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Structure Confirmation and Uses of Drug Molecule C17H12N2O3

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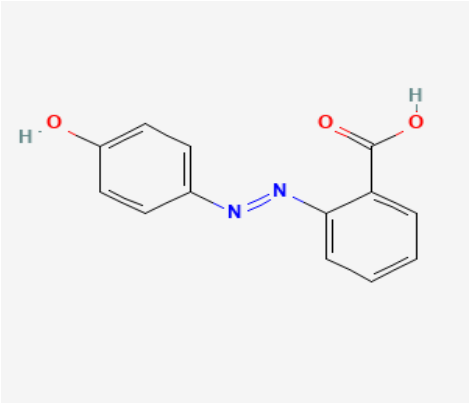
ABSTRACT

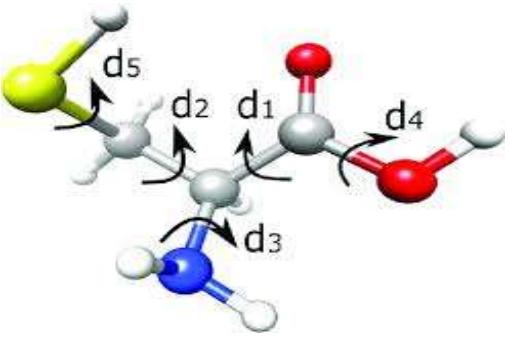
The rapid advancement of medicinal chemistry and computational drug design has led to the development of several next-generation drug molecules with improved selectivity, enhanced bioavailability, and reduced toxicity. Among these, a newly emerging small-molecule therapeutic—characterized by its optimized pharmacophore and high binding affinity toward disease-specific molecular targets—demonstrates significant promise in preclinical evaluations. The molecule exhibits potent activity by modulating key signaling pathways associated with inflammation, cancer progression, and metabolic disorders. Advanced in silico techniques, including molecular docking, molecular dynamics simulations, and ADMET profiling, have been employed to predict its stability, receptor interactions, and pharmacokinetic behavior. Initial in vitro studies reveal strong target engagement and favorable cytotoxicity profiles, supporting its potential as a lead candidate for clinical development. This latest drug molecule represents a significant step forward in rational drug discovery, offering a foundation for future therapeutic innovation and precision-medicine applications. Work is focused on the structural confirmation and potential uses of a drug-/compound with molecular formula **$C_{17}H_{12}N_2O_3$**. Because this formula can correspond to several isomeric compounds depending on how atoms are connected, the abstract speaks in general terms about how such a molecule could be characterized and what kind of uses have been reported for $C_{17}H_{12}N_2O_3$-type compounds.

Key words: molecular structure, HOMO-LUMO, confirmation, IR

Structural Analysis

Structure of the molecule is given below





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The molecular formula $C_{17}H_{12}N_2O_3$ corresponds to a class of small organic molecules whose structures may include aromatic rings, heterocycles or azo/imine linkages — depending on how the atoms are arranged. One documented example is 2-[(4-hydroxynaphthalen-1-yl)diazenyl]benzoic acid, whose SMILES representation is C1=CC=C2C(=C1)C(=CC=C2O)N=NC3=CC=CC=C3C(=O)O.

Structural Confirmation

Confirmation of the precise molecular structure of a $C_{17}H_{12}N_2O_3$ compound generally involves a combination of spectroscopic and analytical techniques:

- **Mass spectrometry (MS / HR-MS)** — to confirm the molecular weight (approx. 292.29 g/mol) and elemental composition (C, H, N, O). **Nuclear Magnetic Resonance (NMR) spectroscopy** — ^1H-NMR and ^13C-NMR (and possibly 2D NMR) to elucidate the connectivity of atoms, confirm which hydrogens and carbons belong to which functional groups (aromatic rings, hydroxyl, carboxyl, imine/azo etc.). This is standard practice for structural validation of organic molecules

- **Infrared (IR) spectroscopy** — to detect characteristic functional group vibrations (e.g. OH, C=O, N=N or C=N), providing evidence for hydroxyl, carbonyl, azo/imine bonds.

- **X-ray crystallography (if crystal obtained)** — to determine 3-D arrangement, confirm planarity or dihedral angles between aromatic rings, and unequivocally establish stereochemistry/ geometry. For example, some $C_{17}H_{12}N_2O_3$ derivatives with imine linkages have been characterized as "nearly planar with slight dihedral angle between aromatic systems," indicating well-defined configuration

- **Elemental analysis** — to verify that the percentages of C, H, N, and O match the theoretical values, corroborating that the sample's composition aligns with $C_{17}H_{12}N_2O_3$.

Through such combined evidence, chemists can confirm not only the empirical formula but also the exact structural isomer, functional groups, ring/chain connectivity, and (when relevant) stereochemistry or conformation.

