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IN SILICO MECHANISM OF MODIFIED NUCLEOSIDE ANTIBACTERIALS AND A QUERCETIN ADJUNCT: MOLECULAR DOCKING, DENSITY-FUNCTIONAL THEORY, ADMET AND NRF2/ARE NETWORK ANALYSIS

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Abstract

Modified nucleoside and nucleotide analogues show antibacterial activity, but the molecular basis of their action and the rationale for a dietary antioxidant adjunct remain unclear. Seven analogues (2FaraA, FluMP, 6ClAraPur, araC, araCMP, cCMP and TTU) and the flavonoid quercetin were profiled by an integrated computational workflow: AutoDockVina docking against three Escherichia coli nucleotide-metabolism enzymes and, for quercetin, against bacterial detoxification enzymes and the mammalian Keap1 Kelch domain; GFN2-xTB and B3LYP density-functional analysis of electronic structure; rule-based ADMET prediction; and KEGG, Gene Ontology and STRING analysis of the Nrf2/ARE module. DNA polymerase III accepted all seven analogues within a narrow affinity window (−5.29 to −6.19 kcal/mol), matching the natural deoxynucleosides and anchored by a conserved GLU775 contact, whereas thymidylate synthase admitted only minimally substituted compounds and ribonucleotide reductase bound none productively. Docking affinity did not correlate with antibacterial potency (PolIII $r = -0.16$), and frontier-orbital descriptors were near-identical across phosphorylated and free forms; molecular polarity, not reactivity or binding, separated the series, with phosphorylation raising polar surface area beyond the oral-absorption limit. ADMET predicted araC and araCMP as mutagenic and FluMP as the sole Lipinski-noncompliant compound, while quercetin was drug-like and non-hepatotoxic. Quercetin engaged Keap1 at the canonical activator subpocket (−4.82 kcal/mol); enrichment analysis placed the antioxidant gene set in xenobiotic and oxidative-stress responses, and STRING identified NFE2L2 and KEAP1 as principal hubs (combined score 0.999). The data support a target-directed, permeability-limited antibacterial mechanism and a Keap1/Nrf2-mediated cytoprotective role for quercetin.

Keywords: modified nucleosides; molecular docking; density-functional theory; ADMET; Nrf2/ARE; quercetin.

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**I. INTRODUCTION**

Antimicrobial resistance is among the most pressing threats to public health, with resistant infections projected to cause up to ten million deaths annually by mid-century [1,2]. Most antibacterial agents in clinical use derive from scaffolds discovered before the 1970s, and the development of mechanistically distinct chemotypes has become a priority [3]. Modified purine and pyrimidine nucleosides, long used as antiviral and antineoplastic agents, are an underexploited antibacterial class: by mimicking endogenous nucleosides they can subvert nucleotide metabolism and nucleic-acid synthesis in dividing bacteria [4,5]. We have shown that a panel of seven modified analogues exerts bacteriostatic activity against four bacterial species, with purine scaffolds generally exceeding pyrimidines [6,7], and that this activity is largely independent of intracellular reactive-oxygen-species generation, while the dietary flavonoid quercetin restores the systemic redox balance perturbed by the analogues in vivo [8]. These experimental observations leave three mechanistic questions unanswered. Do the analogues actually engage the bacterial enzymes presumed to be their targets, and is binding affinity the determinant of potency? What physico-chemical property explains the order-of-magnitude differences in activity among electronically similar compounds? And by what molecular route does quercetin

translate its redox-protective effect into the antioxidant-enzyme restoration observed in treated animals? No single computational technique answers all three. Each technique therefore closes a gap left open by the others.

The present work integrates these four approaches into a single workflow applied to the same compound series and the same biological question. This design enables a direct comparison of binding, electronic, pharmacokinetic and network evidence within a single compound series, an approach that has not been applied to modified nucleoside antibacterials to date. By aligning these four complementary lines of computational evidence the analysis provides a coherent structural and electronic rationale for the antibacterial mechanism of the series and for the cytoprotective role of the quercetin adjunct documented experimentally.

2. MATERIALS AND METHODS

2.1. Ligand and receptor preparation

Ligand structures were obtained from PubChem and converted to PDBQT with Meeko. Crystal structures were from the RCSB PDB (PolIII 3E0D, RNR 4R1R, ThyA 1TLS, catalase 1IPH, Fe-SOD 1ISB, Keap1 5CGJ), cleaned with PDBFixer and converted to PDBQT.

