AFMN BIOMEDICINE

REVIEW ARTICLE

FOLIC ACID AND RELATED FOLATES AS PROPOSED INHIBITORS OF ORTHOPOXVIRUS MRNA CAP METHYLTRANSFERASES: A NARRATIVE REVIEW OF EVIDENCE AND LIMITATIONS

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Monkeypox virus (MPXV) replicates in the host cell cytoplasm and must therefore encode its own messenger RNA capping machinery, which makes the viral cap methyltransferases attractive antiviral targets. Folic acid has recently been proposed as an inhibitor of these enzymes and, on that basis, as a candidate treatment for MPXV. This narrative review examines that proposition and the level of evidence supporting it. PubMed, Scopus, and Google Scholar were searched through May 2026; no original experimental work was undertaken. The proposal rests almost entirely on a single virtual screening study in which folic acid emerged as a high-scoring ligand in molecular docking and molecular dynamics simulations, supported indirectly by reports that folates and folate antagonists modulate the replication of unrelated viruses. No published study demonstrates concentration-dependent inhibition of a purified orthopoxvirus methyltransferase by folic acid, and no kinetic parameters have been determined. Folate biochemistry offers no straightforward mechanism because S-adenosylmethionine, rather than folate, is the methyl donor for these enzymes and folate contributes only indirectly by supplying that pool. Furthermore, reported antiviral activity at millimolar concentrations exceeds achievable plasma exposures by approximately three orders of magnitude. By contrast, sinefungin-derived inhibitors of the MPXV 2′-O-methyltransferase VP39 have been characterized structurally, inhibit the purified enzyme at submicromolar concentrations, and suppress viral replication in cell culture, confirming that this machinery is druggable. Folic acid is therefore best described as a computationally derived hypothesis requiring enzymatic validation, rather than an established methyltransferase inhibitor or a therapy for MPXV.

Keywords: monkeypox virus, MPXV, mRNA cap methyltransferase, VP39, folic acid

Submitted: April 28, 2026 **Revised:** September 9, 2026
Accepted: September 23, 2026
Published online: October 1, 2026
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INTRODUCTION

Monkeypox virus (MPXV) is a double-stranded DNA virus of the genus *Orthopoxvirus*, family *Poxviridae*, alongside variola, vaccinia, and cowpox viruses (1–3). Two clades are recognized: clade I, comprising subclades Ia and Ib, and clade II, comprising subclades IIa and IIb (4,5). These correspond broadly to the historical Congo Basin (Central African) and West African lineages (6). Clade I infection has been associated with more severe disease and higher case fatality than clade II, whereas the 2022 global outbreak was driven predominantly by clade IIb (1,4).

Because orthopoxviruses replicate entirely in the cytoplasm, they cannot use the host nuclear capping apparatus and must encode a complete mRNA capping and cap-methylation system of their own (7,8). The 5′ cap protects viral transcripts from degradation, supports cap-dependent translation, and, through 2′-O-methylation in particular, allows viral mRNA to be read as self and so evade cytoplasmic innate immune sensors (7,9). This dependence makes the viral cap methyltransferases (MTases) an attractive antiviral target, and inhibitors of the MPXV 2′-O-MTase VP39 have already been shown to suppress viral replication in cell culture, as presented in Figure 1 (9–11).

Against this background, a 2024 virtual screening study of Middle Eastern plant-derived compounds reported that folic acid occupied a favorable position within the active site of an MPXV MTase model and achieved better docking and MM-GBSA scores than the pan-MTase reference inhibitor sinefungin (12). That observation has since been reiterated in the secondary literature, sometimes accompanied by progressively stronger claims. The purpose of the present review is therefore narrow and deliberate: to set out what is and is not established about folates as orthopoxvirus MTase ligands, to place that evidence in a defined hierarchy, and to specify the experiments that would be needed before folic acid could reasonably be described as an inhibitor, let alone a treatment.

Epidemiological Context and Clinical Management

First identified in 1958 in captive monkeys in Copenhagen, MPXV gave rise to the name monkeypox; the first human case was recognized in the Democratic Republic of the Congo in 1970 (6,13). For several decades, human infection remained largely confined to forested regions of Central and West Africa (14). In 2022, sustained human-to-human transmission of clade IIb produced a multi-country outbreak affecting more than 100 countries, which the World Health Organization (WHO) designated a public health emergency of international concern (PHEIC) in July 2022 (1,15).

The epidemiological situation has since changed in ways directly relevant to therapeutic development. A novel clade I lineage, designated clade Ib, was identified in eastern DRC in 2023–2024 and spread to neighboring and then more distant countries, prompting a second PHEIC declaration on