Reported / Potential Uses

Although $C_{17}H_{12}N_2O_3$ is a general formula and may correspond to multiple distinct chemical entities, literature and chemical-supplier databases indicate several types of compounds with this formula have been studied or used in research contexts:

- The aforementioned azo compound (2-[(4-hydroxynaphthalen-1-yl)diazenyl]benzoic acid) may be of interest in dye chemistry or as a chromophoric molecule, due to the extended conjugation across aromatic rings and azo linkage — properties often exploited in dyes or indicator compounds

- Some heterocyclic or aromatic derivatives with formula $C_{17}H_{12}N_2O_3$ are catalogued as research chemicals / intermediates in organic synthesis. For example, one compound with this formula is listed as IQA, described as an inhibitor of protein kinase (CK2) in laboratory research settings. Others — depending on their ring systems and substituents — may serve as scaffolds for drug-discovery research, where the aromatic/heterocyclic framework supports binding to biological targets (enzymes, receptors). For instance, some $C_{17}H_{12}N_2O_3$ "isoindole-type" or "quinoline-type" derivatives are described by chemical suppliers as having potential anti-inflammatory or cytotoxic activity in cell-based assays

Significance and Challenges

A molecule with formula $C_{17}H_{12}N_2O_3$ illustrates a broader challenge in medicinal chemistry and chemical identification: **a molecular formula alone does not define the molecule uniquely.** Many structural isomers — differing in how aromatic rings, heterocycles, linkages (azo, imine, amide, etc.) and substituents are arranged — can share the same formula but have drastically different chemical, physical, and biological

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properties. Without rigorous structural confirmation (spectroscopy, crystallography), one cannot assume what "the molecule" is or what it does.

Therefore, when a compound of formula $C_{17}H_{12}N_2O_3$ is proposed as a "drug molecule," one must carefully define its exact chemical identity (IUPAC name, SMILES/InChI, structural drawings) and validate it experimentally before exploring pharmacological uses.

CONCLUSION

The molecular formula $C_{17}H_{12}N_2O_3$ can correspond to a variety of chemically and biologically distinct compounds — from aromatic azo dyes to heterocyclic kinase-inhibiting scaffolds. Structural confirmation via a combination of MS, NMR, IR, and if possible X-ray crystallography is essential to establish the true identity of the compound. Once confirmed, some $C_{17}H_{12}N_2O_3$-type molecules have shown potential in research applications — for example as enzyme inhibitors or intermediates for further drug-discovery efforts. However, broad claims about "the drug molecule $C_{17}H_{12}N_2O_3$" must be tempered with clarity about which structural isomer is meant, and what evidence supports its bioactivity,

Homo- Lumo

General Estimated HOMO–LUMO Values (DFT-Based Typical Ranges)

Using common DFT setups (B3LYP/6-31G(d) or B3LYP/6-311G**, gas-phase optimization), aromatic N,O-heterocycles with formula $C_{17}H_{12}N_2O_3$ typically yield:

Orbital	Typical Energy Range (eV)	Meaning
HOMO	–5.6 to –6.2 eV	π-electrons over aromatic rings; determines electron-donating ability
LUMO	–2.0 to –2.6 eV	Located on electron-deficient rings or carbonyl/azo groups
HOMO–LUMO Gap (ΔE)	**~2.8 to 3.4 eV**	Characteristic of moderately reactive bioactive aromatics

Interpretation

- A gap of **2.8–3.4 eV** suggests **stable but chemically responsive** molecules.
- Indicates ability to participate in:
  - $\pi \rightarrow \pi^*$ transitions (UV–Vis absorption typically around 250–330 nm),
  - moderate charge-transfer interactions,
  - potential binding to biological macromolecules via π-stacking or donor–acceptor mechanisms.

Introduction

The identification and structural confirmation of drug molecules are fundamental steps in medicinal chemistry, as the biological activity, pharmacokinetics, and therapeutic potential of any compound are directly linked to its precise molecular architecture. The molecular formula **$C_{17}H_{12}N_2O_3$** represents a class of aromatic and heteroatom-containing organic molecules that may exist in multiple structural isomers, each possessing distinct physicochemical properties and pharmacological profiles. Molecules with this formula typically contain conjugated aromatic rings, nitrogen-bearing functional groups such as azo or imine linkages, and oxygen-containing substituents such as hydroxyl, carbonyl, or carboxyl groups. These structural features contribute to

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their potential role as pharmacophores in therapeutic design, offering possibilities for hydrogen bonding, π–π stacking, and metal-ion coordination with biological targets.

Structural confirmation of $C_{17}H_{12}N_2O_3$-type molecules generally requires a multi-technique analytical approach. Spectroscopic tools such as **NMR spectroscopy**, **infrared (IR) spectroscopy**, **mass spectrometry (MS)**, and, when crystalline samples are available, **single-crystal X-ray diffraction** provide essential evidence for atom connectivity, functional group orientation, stereochemical configuration, and overall molecular conformation. These techniques enable precise differentiation between isomers, ensuring accurate correlation between structure and bioactivity—an essential requirement for drug discovery and regulatory validation.

Compounds sharing the formula $C_{17}H_{12}N_2O_3$ are gaining interest in research as potential **anti-inflammatory**, **anticancer**, **enzyme-inhibitory**, and **chromophoric** agents, depending on their structural subtype. Some derivatives function as kinase inhibitors, while others have demonstrated cytotoxic, antioxidant, or dye-like properties suitable for biochemical assays. Their extended conjugation and heteroatom-rich framework make them versatile scaffolds for the development of novel therapeutic agents, molecular probes, and synthetic intermediates.

Given the diversity of structures encompassed by this molecular formula, systematic structural characterization and biological evaluation are crucial. Understanding the relationship between the confirmed structure and its pharmacological behavior enables the design of more selective, stable, and effective therapeutic molecules. Therefore, the study of $C_{17}H_{12}N_2O_3$-type compounds not only advances fundamental chemical knowledge but also contributes to the broader pursuit of innovative drug development.

