

3) Design Strategies for Dual PDE4 Antioxidant/ Anti-inflammatory Agents

3.1) Hybrid Molecule Concept

The hybrid molecule approach is to combine antioxidant and PDE4-inhibitory pharmacophores into one bifunctional compound. One good example is the sappanone A-based design, which is a natural PDE4 inhibitor with inherent antioxidant property. In a study, scientists combined the catechol antioxidant unit of sappanone A with the catechol ether template shared by PDE4 inhibitors such as rolipram, roflumilast, and apremilast. This novel hybridization provided 27 hybrids bearing both PDE4 inhibition and radical-scavenging activity, with several performing better than edaravone in vitro and decreasing TNF-α and MDA generation in cell and animal models**[38]**.

In the same manner, dual-target ligand design strategies are illustrated in the design of sEH/PDE4 dual inhibitors, wherein most pharmacophore features of a soluble epoxide hydrolase inhibitor (GSK 2256294) were combined with rolipram's PDE4 moiety to enhance dual bioactivity within a single molecule**[39].**

3.2) Pharmacophore Modeling

Pharmacophore modeling of PDE4 inhibitors has been widely employed to direct hybrid design. Five-feature pharmacophore hypothesis (e.g., hydrophobic/aromatic rings, hydrogen-bond donors/acceptors) was built from active PDE4B inhibitor training sets, which resulted in high predictive accuracy ($R^2 \approx 0.918$, $Q^2 \approx 0.852$). These models assisted in the identification of key features—like aromatic interactions and H-bonding—any hybrid molecule is required to maintain to enable PDE4 inhibitory activity**[40]**.

Pharmacophore modeling of such known inhibitors as rolipram analogs identified essential attributes (e.g. dialkoxyphenyl aromatic rings) that guide how an antioxidant moiety (e.g. catechol) may be integrated spatially without interfering with PDE4 binding**[41]**.

3.3) In Silico Drug Design

In silico methods—docking, molecular dynamics (MD), free energy calculations, and virtual screening—are pivotal in dual-agent design. For example, food polyphenols curcumin, resveratrol, 6-gingerol, and capsaicin were computationally screened as prospective PDE4D inhibitors. Curcumin had the most potent binding (electrostatic and hydrogen-bonding-dominated), propounding that similar natural antioxidant backbones can serve as PDE4 ligands as well**[42]**.

In the sappanone A derivatives example, computationally guided docking and radical scavenging assays established retention of both PDE4 inhibitory and antioxidant activities to inform synthesis towards leading candidates such as compound "6o," which also exhibited favorable ADME and in vivo anti-TNF-α activity**[38]**. Further in silico design involves virtual screening of large chemical libraries against cavity-derived pharmacophore models and docking (e.g., PDE4 model of PDB ID: 7W4X), and subsequent MD simulation and MM-GBSA free energy calculation to filter lead hits with the best binding and ADMET profiles**[43].**

4) Synthesis Approaches to Dual-Acting Agents

4.1) Common Chemical Scaffolds Used

Scientists frequently use chemical scaffolds which naturally facilitate both antioxidant and anti-inflammatory activities. A noteworthy illustration involves trolox and other phenolic acids conjugated with cysteamine or L-cysteine ethyl ester to produce amide and disulfide analogs. Such compounds demonstrated radical-scavenging and lipid-peroxidation inhibition 17-fold superior to parent acids and inhibited acute inflammation by as much as 87%, in addition to providing cytoprotective and hypolipidemic benefits**[44]**.

A second scaffolding class includes 8-aminopurine-2,6-dione derivatives designed as pan-PDE inhibitors. The compounds possessed strong antioxidant and anti-inflammatory activity in various PDE isoforms and demonstrated robust efficacy in models of inflammation and fibrosis of asthma**[45]**.
In addition, new imidazo[2,1-b]thiazole-pyrazoline derivatives offered dual activity, exhibiting similar anti-inflammatory activity to diclofenac but better antioxidant activity than vitamin C in in vitro tests**[46]**.

4.2) Stepwise Synthesis Strategies

A traditional synthetic approach uses co-prodrug design, in which an NSAID is chemically attached to an antioxidant moiety through esterification reactions. Dexibuprofen, for instance, was attached to antioxidant-derived chloroacetyl intermediates (3a–c) through a two-step process—initial preparation of chloroacetyl derivatives through acylation followed by SN2-type coupling with dexibuprofen using triethylamine and KI—to produce co-prodrugs (5a–c), which were subsequently purified and characterized**[47]**.