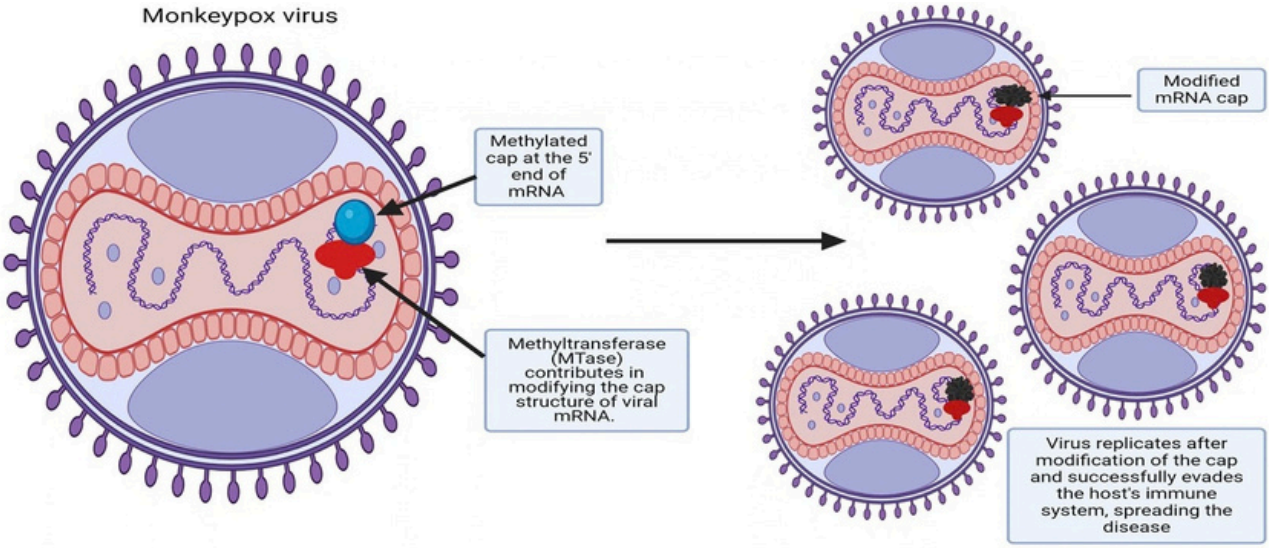


Figure 1. *Monkeypox virus structure and the role of mRNA cap methylation. Methyltransferase-mediated modification of the 5′ cap of viral mRNA supports transcript stability and cap-dependent translation. This modification allows viral mRNA to evade cytoplasmic innate immune sensing, permitting productive replication. The responsible enzymes are identified in Table 1. Image created with BioRender.com.*

14 August 2024 (5,16). That emergency was lifted on 5 September 2025 following sustained declines in case numbers, although WHO advised that this did not signal the end of the threat and extended its standing recommendations for MPXV to 20 August 2026 (16). Clades I and II and their subclades continue to co-circulate, and Africa CDC has maintained its continental emergency designation (16).

Two implications follow for the present review. First, any candidate agent must eventually be evaluated against clade Ia, Ib, and IIb isolates rather than a single laboratory strain. Second, and reassuringly for a cap-MTase strategy, the capping enzymes are among the most highly conserved orthopoxvirus proteins, so target sequence variation between clades is expected to be limited, although this expectation has not been formally tested for any of the folate-related work discussed below, and all published VP39 inhibitor studies to date have used clade IIb isolates (9,11). This is a substantive gap rather than a technicality.

Clinically, MPXV resembles a milder form of smallpox, with lymphadenopathy as a distinguishing feature (1). The incubation period is typically 7–14 days (range 5–21 days), and illness begins with fever, headache, myalgia, backache, lymphadenopathy, and fatigue (17). Rash follows within 1–3 days,progressing through macular, papular, vesicular, pustular, and crusted stages (18). Complications include secondary bacterial infection, keratitis and vision loss, pneumonitis, and encephalitis (1). Disease is usually self-limiting over 2–4 weeks, but severity depends on clade, route of infection, age, and immune status, with people with advanced HIV at particularly high risk (19). Transmission occurs through direct contact with lesions, body fluids, respiratory secretions, or contaminated materials, and, in the 2022 and clade Ib outbreaks, substantially through sexual networks (1,5).

Regarding the current clinical management of MPXV, WHO issued updated guidance on the clinical management of mpox and associated infection prevention and control in 2025 (20). The central message of that guidance is that care is primarily supportive: management of pain, of skin and mucosal lesions, of secondary bacterial infection, of hydration and nutrition, and of underlying conditions, particularly HIV.

Tecovirimat (ST-246, TPOXX), which inhibits the F13 (VP37) envelope protein and thereby blocks extracellular virion formation, was for several years the presumptive antiviral of choice based on in vitro and animal data. That position is no longer tenable. In the randomized, placebo-controlled PALM007 trial of 597 children and adults with laboratory-confirmed clade I MPXV in the DRC, tecovirimat did not reduce time to lesion resolution (median 7 days versus 8 days); overall mortality was 1.6%, substantially lower than typically reported in the region and attributed to hospitalization with high-quality supportive care (21). The STOMP trial in mild-to-moderate clade II MPXV likewise did not demonstrate clinical benefit and was stopped early. The European Medicines Agency initiated a review of tecovirimat in July 2025 in light of these data. In the United States, tecovirimat for MPXV remains investigational and is accessible through an expanded-access protocol for selected patients, rather than as an approved therapy.

Cidofovir and its lipid conjugate brincidofovir inhibit the viral DNA polymerase and have activity in animal models, but no controlled clinical efficacy data exist for MPXV; cidofovir carries significant nephrotoxicity and brincidofovir hepatic and gastrointestinal toxicity (22,23). Vaccinia immune globulin has been used in severe disease without controlled evidence. Vaccines, principally MVA-BN and LC16m8, remain the mainstay of prevention.

The correct summary of the current position is therefore that no antiviral agent has demonstrated clinical efficacy against MPXV in a randomized controlled trial. This strengthens rather than weakens the case for pursuing new mechanistic targets, but it also raises the standard of evidence that any new candidate must meet and makes premature therapeutic claims correspondingly more damaging.

Defining the Target: Orthopoxvirus mRNA Cap Methyltransferases

A recurring weakness of the earlier literature in this field is imprecision about which enzyme is being discussed. Orthopoxvirus cap maturation involves two distinct methylation reactions catalyzed by two distinct enzymes; these reactions must not be conflated (Table 1).