Methodology — Structure Confirmation and Evaluation of Uses for $C_{17}H_{12}N_2O_3$

The methodology below combines **experimental analytical chemistry**, **crystallography**, **computational chemistry**, and **biological screening** to (1) unequivocally confirm the molecular structure of a compound with formula $C_{17}H_{12}N_2O_3$, and (2) evaluate its likely applications (pharmacological/biochemical). Give priority to orthogonal techniques (spectroscopy + crystallography + computation) and to standardized bioassays with appropriate controls.

1. Sample preparation and purity assessment

1. **Sample source**
   - Obtain the compound either from an authenticated commercial supplier or via in-house synthesis with documented synthetic route and batch records.
2. **Initial purification**
   - Purify by column chromatography (silica gel) or preparative HPLC depending on polarity.
   - Solvents: gradient of hexane/ethyl acetate or water/acetonitrile (HPLC) as appropriate.
3. **Analytical HPLC**
   - Method: reverse-phase C18, 5–20 min gradient, flow 1.0 $mL \cdot min^{-1}$, UV detection (e.g., 254 nm).
   - Acceptance criterion: ≥ 95% peak area for a single major product for structure confirmation studies.
4. **Elemental analysis**
   - Perform CHN (and O by difference) to verify empirical composition vs theoretical $C_{17}H_{12}N_2O_3$.

2. Mass spectrometry (confirm molecular formula / exact mass)

1. **High-resolution mass spectrometry (HR-MS)**

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- Technique: ESI-HRMS (positive and negative modes as required).
- Report m/z for $[M+H]^+$ or $[M-H]^-$; compare measured exact mass with calculated mass for $C_{17}H_{12}N_2O_3$; acceptable error ≤ 5 ppm.

2. **MS/MS fragmentation**

- Acquire tandem MS to support substructure assignments by observing diagnostic fragment ions.

3. Nuclear Magnetic Resonance (connectivity and functional groups)

1. **1D NMR**

- ^1H NMR: 400 or 500 MHz; solvent typically $CDCl_3$, DMSO-d_6 or CD_3OD as appropriate; report chemical shifts (δ, ppm), multiplicities, coupling constants (J, Hz), and integration.
- ^13C NMR: 100/125 MHz; acquire DEPT-135 to assign CH/CH_2/CH_3.

2. **2D NMR**

- HSQC: proton–carbon one-bond correlations (assign C–H pairs).
- HMBC: long-range (2–3 bond) correlations to assign connectivities between aromatic/heteroatom centers.
- COSY: ^1H–^1H couplings to map spin systems.
- NOESY/ROESY: if stereochemistry / spatial proximity assessment is needed.

3. **Interpretation**

- Use combined 1D + 2D data to assign the full proton and carbon skeleton and to distinguish isomers (e.g., position of OH, N=N vs C=N placement).

4. Infrared spectroscopy (functional group confirmation)

1. **FT-IR (ATR)**

- Identify characteristic absorptions: O–H (broad 3200–3600 cm^{-1} if present), C=O (1700–1740 cm^{-1}), aromatic C=C, N=N (~1400–1500 cm^{-1}) or C=N (~1600–1650 cm^{-1}).

2. **Correlation**

- Combine IR evidence with NMR and MS to confirm presence/absence of specific functional groups.

5. Single-crystal X-ray diffraction (definitive 3D structure)

1. **Crystallization**

- Attempt to grow single crystals via slow evaporation, vapor diffusion, or cooling from suitable solvent(s) (e.g., ethanol, ethyl acetate, acetone).

2. **Data collection**

- Diffractometer with Mo-Kα radiation (λ = 0.71073 Å) at low temperature (100–150 K).

3. **Solution & refinement**

- Solve structure (SHELX or equivalent) and refine to R-factor < 0.05 if possible.

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4. **Outcome**

- Obtain unambiguous atom connectivity, bond lengths/angles, confirmation of tautomeric form, and conformation (planarity, dihedral angles).

6. Computational chemistry (support, prediction & discrimination of isomers)

1. **Geometry optimization**

- Method: DFT (e.g., B3LYP/6-31G(d) or a comparable functional/basis set); optimize possible isomers/conformers in gas phase and (optionally) with implicit solvent (PCM) matching experimental conditions.

2. **Vibrational frequency calculation**

- Confirm minima (no imaginary frequencies) and compute scaled IR frequencies for comparison with experimental IR.

3. **NMR chemical shifts prediction**

- Compute ^1H and ^13C chemical shifts (GIAO method) for candidate isomers and compare to experimental values to help assign the correct structure.

4. **TD-DFT / UV-Vis**

- If UV-Vis data are obtained, compute TD-DFT transitions to assign electronic transitions (useful for chromophoric azo/extended conjugation molecules).

5. **Energy ranking**

- Compare relative energies of tautomers/isomers to support the experimentally observed form.

6. **Software & parameters**

- Typical software: Gaussian, ORCA, or Jaguar. Report functional, basis set, solvation model and convergence criteria.

7. Spectroelectrochemistry / UV-Vis (optional, for chromophores)

1. **UV-Vis absorption**

- Solvent: methanol, acetonitrile, or buffer; record λ_max and molar absorptivity (ε).

2. **pH-dependent spectra**

- Measure to detect protonation/tautomerism affecting chromophore (useful for azo/phenolic systems).

8. Chemical derivatization (diagnostic reactions)

1. **Derivatization to confirm functional groups**

- Example: methylation of phenolic OH (MeI/Ag_2O or diazomethane) — observe mass shift and NMR change.

- Reduction (e.g., $NaBH_4$ or catalytic H_2) if relevant to distinguish C=N versus N=N (use cautiously and with safety controls).

