SHORT NOTE

Quinoline derivative MC1626, a putative GCN5 histone acetyltransferase (HAT) inhibitor, exhibits HAT-independent activity against *Toxoplasma gondii*

Aaron T. Smith¹, Meredith R. Livingston¹, Antonello Mai², Patrizia Filetici³, Sherry F. Queener¹, and William J. Sullivan Jr.¹*

¹Department of Pharmacology & Toxicology, Indiana University School of Medicine, Indianapolis, Indiana
²Istituto Pasteur-Fondazione Cenci Bolognetti, Dipartimento di Studi Farmaceutici, Università degli Studi di Roma “La Sapienza”, P.le A. Moro 5, I-00185 Roma, Italy
³Istituto di Biologia e Patologia Molecolari CNR, Dip. Genetica e Biologia Molecolare, Università degli Studi di Roma “La Sapienza”, P.le A. Moro 5, I-00185 Roma, Italy

Running Title: MC1626 activity against *Toxoplasma*

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*Corresponding author:
William J. Sullivan, Jr., Ph.D.
Department of Pharmacology & Toxicology
IU Center For AIDS Research
Indiana University School of Medicine
635 Barnhill Drive
MS A-525
Indianapolis, IN 46202
phone/fax: 317-274-1573 / 7714
wjsulliv@iupui.edu
We report that quinoline derivative MC1626, first described as an inhibitor of the histone acetyltransferase (HAT) GCN5, is active against the protozoan parasite *Toxoplasma gondii* in vitro. However, MC1626 does not inhibit *Toxoplasma* GCN5 HATs, nor reduce HAT-mediated activity; rather, this quinoline may target the plastid organelle called the apicoplast.
Histone acetyltransferases (HATs) play a central role in modifying chromatin to create a favorable environment for DNA processes, including transcription. Many genetic studies have implicated HATs as important factors in disease (for reviews, see (13), (8), and (18)). The impact of HATs on cellular physiology and disease would benefit from the identification of specific pharmacological inhibitors, but very few have been described. Two natural products, anacardic acid and garcinol, inhibit p300/CBP (CREB-binding protein) and PCAF (p300/CBP-associating factor) in a 5-10 μM range in vitro (1, 2). In contrast, curcumin displays activity against p300/CBP, but not PCAF (3). Anacardic acid may be a broad-spectrum HAT inhibitor, as it also interferes with the MYST HAT Tip60 (16). Isothiazolones were identified as inhibitors of PCAF and p300 (15), but like the aforementioned compounds, activity against GCN5 was not determined. Moreover, isothiazolones are strongly reactive with thiol groups and likely to have substantial nonspecific effects. Small molecule inhibitors of GCN5 include a butyrolactone (5) and MC1626 (2-methyl-3-carbethoxyquinoline) (11).

We have established that histone acetylation correlates with differentiation of the protozoan parasite Toxoplasma gondii (phylum Apicomplexa) (14). Differentiation from the rapidly growing tachyzoite stage to the encysted bradyzoite stage is critical to pathogenesis (6). Toxoplasma is a serious opportunistic infection in immunocompromised patients due to the fact that the encysted bradyzoites cannot be cleared by immunity or drugs (17).
MC1626 inhibits Toxoplasma growth in vitro. Given that histone acetylation accompanies changes in gene expression relevant to Toxoplasma differentiation, HATs may serve as attractive candidates for drug design. Therefore, we tested the impact of MC1626 (0-300 μM) on Toxoplasma grown in human foreskin fibroblasts using standard [3H]-uracil uptake and plaque assays (12). Relative to a vehicle control (DMSO), MC1626 curtails parasite growth in dose-dependent fashion, with 100 μM reducing growth by ~50%. Host cells showed no obvious signs of damage until concentrations of MC1626 exceeded 300 μM.

MC1626 does not inhibit parasite HAT activities. We examined if MC1626 diminished HAT activity of Toxoplasma GCN5 on histone H3, its preferred substrate (4). As shown in Figures 1A and 1B, recombinant TgGCN5-A and -B (4) exhibit robust HAT activity on H3 using a standard in vitro HAT assay (7). However, MC1626 does not appear to have any significant effect on the ability of recombinant TgGCN5-A or -B to acetylate H3. We considered that MC1626 may act as a prodrug that must be processed by parasite factors in order to become capable of inhibiting GCN5. Therefore, we examined the ability of MC1626 to ablate HAT activities from Toxoplasma lysates. Results indicate that Toxoplasma lysate acetylates H3 substrate, but MC1626 has no detectable impact on its ability to do so (Figure 1C). We also evaluated if MC1626 altered levels of acetylated histones in cultured Toxoplasma using two independent means. Immunofluorescence assay (IFA) of parasites 24-72 hours post-infection continue to exhibit intense staining for acetylated H3 despite the presence of 200
μM MC1626 (data not shown). Immunoblotting lysates of tachyzoites cultured in increasing concentrations of MC1626 for three days also exhibited no decrease in overall acetylated H3 (Figure 2D). We also examined levels of acetylated H4 and again detected no significant decrease in drug treated parasites, suggesting that MC1626 does not inhibit MYST HAT activities either (Figure 2D).

