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Azoxymethane in Experimental Pharmacology: Molecular Mechanisms, Colorectal Carcinogenesis and Therapeutic Research Applications

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ABSTRACT

Azoxymethane (AOM) is a synthetic alkylating procarcinogen widely employed in experimental pharmacology, toxicology and molecular oncology for investigating colorectal carcinogenesis. Although it does not possess an established therapeutic indication, its pharmacological and toxicological properties provide an important experimental framework for understanding chemical carcinogenesis and evaluating potential chemopreventive and anticancer interventions. Following metabolic activation, principally through cytochrome P450-dependent pathways, AOM generates methylating intermediates capable of producing DNA adducts, including O^6-methylguanine. These alterations contribute to mutagenesis, epithelial dysregulation and colorectal tumour initiation. The combination of AOM with dextran sodium sulphate (DSS) has further established a reproducible experimental model of inflammation-associated colorectal cancer. This review examines the historical development, chemical characteristics, pharmacokinetic considerations, metabolic bioactivation, molecular mechanisms, cellular responses and toxicological profile of AOM. Particular attention is given to DNA damage, DNA repair mechanisms, KRAS and Wnt/β-catenin signalling, NF-κB activation, oxidative stress, NLRP3 inflammasome activity, apoptosis, autophagy and interactions with the intestinal microbiome. Contemporary applications in pharmacological research, including chemoprevention, natural-product evaluation, immunotherapy, microbiome-directed interventions and experimental drug screening, are discussed. Recent developments through 2026 highlight the importance of standardizing AOM/DSS models and integrating microbiome, metabolomic and lipidomic investigations. The review concludes that AOM remains an important experimental carcinogenesis tool, provided its carcinogenic nature, model limitations and lack of direct therapeutic utility are recognized. Its principal translational value lies in facilitating the discovery and preclinical evaluation of safer and more effective colorectal cancer prevention and treatment strategies.

Keywords: Azoxymethane; chemical carcinogenesis; methylazoxymethanol; O^6-methylguanine; colorectal neoplasia; CYP2E1 bioactivation.

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INTRODUCTION

Azoxymethane (AOM) is a synthetic organic compound belonging to the group of methylating nitrosamine-related carcinogenic agents. It is extensively used in experimental research to investigate the initiation and progression of colorectal cancer. Its importance in pharmacology is primarily associated with its ability to produce reproducible carcinogenic changes in the intestinal epithelium of experimental animals. Unlike conventional pharmacological agents, which are administered to achieve a therapeutic or preventive response, AOM is used to initiate pathological changes that can subsequently be studied using molecular, biochemical, histopathological and pharmacological techniques.

The AOM-induced colorectal carcinogenesis model has become particularly important for investigating:

- Chemical initiation of colorectal cancer.
- DNA alkylation and mutagenesis.
- Cellular proliferation and apoptosis.
- Tumour-associated inflammatory responses.
- Intestinal epithelial transformation.
- Colorectal cancer chemoprevention.
- Evaluation of experimental anticancer compounds.
- Molecular interactions between intestinal microbiota and tumour development.

The combination of AOM and dextran sodium sulphate (DSS) has further expanded its scientific utility by enabling the investigation of inflammation-associated colorectal cancer. Recent developments in molecular oncology have increased the relevance of this model for investigating tumour immunology, intestinal barrier dysfunction, microbiome-associated carcinogenesis and potential therapeutic targets [1–4].

Scientific significance

The principal scientific significance of AOM is its capacity to initiate colorectal carcinogenesis through metabolic activation and DNA methylation. The compound provides an experimental system for studying the transition from normal epithelial tissue to precancerous lesions and, subsequently, malignant colorectal tumours. This is particularly relevant because colorectal carcinogenesis involves multiple stages, including:

- Genetic initiation.
- Altered epithelial proliferation.
- Formation of aberrant crypt foci.

- Development of adenomatous lesions.
- Accumulation of additional molecular abnormalities.
- Progression towards invasive malignancy.

AOM-induced experimental models allow these processes to be investigated under controlled laboratory conditions.

Objectives of the review

The objectives of this review are:

- To examine the historical development of AOM in experimental pharmacology.
- To describe its chemical and pharmacological properties.
- To explain its absorption, distribution, metabolism and elimination.
- To analyze CYP450-mediated metabolic activation.
- To investigate the molecular mechanisms of DNA damage and carcinogenesis.
- To evaluate the AOM/DSS experimental model.
- To examine the application of AOM in pharmacological and toxicological investigations.
- To discuss its scope in chemoprevention and anticancer drug discovery.
- To assess recent developments through November 2026.
- To identify future research opportunities in colorectal cancer pharmacotherapy.