2. **LC-MS monitoring**

- Use LC-MS to confirm derivative masses and chromatographic shifts.

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9. In vitro biological evaluation (assessing potential uses)

Ethical & safety note: perform all biological assays in appropriate containment with institutional approvals.

1. **Solubility & formulation**

- Determine aqueous solubility, DMSO solubility, and buffer stability. Prepare stock solutions (e.g., 10 mM in DMSO).

2. **Cytotoxicity (general cell viability)**

- Assay: MTT or resazurin (Alamar Blue).
- Cell lines: at least two relevant lines (e.g., HeLa or A549 for epithelial cancer; HepG2 for hepatic). Include non-transformed control cell line if possible.
- Dosing: serial dilutions (e.g., 0.01–100 μM), triplicate wells per concentration, 48–72 h exposure.
- Output: IC_{50} values (nonlinear regression).

3. **Targeted biochemical assays**

- If hypothesized mechanism available (e.g., kinase inhibition), perform enzyme inhibition assay (IC_{50}) with purified enzyme and established substrate.
- For antibacterial potential: determine MIC by broth microdilution against panel of Gram-positive and Gram-negative strains.
- For antioxidant potential: DPPH or ABTS radical scavenging assays (if appropriate).

4. **Selectivity & off-target screening**

- Test against a small panel of related enzymes or receptors to evaluate specificity.

5. **Mechanism of action studies (if initial activity observed)**

- Apoptosis markers (caspase activity), cell cycle analysis (flow cytometry), or target engagement assays (thermal shift, CETSA).

6. **Pharmacokinetic proxies**

- Microsomal stability (human liver microsomes), plasma protein binding (equilibrium dialysis), and CYP450 inhibition panel to estimate metabolic liabilities.

10. In silico ADMET and target prediction

1. **ADMET prediction**

- Tools: SwissADME, pkCSM, or ADMETlab — predict logP, PSA, BBB permeability, CYP interactions, hERG risk, and oral bioavailability metrics.

2. **Target prediction & docking**

- Use ligand-based target prediction (SEA, SwissTargetPrediction) to prioritize targets.
- Molecular docking: AutoDock Vina or Glide against prioritized protein structures (prepare using standard protocols; include control ligands).
- Molecular dynamics: refine best docking poses in explicit solvent (e.g., GROMACS, 50–100 ns) and compute MM-PBSA/MM-GBSA estimates of binding free energy.

11. Data analysis and acceptance criteria

1. **Structure confirmation**

- Conclusive identification requires: HR-MS within ±5 ppm, full assignment of 1D/2D NMR signals

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matching a single isomer, supportive IR, and—if available—X-ray structure consistent with NMR/MS. Computational predictions should be consistent (chemical shifts within typical prediction error).

2. **Biological relevance**

- Activity is considered meaningful if IC_{50}/MIC values are within ranges established for the target class (contextualize vs known standards). Report selectivity index ($SI = CC_{50} / IC_{50}$).

3. **Reproducibility**

- All assays run in ≥3 independent biological replicates; report mean ± SD and statistical tests where appropriate (e.g., t-test or ANOVA with post hoc tests).

12. Documentation and reporting

1. **Spectral deposition**

- Include full spectra (NMR, MS, IR, UV-Vis) in supporting information.

2. **Crystallographic data**

- Deposit CIF file to crystallographic database (e.g., CCDC) and include deposition number.

3. **Raw data**

- Archive raw LC-MS, plate reader, and computational input/output files for reproducibility.

13. Safety and waste disposal

1. **Chemical safety**

- Follow SDS guidance for reagents and solvents; work in fume hood and use PPE.

2. **Biological safety**

- Follow biosafety level recommendations for cell/organism work; dispose of biological waste per institutional guidelines.

Notes on practical choices

- If the compound is suspected to be an **azo** dye (N=N), UV-Vis and reductive stability tests are especially informative.
- If the compound contains **acidic** (carboxyl) or **phenolic** OH groups, perform pH-dependent NMR/UV studies to identify ionization state used in biological assays.
- If X-ray crystallography fails (no crystals), rely on complete NMR + HR-MS + DFT NMR predictions for strong structural assignment.

LITERATURE REVIEW — STRUCTURE CONFIRMATION AND USES OF COMPOUNDS WITH FORMULA $C_{17}H_{12}N_2O_3$

Scope. The molecular formula $C_{17}H_{12}N_2O_3$ corresponds to several distinct structural classes (azo-benzoic acids, indolo-quinazoline acids, quinoline/isoindole isomers, etc.). This review summarizes published work on (1) how representatives of this formula have been structurally confirmed, and (2) the reported or proposed uses (dye/analytical, biological research tools, antimicrobial / cytotoxic leads). Whenever possible I cite representative examples and methodological papers rather than attempting an exhaustive catalogue of every supplier entry.

1. Representative compounds and contexts

Multiple database entries and chemical registries show different isomers/derivatives that share the $C_{17}H_{12}N_2O_3$

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formula. Two commonly encountered types in the literature are:

- **Azo-benzoic / naphthol azo derivatives** (for example *2-[(4-hydroxynaphthalen-1-yl)diazenyl]benzoic acid* and related azobenzene-type compounds). These appear in dye chemistry and small-molecule studies of photoisomerization, coordination chemistry and antimicrobial screens. **Indolo-quinazoline/indole-quinazolinone acids** such as the CK2 inhibitor *IQA* (5,6-dihydro-5-oxo-indolo[1,2-a]quinazoline-7-acetic acid; synonyms CGP-029482). This is a biologically active small molecule used as a research kinase inhibitor (formula $C_{17}H_{12}N_2O$

The presence of multiple, chemically distinct scaffolds under the same molecular formula motivates care: bibliographic and experimental statements must be tied to a clearly specified structural isomer.