Cap-0 formation. The heterodimeric vaccinia capping enzyme comprises the large D1 subunit (D1R; MPXV homologue E1) and the small D12 subunit (D12L; MPXV homologue E12). The N-terminal region of D1 carries RNA 5′-triphosphatase and guanylyltransferase activities, while the C-terminal domain carries the cap (guanine-N7)-methyltransferase activity that converts GpppN-RNA to m^7GpppN-RNA (7,24). D12 is itself catalytically inactive – structurally, it appears to be a 2′-O-MTase that has degenerated – but it binds D1 and stimulates its N7-MTase activity approximately 40- to 50-fold (24). The crystal structure of the MPXV E12 subunit has been solved and used as a basis for inhibitor discovery (25).

Table 1. *Orthopoxvirus mRNA capping enzymes and cap methyltransferases relevant to antiviral targeting.*

Protein (VACV / MPXV)	Enzymatic activity	Reaction catalyzed	Methyl donor	Substrate	Evidence for essentiality	Evidence of folate interaction
D1R / E1 (C-terminal domain)	Cap (guanine-N7)-methyltransferase; N-terminal domain also RTPase + guanylyltransferase	GpppN-RNA → m7GpppN-RNA (cap-0)	SAM	5′ end of nascent viral mRNA	Essential; required for viral gene expression and early transcription termination	None reported; no enzymatic assay with folates published
D12L / E12	Catalytically inactive stimulatory subunit (degenerate 2′-O-MTase fold)	None; allosterically stimulates D1 N7-MTase ~40–50×	n/a	Binds D1	Essential as D1 partner	None reported; MPXV E12 structure available for docking
J3R (VP39) / VP39	Cap-specific 2′-O-methyltransferase; also, poly(A) polymerase processivity subunit	m7GpppN-RNA → m7GpppNm-RNA (cap-1)	SAM	Cap-0 RNA, minimum ~m7GpppGpN	Required for efficient innate immune evasion; validated inhibitor target	None reported; published inhibitors are sinefungin analogues, not folates
A27L / A29L	NOT a methyltransferase — envelope protein mediating heparan sulfate binding and virion attachment	n/a	n/a	Cell-surface glycosaminoglycans	Important for entry; vaccine and diagnostic antigen	Not applicable; earlier attribution of MTase activity to A29L was erroneous and has been withdrawn

VACV, vaccinia virus; MPXV, monkeypox virus; SAM, S-adenosyl-L-methionine; RTPase, RNA 5′-triphosphatase. Sources: (7,9,10,24–27)

Cap-1 formation. VP39 (vaccinia J3R; MPXV VP39) is a separate, monomeric, cap-specific 2′-O-methyltransferase that methylates the ribose 2′-O position of the first transcribed nucleotide, converting cap-0 to cap-1 (26). VP39 is bifunctional and serves as the processivity subunit of the viral poly(A) polymerase. Crystal structures of MPXV VP39 in complex with sinefungin and with a series of sinefungin-derived inhibitors have been reported, and several of these compounds inhibit MPXV replication in cell culture (9–11).

Both reactions use S-adenosyl-L-methionine (SAM) as the methyl donor and release S-adenosyl-L-homocysteine (SAH). Neither uses folate. The distinction between N7-guanine methylation and 2′-O-ribose methylation is not a matter of terminology: the two reactions are catalyzed by different proteins with different folds, different substrate requirements, and different consequences for innate immune evasion, and a compound active against one has no presumptive activity against the other.

At least two distinct cap-modifying methyltransferase activities are encoded in the D1/E1 C-terminal domain and in VP39. A29L is the MPXV homologue of vaccinia A27L, an envelope protein that mediates attachment to cell-surface heparan sulfate and is a well-characterized diagnostic and vaccine antigen (27); it has no described methyltransferase activity.

When evaluating the chemical role of folates within this system, precision about chemical identity is essential here, because the entities discussed in this literature differ substantially in structure, cellular handling, and pharmacology:

- Folic acid (pteroylmonoglutamic acid) — the fully oxidized synthetic form used in supplements and food fortification (Figure 2). It does not occur in appreciable quantities in unfortified foods.
- Reduced folates — dihydrofolate (DHF) and tetrahydrofolate (THF), generated from folic acid by dihydrofolate reductase; these are the metabolically active scaffolds.
- 5-methyltetrahydrofolate (5-methyl-THF) — the principal circulating and transport form of folate in human plasma.
- Folate polyglutamates — intracellular forms bearing a γ-linked polyglutamate tail, which promotes cellular retention and alters enzyme affinity; approximately 95% of intracellular folate is polyglutamated.
- Antifolates — structural analogues that inhibit folate-dependent enzymes, such as methotrexate and pemetrexed (dihydrofolate reductase, thymidylate synthase) and trimethoprim and pyrimethamine (microbial DHFR) (28).

These are not interchangeable. A compound that is added to a culture medium as folic acid is rapidly reduced and polyglutamated on entering the cell, so the species present at any intracellular target is not the species that was added. Equally, a finding that a synthetic folate analogue inhibits an enzyme is not evidence of what the vitamin does.