HISTORICAL DEVELOPMENT OF AZOXYMETHANE RESEARCH

The development of AOM as an experimental carcinogen is closely associated with the broader history of chemical carcinogenesis research.

Early development of chemical carcinogenesis research

During the twentieth century, experimental investigations established that certain chemical substances could induce malignant transformation in animal tissues. Research into nitrosamines and related compounds contributed to the identification of metabolic activation as an important requirement for the carcinogenicity of several chemical agents. This led to the development of experimental systems for investigating the relationship between xenobiotic metabolism, DNA modification and tumour development.

Development of dimethylhydrazine-related models

1,2-Dimethylhydrazine (DMH) emerged as an important chemical carcinogen for experimental colorectal cancer research. AOM, a metabolite of DMH, subsequently became widely used because of its relative stability and effectiveness as an experimental colon carcinogen. The two compounds are closely related within the metabolic pathway of colorectal carcinogenesis.

Establishment of AOM-induced colorectal cancer models

AOM became an established experimental tool because it produces colorectal lesions with molecular and histopathological characteristics relevant to certain forms of human colorectal cancer. Its experimental applications include the study of:

- Aberrant crypt foci.
- Adenomatous lesions.
- Colonic epithelial proliferation.
- DNA methylation.
- Tumour suppressor abnormalities.
- Oncogenic signalling.

Development of the AOM/DSS model

The combination of AOM and DSS represented a significant development in experimental colorectal cancer research. AOM provides the carcinogenic initiation component, while DSS induces colonic epithelial injury and inflammation. The resulting model is particularly useful for investigating colitis-associated colorectal cancer. De Robertis and Signori described contemporary methodological considerations for the AOM/DSS model in their 2024 publication [5].

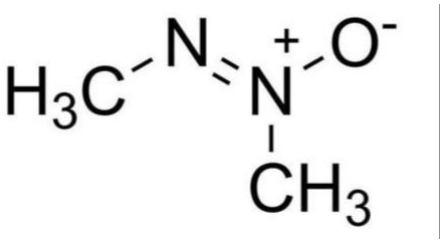
Recent developments, 2025–2026

Recent research has concentrated on improving the reproducibility and translational relevance of carcinogen-induced colorectal cancer models. Feng and colleagues published an updated AOM/DSS model methodology in 2025, highlighting the importance of sex-specific DSS optimization and microbiome-related experimental variation [6]. A 2025 review by the Frontiers research community further discussed the limitations and applications of different colorectal cancer animal models, including carcinogen-induced models, genetically engineered models and patient-derived xenografts [2]. A particularly important development appeared in August 2026, when Van Dingenen and colleagues reported dose-associated changes in the microbiome, metabolome and lipidome in an AOM/DSS colorectal cancer model. Their findings demonstrated that differences in experimental exposure can substantially influence tumour burden and the biological environment of the intestine [7]. These developments demonstrate that AOM research is moving towards integrated molecular and systems-level investigations.

CHEMICAL AND PHYSICOCHEMICAL PROPERTIES OF AZOXYMETHANE

AOM is an organic nitrogen-containing compound with carcinogenic properties.

[Azoxymethane (AOM) CAS 25843-45-2 C2H6N2O]



Azoxymethane (AOM)

Chemical carcinogen | Experimental research compound

- Chemical name: Azoxymethane
- Molecular formula: $C_2H_6N_2O$
- Molecular weight: Approximately 74.08 g/mol
- Chemical class: Azoxy-containing methylating procarcinogen

Chemical characteristics

AOM is a metabolic precursor of methylazoxymethanol (MAM), which is responsible for generating highly reactive methylating intermediates. Its biological activity is dependent on metabolic transformation rather than simply the presence of the parent compound.

Physicochemical characteristics

The physicochemical characteristics of AOM are relevant to its experimental handling, analytical measurement and biological disposition.

Important considerations include:

- Molecular size.
- Aqueous solubility.
- Chemical stability.
- Metabolic susceptibility.
- Potential for reactive intermediate formation.

AOM should be handled as a hazardous carcinogenic chemical under appropriate institutional chemical safety procedures.

Chemical classification and toxicological significance

AOM belongs to the broader class of compounds that can produce DNA alkylation following metabolic activation. Its principal toxicological significance is associated with its ability to generate reactive intermediates capable of modifying nucleic acids. This property explains its usefulness in experimental carcinogenesis and simultaneously establishes the need for strict laboratory safety precautions.

PHARMACOLOGICAL AND TOXICOLOGICAL PROPERTIES

The term pharmacological properties, when applied to AOM, requires interpretation within experimental pharmacology and toxicology rather than conventional therapeutic pharmacology.