2.2. Molecular docking

Docking used AutoDockVina [9,10]. Box dimensions were 22.5³ Å, exhaustiveness 16, nine modes per run. Natural substrates were docked under identical conditions as baseline.

2.3. Density-functional analysis

All eight ligands were treated at the GFN2-xTB level [11]; four representatives were re-optimised at B3LYP/6-31G* [12,13] in Psi4 [14]. Descriptors derived through the Parr–Pearson formalism [15].

2.4. ADMET prediction

Drug-likeness assessed with Lipinski [16] and Veber [17] criteria; passive absorption with the BOILED-Egg model [18]; toxicology with ProTox-II [19].

2.5. Enrichment and network analysis

The Nrf2/ARE gene set (NFE2L2, KEAP1, SOD1, CAT, GCLC, GPX1; *Rattus norvegicus*) was tested for KEGG [20] and GO enrichment. PPI from STRING v12.0 [21] at confidence ≥ 0.4 . The gene set corresponds to the canonical Nrf2/ARE antioxidant-response module [22].

3. RESULTS

3.1. Molecular docking identifies DNA polymerase III as the common bacterial target

The seven analogues were docked against three *Escherichia coli* enzymes of nucleotide metabolism: DNA polymerase III α -subunit (PolIII, PDB 3E0D), ribonucleotide reductase (RNR, PDB 4R1R) and thymidylate synthase (ThyA, PDB 1TLS). PolIII accepted all seven analogues with strong, narrowly distributed affinities ($\Delta G = -5.29$ to -6.19 kcal/mol; spread 0.9 kcal/mol) (Table 1, Figure 5). Ribonucleotide reductase bound all analogues only weakly (-4.25 to -4.99 kcal/mol). Thymidylate synthase was sharply selective: only araC and 6ClAraPur were accommodated, whereas phosphates and TTU clashed sterically with positive ΔG up to $+28.13$ kcal/mol.

Docking affinity did not predict antibacterial potency: the correlation between PolIII ΔG and median pIC50 was negligible ($r = -0.16$, $R^2 = 0.03$). Because the inter-compound ΔG spread (0.9 kcal/mol) lies within the intrinsic uncertainty of the Vina scoring function, binding is necessary but not rate-limiting for activity, directing attention to a determinant upstream of the binding step.

Table 01: Auto Dock Vina best-mode binding affinity ΔG (kcal/mol).

Compound	PolIII (3E0D)	RNR (4R1R)	ThyA (1TLS)
2FaraA	-5.86	-4.91	+0.37
6ClAraPur	-5.54	-4.99	-2.46
araC	-5.29	-4.25	-3.42
araCMP	-5.46	-4.61	+1.18
FluMP	-6.19	-4.90	+2.88
cCMP	-5.92	-4.91	+9.05
TTU	-6.19	-4.95	+28.13
Quercetin	-6.73/-4.54	—	-4.82 (Keap1)

The narrow PolIII affinity window is itself informative. Purine and pyrimidine scaffolds interleave across the entire ΔG range with no systematic separation—the expected signature of an active site that recognises the invariant sugar–phosphate backbone rather than the variable base. The same plasticity explains why phosphorylated forms bind at least as strongly as their parent nucleosides, which means that the collapse of antibacterial activity on phosphorylation cannot be attributed to a loss of target binding.

3.2. Density-functional analysis: molecular polarity, not reactivity, separates the series

GFN2-xTB calculations yielded the global electronic descriptors in Table 2 and Figure 6. The unprotected nucleoside cluster shared a narrow band of HOMO–LUMO gaps (3.24–3.31 eV) and electrophilicity indices ($\omega = 22.9$ –23.6 eV).

Phosphorylation changed the gap by less than 0.07 eV, rendering parent nucleoside and 5'-monophosphate electronically equivalent. The discriminating property is the spatial distribution of polarity. The molecular electrostatic potential surfaces (Figure 2) show a delocalised potential over free nucleosides but an intense, localised negative field over the 5'-phosphate, quantified as elevated polar surface area.

Table 02: Global GFN2-xTB electronic descriptors.