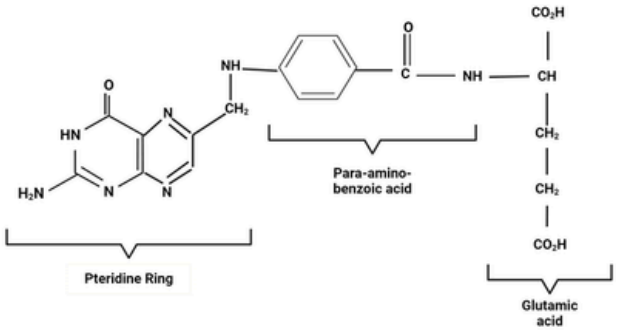


Figure 2. *Chemical structure and physiological state of folic acid (N-pteroyl-L-glutamic acid). It comprises a pteridine ring linked to p-aminobenzoic acid, which is bound via an amide bond to L-glutamic acid. At physiological pH, both glutamate carboxyl groups are ionized, resulting in a dianionic molecule with a high topological polar surface area. Image created with BioRender.com.*

SAM is the methyl donor for essentially all cellular and viral SAM-dependent methyltransferases, including every orthopoxvirus cap MTase as previously described. Folate participates in one-carbon metabolism: 5-methyl-THF donates a methyl group to homocysteine in the methionine synthase reaction (vitamin B-dependent), regenerating methionine, which methionine adenosyltransferase converts to SAM (29,30). Folate therefore contributes to methylation reactions upstream and indirectly, by supplying the SAM pool, and never by transferring a methyl group to a methyltransferase substrate directly.

If folate acted on viral cap methylation through its vitamin function, folate repletion would be expected to increase SAM availability and thereby, if anything, facilitate rather than inhibit viral cap methylation. An inhibitory effect would therefore have to arise from a mechanistically separate property, direct occupancy of the SAM-binding pocket or of the cap-RNA binding site by the intact folic acid molecule, acting as a competitive ligand rather than as a vitamin. It is precisely this second, distinct hypothesis that the docking literature addresses, and stating it explicitly clarifies what an experiment would need to demonstrate (Figure 3).

It should also be noted that folate metabolism intersects with antiviral innate immunity in ways that are neither simple nor uniformly favorable. Folate-dependent one-carbon metabolism supports nucleotide biosynthesis, on which viral genome replication depends, and folate status has been reported to modulate interferon-stimulated antiviral pathways. Antifolates such as methotrexate reduce SARS-CoV-2 replication in cell culture (31), which is the opposite polarity of intervention to folate supplementation. The direction of any net effect on an orthopoxvirus is therefore not predictable *a priori*.

Looking closely at the computational evidence gathered so

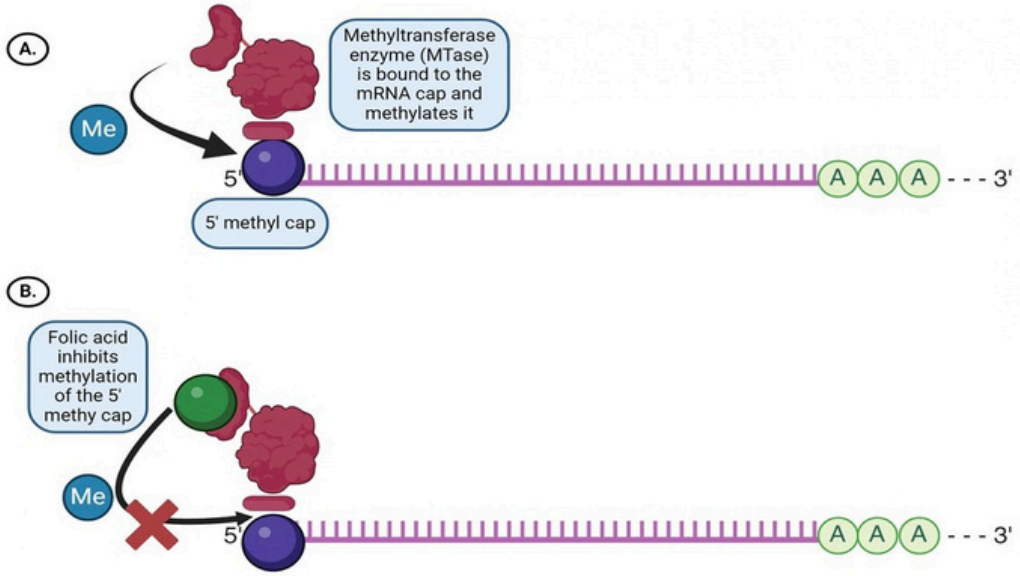


Figure 3. *Proposed mechanism of folic acid interference with orthopoxvirus mRNA cap methylation. (A) A SAM-dependent cap methyltransferase methylates the 5′ cap of viral mRNA. (B) Hypothesized mechanism in which folic acid occupies the enzyme active site, preventing methyl transfer and leaving viral transcripts susceptible to detection by cytoplasmic innate immune sensors. This figure depicts a hypothesis derived solely from molecular docking (12); it does not represent a demonstrated enzymatic result, and no biochemical assay has yet verified this mechanism. Image created with BioRender.com.*

far, the principal source for the folic acid hypothesis is the virtual screening study of Hashim and colleagues (12). In that work, 12,971 compounds from 200 Middle Eastern medicinal plants were reduced to 5,749 unique ligand conformers and screened against an MPXV methyltransferase using high-throughput virtual screening, followed by extra-precision (XP) docking and Prime MM-GBSA rescoring. Folic acid and 1,2,4,6-tetragalloylglucose emerged as the highest-scoring compounds, with MM-GBSA values of −51.87 and −60.61 kcal/mol, respectively, exceeding those of the reference compounds TO1119 (−35.71 kcal/mol) and the pan-MTase inhibitor sinefungin (−31.51 kcal/mol). In molecular dynamics simulations, folic acid was reported to be more stable in the binding site than sinefungin, and to occupy a position that would reduce enzyme interaction with the mRNA substrate.

Two points must be stated plainly. First, these are the results of that study, not of the present authors. Second, the authors of that study themselves concluded that wet-laboratory validation is necessary before any therapeutic inference can be drawn (12). The present review endorses that conclusion.