Concurrently, amide linkages have been utilised: trolox-type antioxidants were amidated using cysteamine or cysteine ethyl ester to prepare hybrid compounds in a controlled fashion. Certain disulfide derivatives have also been prepared but had inferior comparable bioactivity, indicating the role of certain functional groups and permeability in affecting biological results**[44]**.

4.3) Recent Advances and Case Studies

Trolox-Based Amides

These scaffolds were found to possess excellent antioxidant and anti-inflammatory activity, including in vivo protective effects against oxidative liver injury and hypolipidemia**[44]**.

Pan-PDE Dual Inhibitors

The 8-aminopurine-2,6-dione derivatives (compounds 32–35 and 38) of our studies exhibited promising dual anti-inflammatory and antioxidant activity, with broad-spectrum inhibitory effects on PDE1, PDE3, PDE4, PDE7, and PDE8, and effectiveness in human bronchial epithelial cells from asthmatics when exposed to profibrotic cytokines**[45]**.

Imidazo-Thiazole Pyrazolines

These hybrid derivatives exhibited antioxidant and anti-inflammatory activity in vitro, with many of the analogs being equal to or more potent than reference drugs like diclofenac and vitamin C**[46]**.
These investigations highlight the strategic incorporation of two functionalities in one scaffold, frequently via modular or reciprocal prodrug strategies, providing a course towards more efficient and safer therapeutics in oxidative-inflammatory conditions.

5) Biological Evaluation of Dual-Action Molecules

5.1) In Vitro Assays (PDE4 Inhibition, Antioxidant Capacity)

Dual-action compounds are initially evaluated in vitro for PDE4 inhibition and at the same time for the presence of antioxidant activity. For instance, sappanone A derivatives were evaluated for PDE4 inhibitory activity and free radical scavenging ability using the DPPH assay. Some compounds had better PDE4 inhibition compared to the parent compound, with enhanced antioxidant activity compared to that of edaravone in vitro**[38]**.

Other reports comparing dual inhibitors, for example, PDE4 and p38α MAPK inhibition, utilized human peripheral blood leukocytes to assess cytokine (e.g. TNF-α) release suppression and reactive oxygen species. These molecules exhibited higher potency than traditional single-target inhibitors**[48]**.

5.2) In Vivo Models for Chronic Disease

Dual-acting agents are also subjected to testing in animal models to evaluate their therapeutic effectiveness in inflammatory and chronic diseases. For example, a dual sEH/PDE4 inhibitor, MPPA, reached blood levels in rats higher than the IC_{50} for the two targets and exhibited analgesic activity that was commensurate with its pharmacokinetic properties**[49]**.

Dual-acting agents are also evaluated in animal models for their therapeutic activity in inflammatory and chronic diseases. An example is a dual sEH/PDE4 inhibitor, MPPA, that reached blood levels in rats above the IC_{50} for both targets and had analgesic efficacy corresponding to its pharmacokinetic profile**[50]**.

5.3) Pharmacokinetics and Safety Profiles

Extensive evaluation involves pharmacokinetic (PK) profiling and safety evaluations. For compound 36 (the dual PDE4/7 inhibitor), PK experiments in two rat strains (Wistar and Lewis) after IV and IP dosing showed significant parameters: rate constants of absorption (0.103 and 0.172 min^{-1}), volumes of distribution (~4.5 L/kg), rate constants of elimination (0.033 and 0.014 min^{-1}), and elimination half-lives of ~21 min (Wistar) and ~49.5 min (Lewis) with absolute bioavailability of 0.42 and 0.11, respectively**[50]**.

Concurrently, MPPA (dual sEH/PDE4 inhibitor) had quick absorption but also quick elimination. At a 3 mg/kg dose in rats, plasma levels exceeded IC_{50} levels for both targets but decreased markedly within 4 hours**[49]**.

Safety considerations reported that subsequent versions must have enhanced metabolic stability to maintain efficacy and reduce possible side effects of PDE inhibition**[49]**.

6) Current Challenges and Limitations

6.1) Selectivity and Off-Target Effects

Dual-acting agents tend to couple different pharmacophores into one molecule, which can incidentally enhance off-target binding. Such nonspecific binding is problematic since nonspecific binding to antitargets—such as the hERG channel or 5-HT_2B receptor—can lead to cardiotoxicity or other detrimental side effects.