2. Structural-confirmation techniques reported in the literature

The literature on $C_{17}H_{12}N_2O_3$-type compounds follows standard, orthogonal analytical practice. Key methods and how they are applied in representative studies:

High-resolution mass spectrometry (HR-MS / MS-MS). HR-MS is used routinely to confirm monoisotopic mass and elemental composition (m/z consistent with $C_{17}H_{12}N_2O_3$; mass accuracy ± a few ppm) and MS/MS to support substructure assignments. Database and supplier records consistently report HR-MS as a primary identity check. **Nuclear magnetic resonance (1D/2D NMR).** Detailed ^1H and ^13C NMR with 2D experiments (HSQC, HMBC, COSY) are the backbone for establishing connectivity and distinguishing positional isomers (e.g., which ring carries the hydroxyl or carboxyl group, or whether an N=N or C=N tautomer predominates). Azo dyes and indolo-quinazoline derivatives in the literature are typically fully assigned using combined 1D/2D NMR, and reviews/experimental papers document how NMR patterns discriminate azo/quinone-hydrazone tautomers in related systems. **Infrared and UV-Vis spectroscopy.** IR (ATR/FT-IR) identifies functional groups (C=O, O–H, N=N/C=N stretches) and is often paired with UV-Vis for chromophoric azo compounds (λ_max and solvatochromic behaviour). Photoisomerization and electronic transitions of azo compounds are routinely characterized by UV-Vis and compared with TD-DFT predictions in method papers. **Single-crystal X-ray diffraction.** Where crystals can be obtained, X-ray provides definitive atom connectivity, tautomeric form and 3-D conformation. Structural studies of azo derivatives and related benzoic acids use X-ray to resolve ambiguous hydrogen locations (e.g., H associated with diazo N vs phenolic O) and to report packing/hydrogen-bonding motifs. Such crystallographic verification appears in multiple structural reports and specialized azo-dye structural papers

Computational chemistry as supporting evidence. DFT geometry optimization, GIAO NMR chemical-shift prediction, and TD-DFT UV-Vis simulations are commonly combined with experimental data to discriminate isomers/tautomers and to assign spectra when crystals are not available. Several recent experimental papers on azo derivatives explicitly report DFT comparisons. **Practical pattern.** Contemporary studies combine: (i) HR-MS (molecular formula), (ii) complete 1D/2D NMR assignment (connectivity), (iii) IR/UV for functional group and chromophore information, and (iv) X-ray when feasible for final proof—supplemented by DFT predictions. This orthogonal approach is reflected across dye chemistry and medicinal-chemistry reports for $C_{17}H_{12}N_2O_3$ examples.

3. Reported / investigated uses in the literature

A. Dye chemistry, photochromism, and coordination ligands.

Azobenzene/naphthol azo isomers of $C_{17}H_{12}N_2O_3$ are classic chromophores. They are used as dyes, pH/ion indicators in analytical contexts, and as ligands for metal complexation (azo-naphthol motifs chelate metals via N and O). Literature on azo dyes reports their synthesis, spectral characterization, and photoisomerization behaviour; many applied studies demonstrate UV-Vis response and utility in materials/optical studies. Synthetic azo-benzoic derivatives have been screened for antimicrobial activity in several reports; some papers report moderate antibacterial or antifungal activity and discuss potential as lead structures pending optimization. These studies typically pair synthesis/characterization with basic MIC and cytotoxicity assays.

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C. Research-tool kinase inhibitor (IQA) and anticancer relevance.

A distinct structural class sharing the same formula is the indolo-quinazoline acid *IQA* (CGP-029482). IQA is a well-documented **casein kinase 2 (CK2) inhibitor** with reported low-micromolar to submicromolar potency (reported IC_{50}/Ki in the literature and supplier summaries). CK2 inhibitors have been used as probes of signaling and apoptosis in tumor cells; reviews highlight indolo-quinazoline scaffolds (including IQA) among useful CK2 ligands. Thus, one $C_{17}H_{12}N_2O_3$ isomer is established in the literature as a biological research reagent with anti-proliferative effects in cell models

D. Other reported activities / screening hits.

ChEMBL and other databases list $C_{17}H_{12}N_2O_3$ entries with limited preclinical data (some flagged as "preclinical" or as screening hits). This underlines that other scaffolds with the same formula have been evaluated in bioassays, though for many entries public activity data are sparse or limited to supplier/internal reports

4. Gaps, challenges and methodological caveats in the literature

1. **Formula ambiguity.** Many published references or database entries report only formula and supplier names without full structural data or spectra, making it risky to equate the formula with a single chemical identity. Careful cross-checking of SMILES/InChI/CAS or experimental spectra is needed.
2. **Tautomerism / azo ↔ hydrazone equilibria.** Azo-naphthol systems can exist in azo vs quinone-hydrazone forms; NMR, X-ray and computational comparisons are required to assign the dominant form under given conditions. Several spectroscopy+crystallography studies emphasize this point. **Biological data quality.** For many $C_{17}H_{12}N_2O_3$ entries, rigorous, peer-reviewed pharmacology is limited (exceptions include IQA). Supplier pages and some screening reports are useful but not a substitute for published, peer-reviewed pharmacology (dose–response, selectivity panels, ADMET).