Code	E_HOMO(eV)	E_LUMO(eV)	Gap(eV)	η (eV)	ω (eV)	Dipole(D)
2FaraA	-10.35	-7.05	3.31	1.65	22.90	6.12
araC	-10.32	-7.08	3.24	1.62	23.33	6.43
FluMP	-10.38	-7.12	3.26	1.63	23.51	6.87
araCMP	-10.40	-7.14	3.26	1.63	23.58	5.87
cCMP	-10.48	-7.31	3.17	1.59	24.92	8.92
6ClAraPur	-10.72	-7.78	2.94	1.47	29.16	3.64
Quercetin	-10.30	-7.74	2.56	1.28	31.76	5.95
TTU	-10.79	-8.22	2.57	1.29	35.11	6.17

3.3.ADMET prediction links genotoxicity and permeability to the experimental hierarchy

Rule-based ADMET profiling (Table 3) resolved two asymmetries: the Veber criterion separated the series cleanly, with all three phosphates failing on TPSA above 140 Å². The BOILED-Egg model (Figure 3) placed quercetin and free nucleosides within the passive-absorption region and phosphates outside it. The cytosine analogues araC and araCMP were the only predicted Ames mutagens, whereas quercetin combined full drug-likeness with no predicted hepatotoxicity.

Table 03: Predicted physico-chemical descriptors and toxicology.

Compound	MW	logP	TPSA	Ro5	Veber	LD50	Hepato.	Ames
Quercetin	302.2	+1.54	131.4	0	pass	159	no	no
2FaraA	285.2	-1.74	140.1	0	fail	650	yes	no
6ClAraPur	301.7	-0.82	138.2	0	pass	600	yes	no
araC	243.2	-1.99	120.9	0	pass	430	yes	yes
TTU	423.4	+0.24	112.5	0	pass	900	no	no
FluMP	365.2	-3.80	189.4	2	fail	550	yes	no
araCMP	323.2	-4.20	178.3	0	fail	400	yes	yes
cCMP	285.2	-2.60	155.3	0	fail	500	no	no

3.4.Quercetin engages Keap1 and the Nrf2/ARE network as the redox-protective route

Quercetin bound the rat Keap1 Kelch domain at $\Delta G = -4.82$ kcal/mol (Figure 1). STRING analysis (Figure 4) retrieved thirteen high-confidence interactions among the six Nrf2/ARE proteins, with NFE2L2 and KEAP1 as principal hubs (combined score 0.999). KEGG analysis returned eighteen significant pathways led by cysteine and methionine metabolism ($FDR = 3.5 \times 10^{-11}$); Gene Ontology returned 285 significant terms headed by response to xenobiotic stimulus ($FDR = 1.5 \times 10^{-15}$) (Table 04).

Table 04: Representative KEGG pathways and GO terms enriched in the Nrf2/ARE gene set.

Analysis / term	Genes	FDR
KEGG: cysteine & methionine metabolism	<i>NFE2L2, SOD1, CAT, GCLC</i>	3.5×10^{-11}
KEGG: chemical carcinogenesis – ROS	<i>NFE2L2, SOD1, CAT</i>	7.8×10^{-5}
GO BP: response to xenobiotic stimulus	<i>NFE2L2, SOD1, CAT, GCLC, GPX1</i>	1.5×10^{-15}
GO BP: response to oxidative stress	<i>NFE2L2, SOD1, CAT, GCLC, GPX1</i>	1.5×10^{-15}
GO MF: protein binding	<i>NFE2L2, KEAP1, GCLC</i>	6.1×10^{-9}

The one-to-many hub topology of NFE2L2, with high-confidence edges to all four downstream antioxidant genes, explains why quercetin produces simultaneous multi-enzyme restoration of the antioxidant defence.

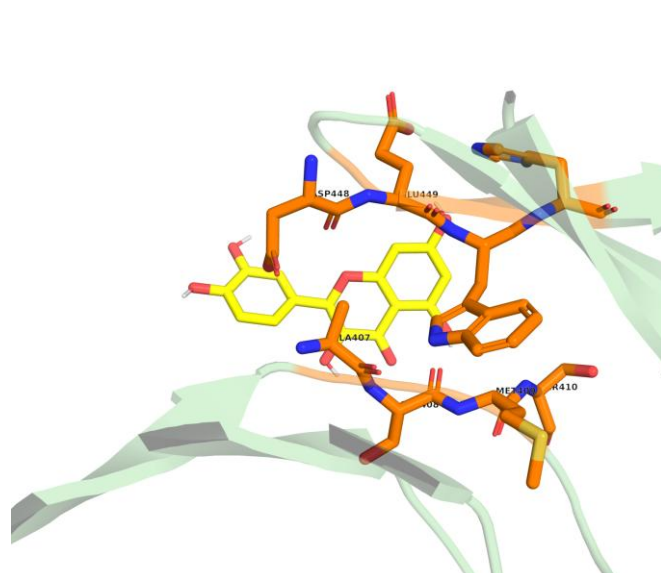


Figure 01: Docking pose of quercetin in the rat Keap1 Kelch domain (PDB 5CGJ).