In addition, chimeric-type dual functional molecules are typically bulky and of high structural complexity (typically >700 Da), which makes selective targeting difficult and increases the potential for binding with unwanted biological targets.

6.2) Bioavailability and Metabolic Stability

Bioavailability Challenges

A recurring problem is the low bioavailability of complex molecules through low solubility, permeability, and extensive first-pass metabolism. These are particularly significant problems for Biopharmaceutics Classification System (BCS) Class II or IV compounds, which tend to be poorly absorbed orally**[51]**.

Many approaches, such as solid dispersions, nanonization, lipid-based dosage forms (such as SEDDS), and prodrug use, have been used to improve solubility and absorption—without any guarantee of success**[52]**

Metabolic Stability Pitfalls.
Just as troublesome is metabolic stability. Most new dual-acting candidates have rapid biotransformation, resulting in short half-lives and inconsistent exposure. Structural manipulations or formulation approaches—such as deuterium inclusion, cyclization, or isosteric replacements—may improve metabolic soft spots.

Yet between-individual variation and constraints on in vitro-to-in vivo extrapolation make metabolic profiling all the more challenging**[53]**

6.3) Regulatory and Clinical Development Issues

From a regulatory perspective, dual-acting agents are subjected to greater barriers: sponsors have to present extensive data for both mechanisms of action, safety, drug-drug interactions, and metabolism routes, greatly increasing development complexity, timelines, and expenses**[54]**.
Regulatory agencies also closely scrutinize off-target safety and long-term risk—particularly where dual activity can span over known risky targets (e.g., COX-2 inhibitors and cardiovascular risk)**[55]**.
Additionally, high molecular weight and chemical complexity of the kind present in dual-function or chimeric compounds may strain drug-likeness and oral bioavailability requirements, creating concerns during review and approval.

7) Future Perspectives and Emerging Trends

7.1) Novel Delivery Systems (Nanocarriers)

Nanotechnology is revolutionizing the delivery of dual-acting therapies. For example, magnetic nanoparticles prepared through plant-mediated approaches—such as $CrFe_2O_4$ nanoparticles encapsulated in rosmarinic acid—were found to increase antioxidant and anti-inflammatory activity coupled with lowering cytotoxicity in vitro (e.g., lower TNF-α, IL-6, IL-1β in LPS-induced macrophages)**[56]**.

In a second example, nanostructured lipid carriers that were co-loaded with the NSAID indomethacin and the phytochemical celastrol were formulated into a dual-delivery transdermal system. This strategy synergistically addressed pain and inflammation in rodent models, without observed renal or reproductive toxicity**[57]**.

These strategies—ranging from magnetic nanoparticles to dual-drug lipid systems—demonstrate the ways in which sophisticated nanocarriers can facilitate targeted delivery, enhance therapeutic indices, and facilitate controlled release.

7.2) Personalised Medicine and Targeted Therapy

Drug delivery advancements increasingly facilitate tailored and targeted therapeutic applications. For instance, mesoporous silica nanoparticles (MSNs) are functionalized with targeting ligands (hyaluronic acid, biotin, galactosylated chitosan) to achieve stimuli-responsive, site-specific drug release in models of colorectal cancer, with enhanced stability and targeted uptake in vivo**[58]**.

These nanocarriers are engineered to take advantage of disease-specific molecular targets, enabling precision-based delivery. Not yet targeted toward dual-acting agents, such platforms provide encouraging paradigms for designing personalized antioxidant and anti-inflammatory therapies with spatial and temporal control**.**

7.3) Combination with Other Therapeutic Modalities

The trend of achieving synergistic therapeutic outcomes, especially in the integration of dual-acting agents with other treatment modalities, is an emerging trend. In rheumatoid arthritis, researchers have developed hydrogel-

nanoparticle composite systems co-delivering conventional small-molecule drugs, such as NSAIDs and DMARDs; nucleic acids; enzymes; or enzyme-like nanozymes to reduce inflammation; enable imaging; and trigger responsive therapy-all in one platform**[59][60]**.

Additionally, co-delivery formulations of antioxidants such as vitamin C derivatives (e.g., L-ascorbate palmitate) with phytochemicals like triptolide have been designed to enhance solubility, lower systemic toxicity, and enhance therapeutic activity in arthritis models**[57]**.

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