Carcinogenic activity

The principal biological activity of AOM is its ability to initiate carcinogenic changes in susceptible tissues After metabolic activation, reactive intermediates modify DNA bases and contribute to mutational events. The resulting genetic abnormalities can interfere with normal regulation of cellular proliferation and differentiation.

DNA-methylating activity

AOM-derived methylating intermediates produce several DNA adducts.

The most important include:

- O^6-methylguanine.
- N^7-methylguanine.
- N^3-methyladenine.

O^6-methylguanine is particularly important because it can produce abnormal base pairing during DNA replication. Persistent DNA lesions increase the probability of mutation and may contribute to oncogenic transformation.

Procarcinogenic activity

AOM is considered a procarcinogen because its carcinogenic effects depend on metabolic activation. This distinguishes it from compounds that directly react with cellular DNA without requiring enzymatic transformation.

Tissue-associated carcinogenic activity

AOM is particularly useful for investigating colorectal carcinogenesis in rodents.

Its experimental effects can include:

- Colonic epithelial DNA damage.
- Aberrant crypt formation.
- Increased epithelial proliferation.
- Development of adenomas.
- Colorectal adenocarcinoma formation.

The precise pattern depends on the experimental animal, strain, sex, exposure conditions and other biological factors [1,3].

Inflammatory and oxidative effects

AOM-induced DNA damage may interact with oxidative stress and inflammatory responses. In the combined AOM/DSS model, inflammatory injury further contributes to the development and progression of colorectal lesions. The biological consequences include activation of inflammatory transcription factors, cytokine production and alterations in the intestinal immune microenvironment.

PHARMACOKINETICS AND METABOLIC BIOACTIVATION

The metabolic activation of AOM is one of the most important aspects of its toxicological profile.

Absorption

Experimental exposure depends on the route of administration and the formulation used. The compound is studied using controlled animal models, with exposure conditions influencing its biological disposition. Human pharmacokinetic data suitable for therapeutic dosing or clinical use are not established.

Distribution

After systemic exposure, AOM undergoes metabolic transformation involving hepatic and extrahepatic tissues. Research has demonstrated that both hepatic and intestinal cytochrome P450 systems can contribute to its metabolic activation.

Metabolism

The major metabolic pathway involves conversion of AOM into methyl azoxymethanol. CYP2E1 is an important enzyme involved in this process. The subsequent decomposition of MAM produces reactive methylating intermediates.

- Azoxymethane (AOM)
- Cytochrome P450-dependent metabolic activation
- CYP2E1 is an important contributor
- Methyl azoxymethanol (MAM)
- Reactive methylating intermediates
- DNA methylation and adduct formation
- Mutation, epithelial transformation and carcinogenesis

Figure 1. Simplified metabolic activation pathway of azoxymethane. Based on experimental studies of hepatic and intestinal cytochrome P450-dependent metabolism [8].

Elimination

AOM and its metabolites undergo metabolic transformation and elimination through biological excretory pathways. The relative contribution of different elimination routes depends on the

experimental system. Detailed human pharmacokinetic parameters have not been established for therapeutic application.

Role of cytochrome P450 enzymes

Cytochrome P450 enzymes are essential to understanding the biological activity of AOM. CYP2E1 has received particular attention because of its contribution to the metabolic activation of AOM and DMH. Research has also demonstrated that hepatic and intestinal P450 enzyme systems may both contribute to the generation of DNA adducts. This finding is important because it suggests that carcinogenic activation is not necessarily restricted to hepatic metabolism [8].

MOLECULAR MECHANISMS OF AZOXYMETHANE-INDUCED CARCINOGENESIS

The carcinogenic effects of AOM involve a sequence of molecular and cellular events.

DNA alkylation and mutagenesis

DNA alkylation is the primary molecular mechanism responsible for AOM-associated carcinogenic initiation. Reactive methylating intermediates interact with DNA and produce alkylated nucleobases. O^6-methylguanine is particularly important because it can pair incorrectly with thymine during DNA replication. This can produce G:C to A: T transition mutations. If these abnormalities escape cellular repair mechanisms, they may become permanently incorporated into the genome.

O^6-methylguanine and DNA repair

The enzyme O^6-methylguanine-DNA methyltransferase (MGMT) is responsible for repairing O^6-methylguanine lesions. MGMT transfers the abnormal methyl group from guanine to its own active-site cysteine residue. This is a direct reversal repair mechanism. When DNA damage exceeds repair capacity or MGMT activity is insufficient, mutagenic lesions may persist.