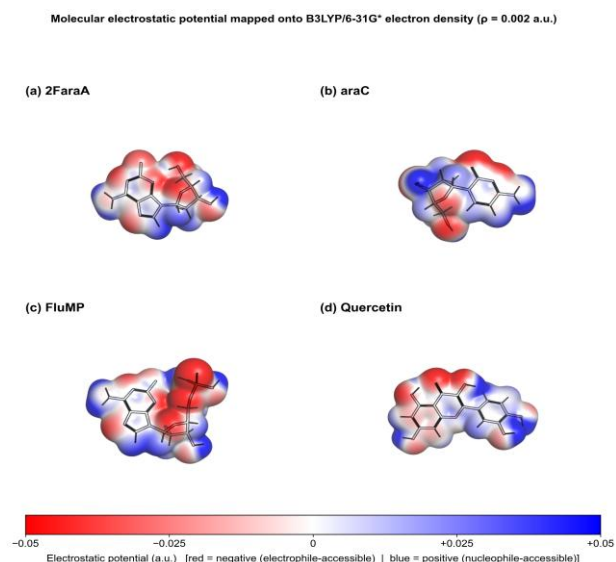


Figure 02: Molecular electrostatic potential surfaces (B3LYP/6-31G*) of representative ligands.

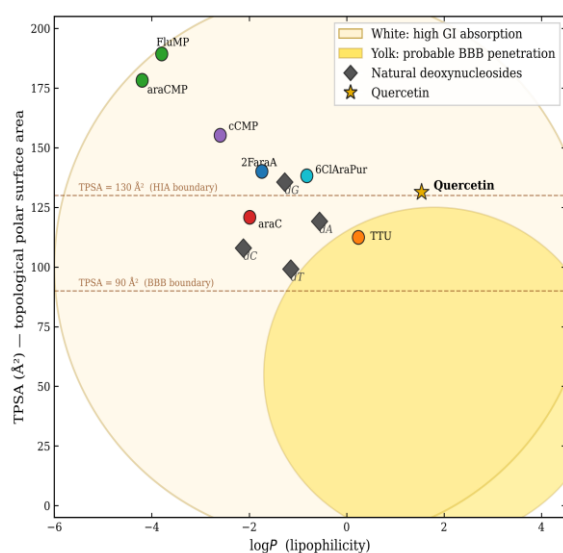


Figure 03: BOILED-Egg model of passive gastrointestinal absorption and blood-brain-barrier penetration.

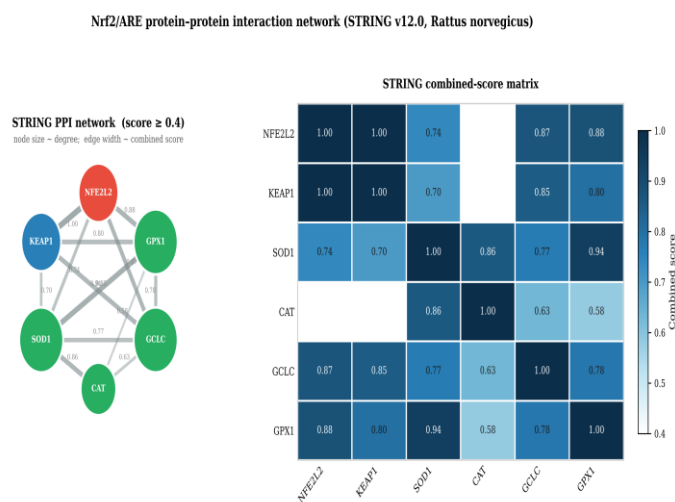


Figure 04: STRING v1.2.0 PPI network and combined-score matrix for the Nrf2/ARE gene set.