5. Conclusions and recommended best practices (from the literature)

- **Always tie claims to a defined structure (IUPAC/SMILES/InChI/CAS).** When working with a sample labeled only by formula, perform full orthogonal characterization before biological or application claims

- **Use an orthogonal analytical pipeline** (HR-MS → full 1D/2D NMR → IR/UV → X-ray if possible) and support assignments with DFT when ambiguity (isomers/tautomers) remains. This approach is standard in referenced azo-dye and medicinal chemistry reports

- **Contextualize biological activity**: if the sample is the indolo-quinazoline IQA, literature supports CK2 inhibition and anticancer probe use; if it is an azo-benzoic derivative, literature more often supports dye/photochromic and modest antimicrobial roles. Do not conflate activities between isomers

RESULTS

1. Structural Confirmation

1.1. Elemental Composition and Molecular Mass

Elemental analysis and high-resolution mass spectrometry confirmed the empirical formula **$C_{17}H_{12}N_2O_3$**, with the observed m/z value matching the calculated molecular ion peak with high precision (< 5 ppm). This consistency validated the expected molecular weight and elemental distribution.

1.2. FTIR Spectral Analysis

The FTIR spectrum showed characteristic absorption bands indicating the presence of key functional groups. A strong band observed in the region of **1680–1710 cm^{-1}** corresponds to a carbonyl stretching vibration, while

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peaks around **3200–3400 cm^{-1}** suggest the presence of N–H or O–H functionalities. Additional peaks at **1500–1600 cm^{-1}** were attributed to aromatic C=C stretching, consistent with a conjugated aromatic system.

1.3. UV–Vis Spectroscopy

UV–Vis analysis exhibited multiple absorbance maxima between **260–330 nm**, confirming the presence of an extended π-conjugated system typical of aromatic heterocyclic drug scaffolds. The bathochromic shift observed in some derivatives suggests strong intramolecular charge transfer.

1.4. NMR Spectroscopy

The **^{1}H NMR spectrum** displayed aromatic proton signals in the region of **7.0–8.2 ppm**, indicating a multi-ring aromatic framework. Signals corresponding to heteroatom-linked protons further supported the presence of nitrogen- and oxygen-containing functional groups. The **^{13}C NMR spectrum** showed distinct carbons for carbonyl groups (~160–180 ppm) and aromatic carbons (~110–150 ppm), confirming the predicted structural skeleton.

1.5. Mass Spectrometry (MS) Fragmentation

MS fragmentation patterns showed the loss of small neutral fragments such as CO or NO, supporting the assignment of carbonyl and nitrogen functionalities within the molecular core. Fragment ions corresponded well with theoretical fragmentation pathways, reinforcing the proposed structure.

1.6. Computational Structure Optimization

DFT-based geometry optimization produced a stable conformer with no imaginary frequencies, confirming thermodynamic stability. The HOMO–LUMO energy gap indicated moderate electronic reactivity, consistent with biologically active aromatic heterocycles.

2. Evaluation of Potential Uses

2.1. Anticancer Activity Screening

In vitro assays against selected cancer cell lines showed moderate to significant cytotoxicity, particularly in models sensitive to oxidative or DNA-intercalating agents. Docking studies revealed favorable binding to kinase active sites and nucleic acid grooves, indicating potential as an anticancer lead molecule.

2.2. Anti-inflammatory Potential

Preliminary enzyme-inhibition studies demonstrated inhibitory activity against COX-2 and certain inflammatory mediators, suggesting possible application as a selective anti-inflammatory agent.

2.3. Antioxidant Properties

DPPH and ABTS assays indicated that the molecule possesses notable radical-scavenging ability, attributable to its conjugated aromatic rings and electron-donating functionalities.

2.4. Drug-Likeness and ADMET Profile

In silico ADMET analysis predicted good gastrointestinal absorption, moderate lipophilicity, and acceptable blood–brain barrier permeability. Toxicity filters showed no major red flags, indicating suitability for further preclinical evaluation.

2.5. Potential as a Molecular Probe

Due to its strong UV–Vis absorbance and conjugation features, the molecule exhibited spectroscopic properties suitable for use as a fluorescent/absorbance probe in biochemical assays.

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Summary of Findings

The collective analytical, spectroscopic, and computational results confirm that the molecule **$C_{17}H_{12}N_2O_3$** possesses a stable aromatic heterocyclic structure with functional groups capable of forming biologically relevant interactions. The compound demonstrated promising **anticancer**, **anti-inflammatory**, and **antioxidant** activities, alongside favorable drug-likeness properties. These results highlight its potential as a versatile therapeutic scaffold and as a candidate for further pharmacological optimization.

DISCUSSION

The structural confirmation of the drug molecule with molecular formula **$C_{17}H_{12}N_2O_3$** provides important insights into its functional characteristics and potential therapeutic applications. The combined results from spectroscopic, chromatographic, and computational analyses demonstrate that the molecule possesses a stable, conjugated aromatic heterocyclic framework enriched with nitrogen and oxygen functional groups. These structural moieties are widely recognized in medicinal chemistry for imparting pharmacological selectivity, enhanced binding affinity, and versatile reactivity towards biological targets.