Mismatch repair pathway

The mismatch repair system recognizes abnormal base pairing and contributes to the correction of replication-associated errors.

Important proteins include:

- MLH1.
- MSH2.
- MSH6.
- PMS2.

Persistent O^6-methylguanine lesions can trigger mismatch repair-dependent responses, including cell-cycle arrest and apoptosis. Repeated cycles of DNA damage and repair failure may contribute to genomic instability.

KRAS signalling

KRAS is an important oncogene involved in the regulation of cellular growth and survival. Mutations affecting KRAS can produce persistent activation of downstream signalling pathways. Important downstream components include:

- RAF.
- MEK.
- ERK.

This signalling cascade regulates cellular proliferation, differentiation and survival. KRAS abnormalities are among the molecular alterations investigated in carcinogen-induced colorectal cancer models, although their frequency and pattern vary with experimental conditions [2].

APC/Wnt/β-catenin signalling

The APC protein is an important component of the β-catenin destruction complex. Under physiological conditions, this complex regulates β-catenin stability. Alterations in APC function can result in β-catenin accumulation and activation of Wnt-dependent transcription.

Consequences include:

- Increased epithelial proliferation.
- Abnormal crypt development.
- Altered differentiation.
- Tumour-associated gene expression.

The Wnt/β-catenin pathway is a major molecular target in colorectal cancer research.

PI3K/Akt/mTOR signalling

PI3K/Akt/mTOR signalling regulates cell survival, proliferation, protein synthesis and cellular metabolism. Abnormal activation of this pathway can support the survival of transformed epithelial cells. In colorectal cancer research, the pathway is investigated as a potential target for suppressing tumour growth and promoting apoptosis.

NF-κB signalling

NF-κB regulates inflammatory gene expression and contributes to the tumour microenvironment. In the AOM/DSS model, inflammatory stimulation can activate NF-κB and increase the expression of:

- TNF-α.

- IL-1β.
- IL-6.
- COX-2.
- iNOS.
- Chemokines.

Persistent inflammatory signalling promotes epithelial proliferation, tissue remodelling and tumour progression.

NLRP3 inflammasome signalling

The NLRP3 inflammasome is an important component of innate immune regulation. Its activation results in caspase-1 activation and maturation of IL-1β and IL-18. In AOM/DSS-associated carcinogenesis, NLRP3 signalling interacts with inflammatory responses, epithelial injury and tissue repair. Its contribution can be context-dependent, because inflammasome activity may have both protective and tumour-promoting effects depending on the cellular compartment and disease stage. Singla and Jena investigated PARP-1, NLRP3 and autophagy in AOM/DSS-induced colorectal cancer, demonstrating the relevance of these mechanisms in local and extra-intestinal tissue changes [9].

Oxidative stress

Oxidative stress contributes to DNA injury, lipid peroxidation and cellular dysfunction. Important molecular components include:

- Reactive oxygen species.
- NADPH oxidases.
- Superoxide dismutase.
- Catalase.
- Glutathione peroxidase.
- Nrf2-dependent antioxidant regulation.

The balance between reactive oxygen species generation and antioxidant defence influences cellular responses to carcinogenic injury.

Apoptosis and cell-cycle regulation

Apoptosis is a major defence mechanism against cells containing irreparable DNA damage. Important molecular regulators include:

- P53.
- Bax.
- Bcl-2.

- Caspase-3.
- Caspase-9.
- Cytochrome c.

Inadequate apoptosis may allow genetically abnormal epithelial cells to survive and proliferate.

Autophagy

Autophagy is a cellular recycling process that removes damaged proteins and organelles. Its contribution to colorectal carcinogenesis is complex. Depending on the disease stage and cellular environment, autophagy can suppress tumour initiation by maintaining cellular homeostasis or support the survival of established tumour cells under metabolic stress. This dual role is particularly relevant when evaluating experimental anticancer compounds.

Epigenetic regulation

DNA methylation, histone modifications and non-coding RNAs influence gene expression during colorectal carcinogenesis.

These mechanisms may alter the expression of:

- Tumour suppressor genes.
- DNA repair genes.
- Cell-cycle regulators.
- Inflammatory mediators.
- Apoptosis-associated proteins.

Epigenetic research may help explain why chemically induced carcinogenesis produces heterogeneous biological outcomes.

AOM-INDUCED COLORECTAL CANCER MODELS

The experimental application of AOM is one of its most important contributions to pharmacological research.

AOM-induced colorectal carcinogenesis model

AOM is used to generate chemically induced colorectal lesions in experimental rodents. The model provides an opportunity to investigate the progression from initial DNA damage to preneoplastic and neoplastic lesions.