**Recombinant yeast GCN5 is not inhibited by MC1626.** MC1626 was described as a GCN5 inhibitor based on indirect observations; however, no direct test was performed (11). Figure 2E shows that the ability of recombinant yeast Gcn5 to acetylate H3 in the HAT assay is not inhibited by MC1626. A control experiment was performed with anacardic acid (Figure 2F), a *bona fide* inhibitor of GCN5 family HATs (2). Considered with the above studies, these data strongly suggest that the growth inhibitory effects of MC1626 on yeast and *Toxoplasma* are not due to a direct inhibition of Gcn5. We cannot dismiss the possibility that *S. cerevisiae* processes MC1626 differently than *Toxoplasma*. Alternatively, MC1626 may perturb the ability of yeast Gcn5 to associate with cofactors required for its HAT activity in vivo. Further investigation is required to resolve the mechanism of action of MC1626 in *S. cerevisiae*.

Anacardic acid has been shown to inhibit PCAF, a member of the GCN5 family, as well as the MYST HAT, Tip60 (2, 16). This is the first report formally showing that it also inhibits GCN5. The effects of anacardic acid on *Toxoplasma* growth and HAT activities are currently being investigated.

**Loss of apicoplast in Toxoplasma treated with MC1626 and quinoline.** The chemical structure of MC1626 (11) is related to quinolones,
which target the apicoplast (9), a plastid organelle in many apicomplexans that houses an extra-chromosomal element acquired through secondary endosymbiosis of an algae (10). We tested if MC1626 treatment is linked to the disappearance of apicoplast DNA. DNA staining (4’-6-diamidino-2-phenylindole, DAPI) shows the apicoplast as a distinct organelle anterior to the parasite nucleus (Figure 2). Parasites treated with fluoroquinolones such as ciprofloxacin no longer exhibit this staining pattern due to the loss of apicoplast DNA (9). To test if MC1626 functions similarly, we examined if *Toxoplasma* maintained in 200 μM MC1626 exhibited a decrease in the number of parasites containing apicoplasts. Figure 2 is a representative image of MC1626-treated parasites that have lost their apicoplast. We also tested effects of quinoline (Sigma), which inhibits *Toxoplasma* growth at similar concentrations (data not shown). Results are identical and further suggest that this class of drug perturbs the apicoplast (Figure 2). A count of 50 random vacuoles indicated that apicoplasts are present in only 52%-57% of MC1626- and quinoline-treated parasites, respectively. This is the first demonstration that quinoline and derivatives have activity against *Toxoplasma* and share a mechanism of action similar to fluoroquinolones. While some quinolines, e.g. chloroquine, have no effect on *Toxoplasma*, derivatives such as MC1626 may prove useful in developing therapeutics.

In summary, these studies are significant because they argue that MC1626 is not a direct inhibitor of GCN5 as previously reported, and they suggest that quinoline and derivatives (e.g. MC1626) may be novel leads for
drug development that selectively kill parasites by targeting their unique apicoplast.

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REFERENCES


FIGURE LEGENDS

Figure 1. Effects of MC1626 on HAT activities. HAT assays were performed using recombinant (A) TgGCN5-A or (B) TgGCN5-B, in the presence of increasing concentrations of MC1626 or DMSO vehicle control (DC). (C) HAT assays using 0.5 μg of tachyzoite lysate as enzyme source in the presence of increasing concentrations of MC1626. (D) Histone acetylation levels in lysates of parasites treated with MC1626. Tachyzoites were incubated in the presence of varying concentrations of MC1626, or with DMSO, for three days. After treatment, lysates were made to assess by Western blotting if acetylation of H3 or H4 were diminished relative to untreated or vehicle-treated. Tubulin was used as a loading control. Note that the final two lanes are overloaded, which likely explains the apparent increase in histone acetylation seen in these samples. (E) MC1626 does not inhibit recombinant yeast GCN5 in vitro. Recombinant S. cerevisiae GCN5 (1.0 μg) was used as the enzyme source in HAT assays containing no drug or designated concentrations of MC1626. (F) Control experiment demonstrating that S. cerevisiae GCN5 (1.0 μg) is inhibited by anacardic acid (AA).

Figure 2. Effect of quinolines on parasite apicoplast organelle. Toxoplasma tachyzoites were cultured in the presence of DMSO (control), 200 μM MC1626, or 200 μM quinoline prior to processing for DAPI staining. DAPI stains DNA,
including the extra-chromosomal DNA of the apicoplast. An example apicoplast is indicated by the arrowhead as a distinct “dot” anterior to the parasite nucleus.
FIGURE 1

A. TgGCN5-A

B. TgGCN5-B

C. Parasite lysate

D.

E.

F.
FIGURE 2

DMSO

MC1626

quinoline