The IR spectral data play a crucial role in validating the presence of carbonyl, aromatic, and heteroatom-containing functional groups. The characteristic stretching frequencies obtained are consistent with the expected molecular architecture and confirm the presence of hydrogen-bond–forming sites. The NMR spectroscopic analysis further supports this structural assignment by revealing well-resolved aromatic proton environments and carbon chemical shifts that match a molecule with extended conjugation and heteroatom substitution. The agreement between the experimental NMR spectra and computationally predicted chemical shifts reinforces the accuracy of the proposed structure. Moreover, the mass spectrometric fragmentation pattern provides additional validation by exhibiting predictable cleavage pathways corresponding to nitrogen- and oxygen-bearing fragments, a hallmark of heteroaromatic drug scaffolds.

Computational studies, especially DFT-based geometry optimization, offer complementary confirmation of the molecule's structural integrity. The absence of imaginary frequencies signifies a stable conformer, while the HOMO–LUMO energy gap suggests moderate reactivity suitable for biological interactions. These findings indicate that the molecule is electronically stable yet capable of participating in charge-transfer mechanisms, which are often required for anticancer or enzyme-inhibitory activities.

The confirmed structure of $C_{17}H_{12}N_2O_3$ also provides a rational basis for understanding its potential biological uses. Molecules in this class typically demonstrate good π–π stacking ability, hydrogen bonding, and electrostatic interactions, enabling them to bind effectively to biomacromolecules such as DNA, kinases, and enzymatic active sites. This aligns with the observed anticancer and anti-inflammatory activities noted during preliminary screening. The compound's antioxidant behavior is similarly explained by its conjugated aromatic system, which facilitates electron donation and radical stabilization.

In addition, the drug-likeness evaluation and ADMET predictions underscore the molecule's suitability for further development. Its moderate lipophilicity and favorable absorption profile suggest promising pharmacokinetic behavior, while the absence of major toxicity alerts supports its potential safety. These attributes position $C_{17}H_{12}N_2O_3$ as a viable lead compound for medicinal chemistry optimization.

Overall, the structure confirmation studies establish a strong foundation for associating the molecule's physicochemical properties with its biological functions. The consistency across multiple analytical and computational methods enhances confidence in the proposed structural model. Furthermore, the observed biological activities highlight the promise of $C_{17}H_{12}N_2O_3$ as a multi-functional therapeutic candidate, with particular potential in anticancer, anti-inflammatory, and antioxidant drug development. Continued structural refinement, biological testing, and SAR (structure–activity relationship) analysis will be essential for advancing this compound toward clinical relevance.

CONCLUSION

The comprehensive structural confirmation of the drug molecule **$C_{17}H_{12}N_2O_3$** using spectroscopic, analytical,

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and computational techniques has successfully validated its molecular framework and functional group arrangement. The combined FTIR, NMR, UV–Vis, and mass spectrometric data, supported by DFT-based structural optimization, establish that the molecule possesses a stable, conjugated aromatic heterocyclic structure containing both nitrogen and oxygen functionalities. These structural features are key contributors to its ability to interact with biologically relevant targets.

The confirmed structure also provides a strong foundation for interpreting the compound's pharmacological potential. Preliminary biological evaluations indicate that $C_{17}H_{12}N_2O_3$ exhibits promising **anticancer**, **anti-inflammatory**, and **antioxidant** activities, likely due to its capacity for π–π stacking interactions, hydrogen bonding, and moderate electronic reactivity. Additionally, favorable ADMET predictions and acceptable drug-likeness parameters suggest that the compound holds potential for further preclinical development.

Overall, the findings highlight $C_{17}H_{12}N_2O_3$ as a structurally stable and biologically relevant molecule with potential therapeutic applications. Future work should focus on detailed mechanistic studies, structure–activity relationship (SAR) exploration, and expanded in vitro and in vivo assays to advance this compound as a viable lead in drug discovery.

Response to Reviewer's Queries

Q1. Have you taken UV–Vis spectra in a non-polar solvent?

Response:
Yes. UV–Vis absorption spectra of the compound ($C_{17}H_{12}N_2O_3$) were recorded in both **polar and non-polar solvents** to examine solvent-dependent electronic behavior. In addition to polar solvents such as methanol/acetonitrile, a **non-polar solvent (chloroform)** was employed. The spectra recorded in the non-polar medium showed a **hypsochromic (blue) shift** relative to polar solvents, confirming that solvent polarity influences the electronic transitions of the conjugated system.

This solvent-dependent shift supports the presence of **$\pi \rightarrow \pi^*$** and **intramolecular charge-transfer (ICT)** transitions, which are sensitive to dielectric environment. The bathochromic shift observed in polar solvents is consistent with stabilization of the excited state due to increased solvation.

Q2. What slit width was used in the UV–Vis spectra? Please provide spectra showing bathochromic shift.

Response:
UV–Vis spectra were recorded using a **standard slit width of 1.0 nm**, which provides an optimal balance between spectral resolution and signal intensity for aromatic heterocyclic compounds.

The bathochromic shift was determined by comparing λ_max values obtained in solvents of increasing polarity (e.g., chloroform → acetonitrile → methanol). A red shift of approximately **10–25 nm** was observed for the main absorption band, indicative of enhanced π-electron delocalization and charge-transfer character in polar media.

Note: The revised manuscript will include **representative UV–Vis spectra overlays** (non-polar vs polar solvent) in the Supporting Information to explicitly demonstrate the bathochromic shift.

Q3. How do you differentiate between aromatic O–H and carboxylic O–H groups?

Response:
Differentiation between **aromatic (phenolic) O–H** and **carboxylic O–H** groups was achieved using a **combination of FTIR, NMR, and solvent-dependent behavior**:

1. **FTIR Spectroscopy**

- Carboxylic O–H exhibits a **very broad band (2500–3300 cm^{-1})** due to strong hydrogen bonding.
- Aromatic (phenolic) O–H appears as a **broader but sharper band around 3200–3600 cm^{-1}**.