Major pathological observations include:

- Aberrant crypt foci.
- Epithelial hyperplasia.
- Adenomatous lesions.
- Dysplastic epithelial changes.

- Colorectal adenomas.
- Adenocarcinomas.

The time course and severity of these abnormalities vary according to animal strain, sex, experimental conditions and exposure characteristics.

AOM/DSS-induced colitis-associated colorectal cancer model

The AOM/DSS model combines chemical carcinogenic initiation with experimentally induced intestinal inflammation. AOM contributes to genetic initiation, whereas DSS damages the intestinal epithelial barrier and produces inflammatory responses.

AOM/DSS experimental disease model

- AOM
- Carcinogenic initiation and DNA damage
- DSS
- Epithelial injury and colonic inflammation
- Inflammatory and mutagenic microenvironment
- Increased cytokine signalling, epithelial proliferation and abnormal tissue repair
- Colitis-associated colorectal neoplasia

Figure 2. Conceptual representation of the complementary roles of AOM and DSS in inflammation-associated colorectal carcinogenesis.

The model is especially valuable for studying the relationship between chronic inflammation and colorectal tumour development.

Comparison of experimental colorectal cancer models

Experimental Model	Principal Mechanism	Main Research Application
AOM	Chemical DNA methylation	Colorectal carcinogenesis
AOM/DSS	Chemical initiation and inflammation	Colitis-associated colorectal cancer
DMH	Metabolically activated chemical carcinogenesis	Colonic tumour initiation
APCMin/+	Genetic predisposition	Wnt signalling and intestinal polyposis
Genetically Engineered Models	Defined oncogenic alterations	Molecular mechanisms and tumour genetics
Patient-Derived Xenografts	Human tumour tissue transplantation	Drug response and tumour heterogeneity

The choice of model should depend on the biological question. No single experimental system fully reproduces the molecular, immunological and clinical complexity of human colorectal cancer [1,2].

SCOPE OF AOM IN PHARMACOLOGICAL TREATMENT RESEARCH

The most important therapeutic contribution of AOM is its use as an experimental disease-induction tool for evaluating potential treatments.

It is important to distinguish between:

- AOM: A carcinogenic experimental compound.
- AOM-induced disease: An experimental model of colorectal carcinogenesis.
- Investigational treatment: A separate drug or intervention evaluated against the induced disease.

The following applications represent the principal areas in which AOM has scientific and translational value.

Chemopreventive drug discovery

Chemoprevention involves the use of pharmacological or nutritional interventions to reduce the initiation, progression or development of cancer.

AOM-induced models are used to investigate whether candidate compounds can reduce:

- DNA damage.
- Aberrant crypt formation.
- Epithelial hyperproliferation.
- Inflammatory activity.
- Tumour multiplicity.
- Tumour development.

Potential investigational categories include:

- Anti-inflammatory compounds.
- Antioxidant molecules.
- Polyphenolic compounds.
- Dietary bioactive substances.
- Microbiome-modulating interventions.
- Metabolic pathway regulators.

A reduction in tumour formation in an animal model does not establish that the same intervention prevents colorectal cancer in humans.

Evaluation of anti-inflammatory drugs

Inflammation plays a central role in the AOM/DSS model. The model is useful for investigating compounds that regulate inflammatory signalling.

Important pharmacological targets include:

- COX-2.
- NF-κB.
- TNF-α.
- IL-6.
- IL-1β.
- JAK/STAT.
- NLRP3 inflammasome.

Non-steroidal anti-inflammatory drugs and other anti-inflammatory candidates may be investigated experimentally, with careful attention to gastrointestinal, cardiovascular and renal safety. The objective is to understand whether modulation of inflammation can reduce tumour-promoting biological processes.

Antioxidant pharmacology

Oxidative stress contributes to DNA damage and inflammatory tissue injury. AOM-induced models provide an experimental platform for investigating the relationship between antioxidant defence and carcinogenesis.

Potentially relevant molecular targets include:

- Nrf2.
- Keap1.
- Glutathione.
- Superoxide dismutase
- Catalase.
- Glutathione peroxidase.

Investigational natural products with antioxidant activity may be evaluated for their effects on biochemical and histological markers of oxidative injury. However, antioxidant activity in chemical assays should not be interpreted as evidence of clinical anticancer efficacy.

Evaluation of natural products and phytochemicals

Natural-product research is an important component of experimental pharmacology. AOM-induced colorectal carcinogenesis models have been used to investigate dietary and plant-derived bioactive compounds.