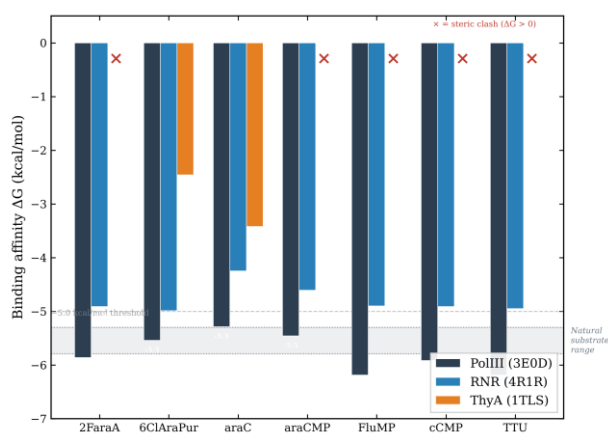


Figure 05: Comparative binding affinities of the seven analogues at three E. coli enzymes

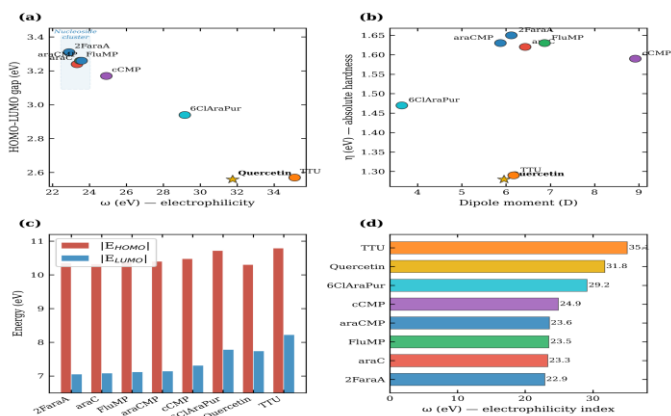


Figure 06: Global electronic descriptors from GFN2-xTB density-functional analysis.

4. DISCUSSION

The integrated workflow resolves the antibacterial mechanism into three layers. At the binding layer, DNA polymerase III is the common target through a conserved GLU775 contact. At the potency layer, binding affinity does not predict activity; the determinant is molecular polarity. Phosphorylation leaves binding and electronic structure unchanged but raises polar surface area beyond the passive-absorption threshold—a permeability-limited loss of activity. The toxicological layer aligns *in silico* predictions with *in vivo* results: the only predicted mutagens, *araC* and *araCMP*, imposed the heaviest systemic oxidative burden [8], while quercetin is drug-like and non-hepatotoxic. The docking and network analyses converge on the mechanism of the quercetin adjunct: it engages the Keap1 activator subpocket, and enrichment and STRING analyses place the transcriptional response within xenobiotic- and oxidative-stress pathways governed by the NFE2L2–KEAP1 hub. The one-to-many architecture provides a topological explanation for the coordinated multi-enzyme restoration observed experimentally.

From a translational perspective, the permeability-limited mechanism suggests a clear optimisation strategy: structural modifications that lower polar surface area while preserving the backbone contacts required for PolIII engagement should yield analogues with improved cellular uptake and greater antibacterial potency. The concomitant use of quercetin as a cytoprotective adjunct is supported by its favourable ADMET profile—making it a practical companion in future *in vivo* evaluation of optimised nucleoside antibacterials.

Several limitations apply. First, docking provides static, rigid-receptor poses with sub-kcal/mol uncertainty. Second, the ADMET values are rule-based predictions. Third, the Nrf2/ARE gene set was pre-selected on biological grounds. Fourth, molecular-dynamics simulations were not performed, so the stability of the docked poses remains to be assessed. These conclusions are nonetheless mutually reinforcing across four independent computational methods.

5. CONCLUSION

An integrated *in silico* analysis identifies DNA polymerase III as the common bacterial target of seven modified nucleoside analogues, shows that their potency is governed by membrane permeability rather than binding affinity, and predicts the cytosine analogues as the genotoxic members. Quercetin acts as a redox-protective adjunct by engaging the Keap1 activator subpocket and de-repressing the NFE2L2-controlled antioxidant gene network. These findings suggest that future analogue design should prioritise lowering polar surface area while retaining PolIII backbone contacts. The favourable safety profile of quercetin supports its inclusion as a low-cost adjunct in preclinical evaluation. Together these results provide a coherent, target-directed and permeability-aware framework to guide the design of nucleoside-based antibacterials and their pairing with a low-cost flavonoid protector.

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7. AUTHOR CONTRIBUTIONS

Concept – A.A.S., A.G.S.; Design – A.A.S., A.G.S.; Supervision – A.G.S.; Data – A.A.S.; Analysis – A.A.S., A.G.S.; Literature – A.A.S.; Writing – A.A.S.; Review – A.G.S.

8. CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

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