In contrast to these computational models, the biochemical and cell-based evidence reveals a stark gap: no published study reports concentration-dependent inhibition of a purified orthopoxvirus methyltransferase by folic acid, folate polyglutamates, or reduced folates. No IC_{50}, K_i or equivalent kinetic parameter has been determined for any folate against D1/E1, D12/E12, or VP39. No study demonstrates intracellular target engagement of a viral MTase by folate in infected cells. This absence is the central finding of the present review and is stated here without qualification. What can legitimately be said is more limited. Folic acid has been reported to bind the SARS-CoV-2 nucleocapsid protein in a combined computational and biochemical study (32), and folate supplementation has been proposed to restrict SARS-CoV-2 entry through effects on ACE2 (33). These are observations about a different virus and different targets; they establish that folates can act as protein ligands, which is not in dispute, but they carry no weight regarding orthopoxvirus cap methylation. Conversely, the antifolate methotrexate reduces coronavirus replication in cell culture (31), illustrating that folate-pathway modulation in either direction can affect viral replication for reasons unrelated to viral methyltransferase inhibition.

This lack of empirical proof contrasts sharply with validated inhibitors of the same target class, which provide a concrete template for what adequate evidence looks like. Silhan and colleagues determined crystal structures of MPXV VP39 in complex with sinefungin and with a series of derived inhibitors, several of which inhibit the purified enzyme at sub-micromolar concentrations, and showed that the sinefungin binding mode is conserved across unrelated viral 2′-O-MTases, including SARS-CoV-2 nsp14 (9). Skvara and colleagues defined the structural basis for RNA-cap recognition and methylation by VP39 (10). Zgarbová and colleagues then demonstrated that these VP39 inhibitors suppress MPXV replication in cell culture (11). In parallel, the MPXV E12 crystal structure has enabled inhibitor discovery against the N7-MTase heterodimer (25). This body of work establishes the orthopoxvirus cap methylation machinery as a genuinely druggable target and, importantly, sets the evidentiary standard against which folate-related claims should be judged: a defined enzyme, a purified protein preparation, a defined substrate and methyl donor, concentration–response data, structural confirmation of the binding mode, and antiviral activity in infected cells.

Limitations, Gaps, and Future Research Directions

These dynamics highlight several profound limitations of the current evidence base (Table 2). First, docking generates hypotheses about binding poses but does not establish binding affinity, enzymatic inhibition, selectivity, or cellular target engagement. Docking scores correlate only weakly with measured affinities, and end-point free energy methods such as MM-GBSA are known to be sensitive to force field, solvation model, protonation state assignment, and simulation protocol, with reported errors that frequently exceed the differences used to rank compounds (34). Highly charged ligands are handled particularly poorly, because desolvation penalties are systematically underestimated. Folic acid, bearing two ionized carboxylates at physiological pH, is precisely the class of ligand for which such scores should be treated with caution, and polar, multiply charged molecules are recognized as recurrent artefactual hits in virtual screens against SAM-binding pockets, which readily accommodate polar heterocyclic scaffolds. Two further limitations apply specifically to the study underpinning the folic acid hypothesis. Selectivity was not addressed: the human cap methyltransferase RNMT and the 2′-O-MTase CMTR1 perform analogous reactions with conserved SAM-binding architecture, and no counter-screen against them was reported. Furthermore, the identity of the target structure is not stated with sufficient precision to determine whether the N7-MTase or VP39 was modelled. This point must be resolved before the result can be interpreted at all.

Table 2. *Evidence level for compounds proposed to act on orthopoxvirus methyltransferases and for currently used mpox therapeutics.*

Candidate	Proposed target	Highest level of evidence	Experimental system	Key limitation
Folic acid	MPXV mRNA cap MTase (enzyme not specified in source)	In silico only	Glide XP docking, Prime MM-GBSA, MD simulation (2)	No enzymatic assay; no IC50/Ki; docking score is not inhibition; dianionic at physiological pH with poor expected passive permeability; target enzyme not unambiguously specified
1,2,4,6-tetragalloylglucose	MPXV mRNA cap MTase	In silico only	Same screening study (12)	As above; polyphenolic galloyl esters are recognized frequent hitters in screening assays
Sinefungin	Pan-MTase, SAM-competitive	Biochemical + structural + cell culture	Purified MPXV VP39; crystallography; MPXV-infected cells (9–11)	Poor selectivity across host and viral MTases; historical toxicity limits therapeutic use
Sinefungin analogues (e.g. TO507)	MPXV VP39 (2′-O-MTase)	Biochemical + structural + cell culture	Purified VP39; crystal structures; MPXV replication assays (9,11)	No published in vivo efficacy or pharmacokinetic data; tested against clade IIb only
E12-directed compounds	MPXV E12 / N7-MTase heterodimer	Structural + biochemical	Crystal structure of MPXV E12; inhibitor screening (25)	Early stage; antiviral activity in infected cells not established
Tecovirimat	F13 (VP37) envelope protein	Randomized controlled trials	PALM007 (clade I, n = 597); STOMP (clade II) (21)	No reduction in time to lesion resolution in either trial; efficacy not demonstrated
Cidofovir / brincidofovir	Viral DNA polymerase (E9)	Animal models; observational case series	Nonclinical models; uncontrolled clinical use (22,23)	No randomized efficacy data in mpox; nephrotoxicity (cidofovir); hepatic and GI toxicity (brincidofovir)

MTase, methyltransferase; MD, molecular dynamics; MM-GBSA, molecular mechanics generalized Born surface area; SAM, S-adenosyl-L-methionine.