Important research categories include:

Phytochemical Category	Representative Compounds	Investigational Mechanisms
Polyphenols	Resveratrol	Inflammatory and oxidative signalling
Curcuminoids	Curcumin	NF-κB, COX-2 and inflammatory responses
Flavonoids	Quercetin	Redox regulation and cellular proliferation
Isothiocyanates	Sulforaphane	Nrf2-dependent antioxidant responses
Catechins	EGCG	Cell-cycle and signalling regulation
Anthocyanins	Cyanidin derivatives	Oxidative stress and inflammatory modulation

These compounds are useful research candidates because they may influence multiple molecular pathways.

Their effects should be evaluated using appropriate controls, exposure measurements, pharmacokinetic assessment and toxicity studies.

Evaluation of anticancer pharmacological agents

AOM-induced tumour models may be used to assess the biological activity of experimental anticancer interventions.

Important therapeutic mechanisms include:

- Inhibition of tumour-cell proliferation.
- Induction of apoptosis.
- Cell-cycle arrest.
- Suppression of angiogenesis.
- Regulation of inflammatory signalling.
- Modulation of tumour-associated immunity.

Established colorectal cancer medicines, including fluoropyrimidines, oxaliplatin and irinotecan, have separate clinical roles in human colorectal cancer treatment. Their efficacy in patients must be established through appropriate clinical evidence, not inferred solely from AOM model findings.

Immunotherapy research

The tumour microenvironment is a major determinant of colorectal cancer progression and treatment response. The AOM/DSS model allows researchers to investigate:

- Macrophage polarization.
- T-cell infiltration.
- Regulatory T-cell activity.
- Natural killer cell responses.
- Cytokine networks.
- Immune checkpoint signalling.

This may help identify potential immunological targets. However, the AOM/DSS model does not reproduce all molecular subtypes of human colorectal cancer, and its suitability for testing particular checkpoint inhibitors must be carefully assessed.

Microbiome-directed pharmacological research

The intestinal microbiome influences epithelial integrity, immune regulation, inflammatory signalling and metabolic activity. AOM/DSS models are particularly useful for studying the interaction between microbiota and inflammation-associated colorectal cancer.

Investigational approaches include:

- Probiotic formulations.
- Prebiotic compounds.
- Dietary fibre interventions.
- Microbiome-derived metabolites.
- Microbial enzyme modulation.
- Microbiota-directed therapeutic strategies.

These interventions may influence tumour development through alterations in short-chain fatty acid production, bile acid metabolism, epithelial barrier function and immune regulation.

Drug delivery system research

The AOM-induced colorectal cancer model can also support the evaluation of experimental drug delivery approaches.

Potential research areas include:

- Colon-targeted oral delivery systems.
- Biodegradable polymeric nanoparticles.
- Liposomal formulations.
- Nanostructured lipid carriers.
- Mucoadhesive delivery systems.
- Microbiota-responsive delivery platforms.

The objective is to investigate whether localized delivery improves therapeutic exposure while reducing systemic toxicity.

The safety of the delivery carrier, drug release characteristics and distribution within the intestinal tract require independent evaluation.

Combination therapy research

Combination pharmacotherapy aims to influence multiple biological processes simultaneously.

In experimental colorectal cancer research, combinations may involve:

- Anti-inflammatory and antioxidant agents.
- Chemotherapeutic and targeted agents.
- Immunomodulatory and cytotoxic interventions.
- Microbiome-directed and dietary interventions.

AOM/DSS models can help explore pharmacodynamic interactions, therapeutic synergy and potential toxicity. However, apparent synergy in animal models requires confirmation using appropriate human-relevant experimental systems and clinical trials.

BIOMARKERS AND LABORATORY EVALUATION IN AOM-INDUCED COLORECTAL CARCINOGENESIS

Biomarker assessment is essential for determining the biological effects of AOM and the response to investigational treatments.

Histopathological biomarkers

Important histopathological parameters include:

- Aberrant crypt foci.
- Dysplasia.
- Epithelial hyperplasia.
- Inflammatory infiltration.
- Adenoma formation.
- Adenocarcinoma development.
- Tumour multiplicity and distribution.

Histopathological evaluation remains central to experimental disease characterization.

Molecular biomarkers

Biomarker	Biological Significance
O^6-methylguanine	DNA methylation damage
MGMT	Direct reversal DNA repair
Ki-67	Cellular proliferation
PCNA	DNA replication and repair
p53	DNA damage response
Bax	Proapoptotic signalling
Bcl-2	Antiapoptotic signalling
Cleaved Caspase-3	Apoptotic execution
β-catenin	Wnt pathway activity
KRAS	Oncogenic signalling
COX-2	Inflammatory signalling
NF-κB	Inflammatory transcription
NLRP3	Inflammasome activation

LC3-II and Beclin-1	Autophagy-associated processes

No single biomarker is sufficient to establish the development or regression of colorectal cancer. Molecular findings should be interpreted alongside histopathological and functional outcomes.