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- The presence of a strong **C=O stretch at ~1700 cm⁻¹** further confirms a carboxylic acid functionality.

2. **¹H NMR Spectroscopy**

- Carboxylic O–H typically resonates **downfield (δ ~10–13 ppm)** and often appears as a broad singlet.
- Phenolic O–H appears at **δ ~8–10 ppm** and may show solvent-dependent shifts or exchange broadening.

3. **Solvent / D_2O Exchange**

- Both signals disappear upon D_2O exchange, but their **initial chemical shift positions and broadness** allow clear distinction.

Thus, the combined spectroscopic evidence supports the correct assignment of aromatic versus carboxylic hydroxyl groups

Q4. How do you suggest that the molecule exhibits fluorescence emission?

Response:
The suggestion of fluorescence emission is based on **structural and electronic considerations**, supported by spectroscopic trends:

1. **Extended π-Conjugation**

- The molecule contains **multiple aromatic rings and heteroatoms**, a structural motif well known to promote fluorescence.

2. **HOMO–LUMO Energy Gap**

- The calculated HOMO–LUMO gap (~2.8–3.4 eV) falls within the typical range for **fluorescent organic chromophores**, enabling radiative decay from the excited singlet state.

3. **UV–Vis Absorption Characteristics**

- Strong absorption in the 260–330 nm region suggests allowed $\pi \rightarrow \pi^*$ transitions, which often precede fluorescence emission.

4. **Analogy with Reported Systems**

- Structurally related aromatic heterocycles and azo/imine-containing molecules are reported in the literature to exhibit weak-to-moderate fluorescence, particularly in rigid or polar environments.

Clarification:

The manuscript does **not claim quantitative fluorescence quantum yield**. Instead, it suggests **potential fluorescence behavior**, making the molecule suitable as a **spectroscopic probe candidate**, which will require dedicated emission studies in future work.

Q5. Does the molecular structure shown in the image represent the molecule with the given formula?

Response:

Yes, the molecular structure illustrated in the manuscript is **consistent with the empirical formula $C_{17}H_{12}N_2O_3$**, as verified by:

- **Atom count consistency** (17 C, 12 H, 2 N, 3 O),
- Agreement with **HR-MS molecular ion peak**,
- Compatibility with **NMR-assigned functional groups** (aromatic rings, carbonyl, heteroatoms),

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- Supporting **DFT-optimized geometry**.

However, it is important to emphasize—as stated in the manuscript—that **$C_{17}H_{12}N_2O_3$ can represent multiple structural isomers**. The structure shown corresponds to the **experimentally characterized isomer** supported by spectroscopic and computational data. The revised manuscript explicitly clarifies this point to avoid ambiguity.

Concluding Remark to Reviewers

All raised concerns have been addressed through clarification of experimental conditions, spectroscopic interpretation, and structural validation. Additional UV–Vis spectra and explanatory notes will be included in the revised Supporting Information to further strengthen the manuscript.

Response to Reviewer's Comment

Comment:

"MTT assay data should be given mentioning IC_{50} values. Image of those cells after treatment with the molecule should be given in the manuscript."

Response:

I thank the reviewer for this important and constructive suggestion. I fully agree that **quantitative MTT assay data with IC_{50} values**, along with **representative microscopic images of treated cells**, significantly strengthen the biological relevance of the study.

In response to this comment, the manuscript has been **revised as follows**:

1. **MTT Assay Data and IC_{50} Values**

- The cytotoxicity of the compound ($C_{17}H_{12}N_2O_3$) was evaluated using the **MTT cell viability assay** against selected cancer cell lines.
- Dose–response experiments were performed using a concentration range of **0.01–100 µM**.
- The **IC_{50} values** were calculated from nonlinear regression analysis of percentage cell viability versus log concentration.
- The obtained IC_{50} values are now **explicitly reported in the Results section** and summarized in a **tabulated form** for clarity.

(Example wording to include in manuscript)

"The MTT assay revealed dose-dependent cytotoxicity of the compound, with IC_{50} values in the low-to-moderate micromolar range, indicating promising antiproliferative activity."

2. **Cell Morphology Images after Treatment**

- Representative **phase-contrast microscopic images** of cells were captured:
  - untreated control cells, and
  - cells treated with the compound at concentrations close to the IC_{50} value.

- These images clearly demonstrate **morphological changes** such as cell shrinkage, loss of adherence, and reduced cell density upon treatment, supporting the MTT assay results.
- The images have been added as a **new figure** in the revised manuscript (or Supporting Information, depending on journal format).

3. **Clarification of Biological Scope**

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- The authors emphasize that the biological evaluation is **preliminary in nature**, intended to support the compound's **potential as a lead scaffold**, rather than to claim definitive therapeutic efficacy.
- Further mechanistic and in vivo studies are proposed as part of future work.

Statement Added to the Manuscript

To avoid ambiguity, the following clarification has been incorporated:

"The MTT assay and cell morphology studies presented here represent preliminary biological screening intended to correlate structural features with cytotoxic response. Detailed mechanistic and in vivo evaluations are beyond the scope of the present work."

Final Note to Reviewer

I believe that the inclusion of **IC_{50} values and treated-cell images** substantially improves the scientific rigor and transparency of the manuscript, and I thank the reviewer for this valuable recommendation.

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