Furthermore, the pharmacological plausibility of these models is highly questionable. The concentrations at which antiviral effects have been attributed to folic acid cannot be reconciled with achievable human exposures, and this deserves emphasis rather than a footnote. Physiological plasma folate concentrations lie in the range of approximately10–45 nmol/L; supplementation at 400 µg/day raises this modestly, and even therapeutic dosing at 5 mg/day yields total plasma folate in the high nanomolar to low micromolar range, constrained by saturable intestinal absorption and renal handling. Activity reported at millimolar concentrations is therefore roughly three orders of magnitude above anything attainable systemically. A compound requiring millimolar concentrations for an effect in vitro is not a viable systemic antiviral, irrespective of how favorable its safety and cost profile may appear at nutritional doses. A selectivity index of approximately 10, as reported in some of the material previously cited, sits at the absolute minimum threshold conventionally regarded to indicate a genuine antiviral effect rather than generalized cytostatic or cytotoxic activity. Typically, values of at least 10 and preferably above 100 are usually expected before a hit compound is advanced. Finally, the assumption that folic acid is intrinsically safe at any dose does not survive scrutiny. High-dose parenteral folic acid is an established and widely used method of inducing acute kidney injury and renal fibrosis in rodents (35). Folic acid also interacts with anticonvulsant therapy and with methotrexate regimens, and elevated unmetabolized folic acid at high intakes remains an area of ongoing investigation. The implication that pharmacological antiviral dosing would be free of risk was not supportable and has been removed. Even if folic acid inhibited a viral MTase in a purified system, delivering it to the cytoplasmic viral factory would be non-trivial. Folates enter cells via the reduced folate carrier, proton-coupled folate transporter, and folate receptors, all of which are saturable and subject to competition from physiological folates. Once internalized,

folic acid is reduced and polyglutamated, so the free monoglutamate species modelled in docking studies would be a minor and transient intracellular component. The relationship between an extracellular concentration and the concentration of the docked species at the target is therefore neither linear nor well characterized.

To systematically resolve these evidence gaps, several clear research directions are required. The literature reviewed above leaves the central claim untested rather than refuted, and the gaps are specific. First, no purified orthopoxvirus cap methyltransferase has been exposed to folic acid in a defined enzymatic assay, so no IC_{50} or K_i has been reported and the mode of inhibition relative to S-adenosylmethionine (SAM) and to the RNA substrate is unknown. Second, direct binding has not been demonstrated by any biophysical method independent of docking, and no experimental structure supports the predicted binding pose. Third, selectivity is entirely uncharacterized: the human cap methyltransferases RNMT and CMTR1 use the same methyl donor and a related fold, yet no comparative data exist. Fourth, where antiviral effects have been attributed to folates in cell culture, target engagement has been inferred from replication readouts rather than measured, and cap-0 and cap-1 species on viral mRNA have not been quantified. Fifth, the clade dependence of any such effect is unknown, and no in vivo data exist. Finally, the single computational study underpinning the hypothesis has not been reproduced by an independent group using different scoring functions and force fields, a step that end-point free energy methods such as MM/GBSA particularly require given their documented variability across systems (34).

Addressing these deficiencies calls for a specific sequence of experimental validations. Enzymatic characterization is the necessary first step: the recombinant MPXV E1 C-terminal domain in complex with E12, and separately purified VP39, assayed against a defined capped RNA substrate with radiolabeled or non-radiometric detection of methyl transfer, would establish whether folic acid inhibits at all. Folic acid, 5-methyl-THF, THF, representative folate polyglutamates and relevant antifolates should be tested individually across a concentration range, with sinefungin as a positive control and an inactive structural analogue as a negative control, since the folate species modelled in silico is not the species that predominates intracellularly. Parallel assays against RNMT and CMTR1 would define the selectivity window, which matters because inhibition of host cap methylation would be expected to be poorly tolerated. Direct binding would then require independent confirmation by isothermal titration calorimetry, surface plasmon resonance, or thermal shift analysis, ideally supported by a co-crystal or cryo-electron microscopy structure of the kind already obtained for sinefungin-derived VP39 inhibitors and for the E12 subunit (9,11,25). Only once inhibition and binding are established do cell-based experiments become interpretable: MPXV replication assays with paired cytotoxicity determination, EC_{50}, CC_{50} and a selectivity index, time-of-addition studies, and direct measurement of target engagement by cellular thermal shift assay or by mass-spectrometric quantification of cap-0 and cap-1 on viral transcripts. Testing across clade Ia, Ib and IIb isolates, with sequence comparison of the target enzymes, would establish generality, and established animal models of orthopoxvirus infection with accompanying pharmacokinetic data would be required before any therapeutic claim could be entertained (36,37).

Two priorities follow from this analysis. The first is that the burden of proof sits at the enzymatic level: a single well-controlled assay on a purified enzyme would either bring the hypothesis into the experimental domain or close it, and such an experiment is inexpensive relative to the attention the claim has already attracted. The second is that the folate question is best pursued within a broader program rather than in isolation. The wider and more productive direction is the systematic exploration of orthopoxvirus cap methylation as a drug target, where validated chemical matter already exists, structures are available for both the N7 and the 2′-O-methyltransferases, and the principal unmet needs are selectivity over the host enzymes, oral bioavailability, and activity across clades. Should folic acid or a reduced folate prove to inhibit these enzymes, the more informative follow-up question would concern the folate-dependent supply of SAM and whether modulating that pool alters viral cap methylation, since such a mechanism is consistent with folate biochemistry in a way that direct competitive inhibition is not. Until at least the enzymatic step has been completed, the accurate description of folic acid remains a computationally generated hypothesis, and reviews, commentaries, and public communication should characterize it as such.