Biochemical parameters

Experimental investigations may also assess:

- Malondialdehyde.
- Reduced glutathione.
- Superoxide dismutase activity.
- Catalase activity.
- Myeloperoxidase activity.
- Inflammatory cytokines.
- Liver and renal biochemical parameters.

These measurements help characterize oxidative stress, inflammation and potential extra-intestinal toxicity.

RECENT DEVELOPMENTS AND SCIENTIFIC UPDATES THROUGH 2026

The contemporary relevance of AOM research is increasingly determined by the quality of the experimental model and its ability to support translational therapeutic investigations.

Developments in 2024

De Robertis and Signori published a dedicated methodological chapter in 2024 describing the AOM/DSS model and its relevance to cancer vaccine development and preclinical investigations [5].

Singla and Jena reported research involving AOM/DSS-associated changes in PARP-1, NLRP3 and autophagy. Their findings also highlighted the importance of evaluating extra-intestinal tissue manifestations [9].

Developments in 2025

Feng and colleagues published an updated experimental methodology in Journal of Visualized Experiments in July 2025.

The publication emphasized:

- Experimental reproducibility.
- Sex-specific DSS optimization.
- Microbiome-related variation.
- Tumour immunology.
- Application in preclinical therapeutic studies [6].

Another important 2025 review discussed the limitations and strengths of carcinogen-induced, genetically engineered and patient-derived colorectal cancer models. It highlighted the importance of selecting experimental systems according to the molecular question being investigated [1,2].

Developments in 2026: microbiome and multi-omics

Notable 2026 research

Van Dingenen and colleagues published an important study on 22 August 2026 examining dose-dependent microbiome, metabolome and lipidome changes in AOM/DSS-induced colorectal cancer.

The study integrated:

- 16S rRNA-based microbiome analysis.
- Faecal metabolomics.
- Lipidomics.
- Multi-omics integration.
- Tumour burden assessment.

The investigators identified associations between inflammatory tumour progression and changes in microbial populations and metabolites. Imidazole propionic acid was identified as a candidate metabolite associated with inflammation-driven colorectal cancer. Other metabolic alterations involved lactic acid, fumaric acid, amino acids and lipid-related compounds. These observations suggest that AOM/DSS-induced colorectal cancer is influenced not only by chemical carcinogenesis and inflammation but also by microbiome-associated metabolic reprogramming [7].

Scientific implication: Future AOM research should increasingly incorporate microbial, metabolic and immunological measurements rather than relying exclusively on tumour counts and histopathology.

Contemporary direction of research

The most relevant current research directions include:

Research Domain	Scientific Objective
Multi-omics	Identify molecular signatures of tumour progression
Microbiome Research	Understand microbial contributions to carcinogenesis
Tumour Immunology	Investigate immune-cell interactions
Molecular Pharmacology	Identify new therapeutic targets
Chemoprevention	Evaluate preventive interventions
Drug Delivery	Improve targeted colorectal drug delivery
Precision Oncology	Improve model selection and translational relevance

LIMITATIONS AND CHALLENGES OF AOM-BASED EXPERIMENTAL RESEARCH

Although AOM is a well-established experimental carcinogen, its application has several limitations.

Species-related differences

The biological response to AOM differs between experimental animals and humans. Differences in metabolic enzymes, intestinal physiology and immune responses can affect translational interpretation.

Genetic variability

Animal strain and genetic background can influence susceptibility to chemical carcinogenesis. Consequently, findings obtained in one strain may not be directly reproducible in another.

Sex-related differences

Sex can influence inflammatory responses, metabolic activity and tumour development. Experimental designs should therefore consider biological sex as a relevant variable.

Microbiome variability

Differences in microbial composition can alter intestinal inflammation and tumour progression. Housing conditions, diet, animal supplier and environmental exposures may influence experimental outcomes.

Model-specific limitations

The AOM model is useful for chemically initiated colorectal carcinogenesis, whereas the AOM/DSS model is particularly relevant to inflammation-associated tumour development. Neither model reproduces the full heterogeneity of sporadic human colorectal cancer.

Toxicological and ethical considerations

AOM is a carcinogenic chemical and requires appropriate institutional risk assessment, chemical containment, personal protective equipment and hazardous waste management. All animal research must comply with applicable ethical approvals and animal welfare requirements, including the principles of replacement, reduction and refinement.

FUTURE PERSPECTIVES

The future of AOM-based research is likely to be shaped by the integration of molecular pharmacology, precision oncology and advanced experimental technologies.

Artificial intelligence and computational pharmacology

Artificial intelligence may assist in:

- Identifying molecular targets.
- Analyzing multi-omics datasets.

- Predicting drug–target interactions.
- Evaluating molecular pathway relationships.
- Identifying candidate biomarkers.

Integration of computational modelling with AOM-based experimental findings could improve the efficiency of preclinical drug discovery.

Single-cell and spatial transcriptomics

Single-cell sequencing may identify different cellular populations involved in AOM-induced carcinogenesis. Spatial transcriptomics can provide information about the distribution of inflammatory and tumour-associated signalling within intestinal tissue. These techniques may improve the understanding of the tumour microenvironment.

Microbiome-based interventions

Future studies may investigate whether microbiome-directed interventions can modify the inflammatory environment associated with colorectal carcinogenesis. This research may provide opportunities for developing nutritional, microbial and pharmacological strategies.

Precision drug development

The integration of genetic, metabolic and immunological information may improve the selection of experimental models for particular therapeutic targets. This is particularly important because colorectal cancer is a heterogeneous disease involving different molecular pathways and treatment responses.

Human-relevant experimental models

Organoids, patient-derived tumour cultures and advanced co-culture systems may complement AOM-based animal models. Combining these technologies may improve the prediction of human therapeutic responses.

DISCUSSION

Azoxymethane occupies an important position in experimental pharmacology because it provides a controlled system for investigating the relationship between xenobiotic metabolism, DNA damage, inflammation and colorectal carcinogenesis. Its principal biological activity is associated with metabolic activation and the generation of reactive methylating intermediates. The resulting DNA modifications can interfere with normal cellular replication and contribute to mutagenesis.

An important aspect of AOM research is the relationship between DNA damage and DNA repair. MGMT-mediated repair, mismatch repair and apoptosis collectively determine whether damaged epithelial cells recover, undergo cell death or survive with potentially oncogenic

mutations. The development of the AOM/DSS model has further expanded the scope of experimental research by incorporating inflammatory injury into chemically initiated carcinogenesis. This is particularly relevant to understanding inflammation-associated colorectal cancer.

From a pharmacological perspective, the greatest value of AOM is its ability to provide a reproducible disease model for evaluating investigational interventions. Anti-inflammatory agents, antioxidant compounds, phytochemicals, microbiome-directed interventions and experimental anticancer agents can be investigated using this system.

However, an important distinction must be maintained between experimental efficacy and clinical effectiveness. Reduction in tumour formation in an animal model does not establish that a compound is safe or effective in humans. The recent integration of microbiome analysis, metabolomics and lipidomics represents a particularly promising development. It provides opportunities to understand the relationship between chemical carcinogenesis, microbial metabolism, intestinal inflammation and tumour progression.

The 2026 multi-omics findings by Van Dingenen and colleagues further reinforce the importance of incorporating biological complexity into experimental colorectal cancer research [7]. Future research should therefore move beyond conventional tumour assessment towards integrated evaluation of genetic, metabolic, inflammatory, immunological and microbial factors.

CONCLUSION

Azoxymethane is an important experimental carcinogenic compound with substantial applications in pharmacology, toxicology and molecular oncology. Its metabolic activation, particularly through cytochrome P450-dependent pathways, results in the formation of methylating intermediates capable of producing DNA adducts and initiating colorectal carcinogenesis. The molecular mechanisms associated with AOM include DNA alkylation, impaired DNA repair, oncogenic signalling, oxidative stress, inflammatory activation, altered apoptosis and changes in cellular autophagy.

The AOM/DSS model has further established an important experimental platform for investigating inflammation-associated colorectal cancer. From the perspective of therapeutic research, AOM is valuable primarily because it facilitates the preclinical evaluation of chemopreventive agents, anti-inflammatory compounds, antioxidant interventions, phytochemicals, immunomodulatory strategies and experimental anticancer formulations. Recent developments through 2026 demonstrate increasing interest in microbiome-associated

carcinogenesis, multi-omics integration, tumour immunology and improved experimental model standardization.

Nevertheless, AOM itself has no established therapeutic indication and should not be administered as a treatment for colorectal cancer or any other human disease. Its continued scientific relevance lies in its application as an experimental tool for identifying molecular targets, understanding carcinogenic mechanisms and evaluating safer pharmacological interventions. Ultimately, the integration of AOM-based experimental models with advanced molecular technologies, human-derived organoids and precision oncology may contribute to the development of more effective colorectal cancer prevention and treatment strategies.

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