Taken together, this review demonstrates that orthopoxvirus mRNA cap methyltransferases are legitimate and well-validated antiviral targets, and the case for pursuing them has strengthened now that tecovirimat has failed to demonstrate clinical efficacy in randomized trials against both clade I and clade II MPXV. Inhibitors of the MPXV 2′-O-methyltransferase VP39 have been characterized structurally, shown to inhibit the purified

enzyme, and shown to suppress viral replication in cell culture, providing proof of principle for the target class.

The status of folic acid within this landscape is much more limited. It has been identified as a high-scoring ligand in a single computational screen against an MPXV methyltransferase model, and that observation is worth following up. It has not been shown to inhibit any purified orthopoxvirus methyltransferase; no kinetic parameters have been reported; no target engagement has been demonstrated in infected cells; and the concentrations at which antiviral effects have been attributed to it exceed achievable human plasma exposures by approximately three orders of magnitude. Folate biochemistry offers no straightforward mechanistic support, since SAM rather than folate is the methyl donor for these enzymes and folate repletion would if anything be expected to increase SAM availability.

Folic acid should therefore be described as a computationally derived candidate warranting enzymatic evaluation, no more and no less. Describing it as the first small-molecule inhibitor of MPXV, or as a promising treatment for MPXV, is not supported by the available evidence. Beyond the requirements of scientific accuracy, such claims carry a practical risk: MPXV outbreaks disproportionately affect settings where an inexpensive, widely available vitamin marketed as an antiviral could readily displace supportive care and vaccination, which are the interventions currently known to help.

Acknowledgements

The study was not supported by any sponsor or funder.

Author Contributions

Conceptualization, A.A., L.A., and M.O.O.A.; Literature search, A.A., L.A., and M.O.O.A.; Writing—original draft preparation, A.A., L.A., and M.O.O.A.; Writing—review and editing, A.A., L.A., and M.O.O.A. All authors have read and approved the published version of the manuscript.

Statement of Ethics

Not applicable.

Statement of Competing Interest

The authors declare no relevant conflicts of interest.

Statement of Data Availibility

No new datasets were generated or analyzed during this study. All analyzed data are included in this published article and its cited references.

Statement of Generative AI Use

An AI tool was used primarily for language editing and rephrasing selected passages. The tool was not used to generate experimental data, alter raw data, fabricate results, or independently determine the scientific conclusions of the study. The authors critically reviewed, verified, and revised all AI-edited content.

Manuscript data were cross-checked against the relevant scientific literature. The authors retain full responsibility for the accuracy, integrity, interpretation, and final wording of the manuscript. Regarding privacy and compliance, no patient-level, human-subject, or personally identifiable research data were shared with the AI system; only information necessary for language editing was processed.

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REFERENCES

1. Huang Y, Mu L, Wang W. Monkeypox: epidemiology, pathogenesis, treatment and prevention. Signal Transduct Target Ther. 2022;7(1):373. [CrossRef]

2. Lansiaux E, Jain N, Laivacuma S, Reinis A. The virology of human monkeypox virus (hMPXV): a brief overview. Virus Res. 2022;322:198932. [CrossRef]

3. Petersen E, Kantele A, Koopmans M, Asogun D, Yinka-Ogunleye A, Ihekweazu C, et al. Human monkeypox. Infect Dis Clin North Am. 2019;33(4):1027–43. [CrossRef]

4. Likos AM, Sammons SA, Olson VA, Frace AM, Li Y, Olsen-Rasmussen M, et al. A tale of two clades: monkeypox viruses. J Gen Virol. 2005;86(10):2661–72. [CrossRef]

5. Vakaniaki EH, Kacita C, Kinganda-Lusamaki E, O'Toole Á, Wawina-Bokalanga T, Mukadi-Bamuleka D, et al. Sustained human outbreak of a new MPXV clade I lineage in eastern Democratic Republic of the Congo. Nat Med. 2024;30:2791–5. [CrossRef]

6. Van Dijck C, Hoff NA, Mbala-Kingebeni P, Low N, Çevik M, Rimoin AW, et al. Emergence of mpox in the post-smallpox era — a narrative review on mpox epidemiology. Clin Microbiol Infect. 2023;29(12):1487–92. [CrossRef]

7. Decroly E, Ferron F, Lescar J, Canard B. Conventional and unconventional mechanisms for capping viral mRNA. Nat Rev Microbiol. 2011;10(1):51–65. [CrossRef]

8. Ramdhan P, Li C. Targeting viral methyltransferases: an approach to antiviral treatment for ssRNA viruses. Viruses. 2022;14(2):379. [CrossRef]

9. Silhan J, Klima M, Otava T, Skvara P, Chalupska D, Chalupsky K, et al. Discovery and structural characterization of monkeypox virus methyltransferase VP39 inhibitors reveal similarities to SARS-CoV-2 nsp14 methyltransferase. Nat Commun. 2023;14(1):2259. [CrossRef]

10. Skvara P, Chalupska D, Klima M, Kozic J, Silhan J, Boura E. Structural basis for RNA-cap recognition and methylation by the mpox methyltransferase VP39. Antiviral Res. 2023;216:105663. [CrossRef]

11. Zgarbová M, Otava T, Silhan J, Nencka R, Weber J, Boura E. Inhibitors of mpox VP39 2′-O methyltransferase efficiently inhibit the monkeypox virus. Antiviral Res. 2023;218:105714. [CrossRef]

12. Hashim HO, Al-Shuhaib JMB, Mohammed MK, Al-Shuhaib MBS. Targeting monkeypox virus methyltransferase: virtual screening of natural compounds from Middle-Eastern medicinal plants. Mol Biotechnol. 2025;67(8):3194–216. [CrossRef]

13. Rabaan AA, Al-Shwaikh SA, Alfouzan WA, Al-Bahar AM, Garout M, Halwani MA, et al. A comprehensive review on monkeypox viral disease with potential diagnostics and therapeutic options. Biomedicines. 2023;11(7):1826. [CrossRef]

14. Bunge EM, Hoet B, Chen L, Lienert F, Weidenthaler H, Baer LR, et al. The changing epidemiology of human monkeypox — a potential threat? A systematic review. PLoS Negl Trop Dis. 2022;16(2):e0010141. [CrossRef]

15. Nuzzo JB, Borio LL, Gostin LO. The WHO declaration of monkeypox as a global public health emergency. JAMA. 2022;328(7):615–7. [CrossRef]

16. World Health Organization. Broader transmission of mpox due to clade Ib MPXV — global situation. Disease Outbreak News. Geneva: WHO; 2025.

17. Shehryar A, Nagaraj RH, Kanwal F, Reddy SM, Grezenko H, Raut Y, et al. Unravelling monkeypox: an emerging threat in global health. Cureus. 2023;15(8):e43961. [CrossRef]

18. Jaiswal V, Nain P, Mukherjee D, Joshi A, Savaliya M, Ishak A, et al. Symptomatology, prognosis, and clinical findings of monkeypox-infected patients during the COVID-19 era: a systematic review. Immun Inflamm Dis. 2022;10(11):e722. [CrossRef]

19. Reynolds MG, Yorita KL, Kuehnert MJ, Davidson WB, Huhn GD, Holman RC, et al. Clinical manifestations of human monkeypox influenced by route of infection. J Infect Dis. 2006;194(6):773–80. [CrossRef]

20. World Health Organization. Clinical management and infection prevention and control for mpox: interim rapid response guidance. Geneva: WHO; 2025.

21. PALM007 Writing Group. Tecovirimat for clade I MPXV infection in the Democratic Republic of Congo. N Engl J Med. 2025;392(15):1484–96. [CrossRef]

22. Rizk JG, Lippi G, Henry BM, Forthal DN, Rizk Y. Prevention and treatment of monkeypox. Drugs. 2022;82(9):957–63. [CrossRef]

23. Eslami A, Alimoghadam S, Khoshravesh S, Shirani M, Alimoghadam R, Alavi Darazam I. Mpox vaccination and treatment: a systematic review. J Chemother. 2024;36(2):85–109. [CrossRef]

24. De la Peña M, Kyrieleis OJP, Cusack S. Structural insights into the mechanism and evolution of the vaccinia virus mRNA cap N7 methyltransferase. EMBO J. 2007;26(23):4913–25. [CrossRef]

25. Wang DP, Zhao R, Wang HF, Wang MY, Hu WS, Lin MM, et al. Crystal structure of mRNA cap (guanine-N7) methyltransferase E12 subunit from monkeypox virus and discovery of its inhibitors. Int J Biol Macromol. 2023;253:127565. [CrossRef]

26. Hodel AE, Gershon PD, Shi X, Quiocho FA. The 1.85 Å structure of vaccinia protein VP39: a bifunctional enzyme that participates in the modification of both mRNA ends. Cell. 1996;85(2):247–56. [CrossRef]

27. Chung CS, Hsiao JC, Chang YS, Chang W. A27L protein mediates vaccinia virus interaction with cell surface heparan sulfate. J Virol. 1998;72(2):1577–85. [CrossRef]

28. Shamshad H, Bakri R, Mirza AZ. Dihydrofolate reductase, *thymidylate synthase*, and serine hydroxymethyltransferase: successful targets against some infectious diseases. Mol Biol Rep. 2022;49(7):6659–91. [CrossRef]

29. Zheng Y, Cantley LC. Toward a better understanding of folate metabolism in health and disease. J Exp Med. 2019;216(2):253–66. [CrossRef]

30. Shulpekova Y, Nechaev V, Kardasheva S, Sedova A, Kurbatova A, Bueverova E, et al. The concept of folic acid in health and disease. Molecules. 2021;26(12):3731. [CrossRef]

31. Stegmann KM, Dickmanns A, Gerber S, Nikolova V, Klemke L, Manzini V, et al. The folate antagonist methotrexate diminishes replication of the coronavirus SARS-CoV-2 and enhances the antiviral efficacy of remdesivir in cell culture models. Virus Res. 2021;302:198469. [CrossRef]

32. Chen Y, Wei J, Qin R, Hou J, Zang G, Zhang G, et al. Folic acid: a potential inhibitor against SARS-CoV-2 nucleocapsid protein. Pharm Biol. 2022;60(1):862–78. [CrossRef]

33. Zhang Y, Pang Y, Xu B, Luo X. Folic acid restricts SARS-CoV-2 invasion by methylating ACE2. Front Microbiol. 2022;13:980903. [CrossRef]

34. Genheden S, Ryde U. The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. Expert Opin Drug Discov. 2015;10(5):449–61. [CrossRef]

35. Yan L. Folic acid-induced animal model of kidney disease. Anim Model Exp Med. 2021;4(4):329–42. [CrossRef]

36. Rosa RB, Ferreira de Castro E, da Silva MV, Paiva Ferreira DC, Jardim ACG, Santos IA, et al. *In vitro* and *in vivo* models for monkeypox. iScience. 2022;26(1):105702. [CrossRef]

37. Wei ZK, Zhao YC, Wang ZD, Sui LY, Zhao YH, Liu Q. Animal models of mpox virus infection and disease. Infect Med. 2023;2(3):153–66. [CrossRef]