



Drug Repurposing Against *Toxoplasma gondii* Through Comparative Analysis of Compound Potency, Selectivity, and Host-Cell Toxicity

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Abstract

Repurposing of drugs is a feasible approach to identify anti-*Toxoplasma gondii* candidates but potency is not enough if there is an overlap of activity with mammalian-cell toxicity or when activity is different between the different parasite stages. This study was a secondary comparative analysis of the open LOPAC screening dataset reported by Uddin et al. (2025) that screened 1280 pharmacologically active compounds and found 44 compounds that inhibited bradyzoites by more than 50%. The focus of the quantitative analysis was on the prioritized panel for which EC₅₀ values were available for the tachyzoites (Tg68, ME49) and alkaline- and glutamine-induced bradyzoites (APTg68, APTg49) as well as CC₅₀ values in HepG2 and differentiated THP-1 cells. Geometric means were used to summarize potency at each stage and overall; CC₅₀ were used to evaluate the host cell safety; and minimum selectivity index across host cell models were used to represent robustness of selectivity. Of the main hits for repurposing, diphenyleneiodonium sulfate was the most potent overall (geometric-mean EC₅₀ 0.036 μ M) and most selective (minimum SI 96). T0070907 was less potent (0.708 μ M) and exhibited a good host-cell selectivity (SI 24–29). Brefeldin A, on the other hand, had a high apparent potency and a high cell-line dependent toxicity that resulted in a low SI of only 1. The analysis shows that prioritization of candidates differs significantly when the potency of the parasite and the toxicity to the host cells are considered simultaneously instead of using EC₅₀ only. These results suggest a multi-parameter approach for the selection of anti-*Toxoplasma* confirmatory development compounds from the library of compounds that can be used for these purposes. Conservative cross-cell selectivity and consistency in development stage are used as a focus point and also a separation of compounds with consistent experimental windows from those that are only seemingly promising based on one positive assay. This will help increase transparency in decisions about repurposing in the early stages, and direct efficient allocation of confirmatory experimental resources. *Toxoplasma gondii*, Drug repurposing, EC₅₀, CC₅₀, Selectivity Index, Host-cell toxicity.

Keywords: *Toxoplasma gondii*; drug repurposing; EC₅₀; CC₅₀; selectivity index; host-cell toxicity

1. Introduction

T. gondii is an obligate intracellular apicomplexan parasite that has a wide host range and is of great public health importance. At the population level, syntheses always report high levels of human exposure, but with considerable regional, age, food system, and environmental variations (Molan et al., 2019; Rostami et al., 2020).

While most immunocompetent infections are either asymptomatic or self-limited, congenital infection, ocular toxoplasmosis, and reactivation in immunocompromised patients may result in serious neurologic, visual or systemic disease. Tissue cysts also help to maintain the infection clinically relevant after the acute stage (Zhao & Ewald, 2020). Therapeutic problem is closely related with the life cycle of the parasite. Acute infection is predominantly due to the rapid growing tachyzoites and the slower growing bradyzoites are found in tissue cysts and are responsible for chronic infection. Unlike the tachyzoites, Bradyzoites are metabolically and developmentally different and their decreased susceptibility to current therapies is a significant contributor to the lack of sterilization of chronic infection by current therapies (Cerutti et al., 2020). Anti-Toxoplasma treatment with pyrimethamine-sulfadiazine and other treatment regimens have been reviewed and noted as being limited by toxicity, duration of treatment, lack of activity against tissue cysts and the need for safer treatment for vulnerable populations (Dunay et al., 2018; Alday & Doggett, 2017).

Toxoplasmosis has thus made the drug re-targeting approach an attractive option. In contrast to starting with uncharacterized chemical matter, screenings are done on drugs already approved for human use as well as clinical compounds and pharmacologically annotated molecules, to look for unexpected antiparasitic activity. This can lead to an earlier discovery in some cases since the chemical identity, prior pharmacology, if not target-class information, is already known. A proof-of-principle screen of 1,120 compounds found a number of compounds that inhibit the growth of *T. gondii* at micromolar or submicromolar concentrations, and found that known drugs can have novel modes of action (Dittmar et al., 2016). This approach has been further expanded by open libraries: Pathogen Box, Malaria Box, Pandemic Response Box, COVID Box and other collections have delivered hits against Toxoplasma with various structures and mechanisms (Spalenka et al., 2018; dos Santos et al., 2023a, 2023b). The key methodological problem is that if an antiparasitic hit is a therapeutically viable lead is not guaranteed. The level of potency needs to be read in conjunction with mammalian-cell toxicity and the resulting therapeutic selectivity. The half-maximal effective concentration (EC₅₀) or half-maximal inhibitory concentration (IC₅₀) is the concentration at which the growth of parasites is reduced by 50% while the half-maximal cytotoxic concentration (CC₅₀) is the concentration at which the viability of the host cells is reduced by 50%. They can be expressed as the ratio, which is often referred as the selectivity index ($SI = CC_{50}/EC_{50}$) and is a rough approximation of the separation of antiparasitic activity and host-cell toxicity. The SI value of compounds with similar EC₅₀ can be very different, thus very different potential for development (Cajazeiro et al., 2022; Moura & Reimão, 2025).

Another difficulty is the stage specificity. A compound active against tachyzoites might not be as active against bradyzoites, or another compound might show the same or even better activity under bradyzoite inducing conditions. Clinically significant since bradyzoites and tissue cysts maintain chronic infection. Systematic evidence was also previously obtained, showing that only a relatively small number of compounds exhibited significant activity against cysts, thus emphasizing the importance of screening against the tachyzoite as well as stage-specific activity (Montazeri et al., 2018). Similarly, the development of anti-Toxoplasma leads in more recent chemical biology programs has focused on stage-specific phenotypes and brain-exposure needs. More recently, chemical biology programs have also placed emphasis on stage-specific phenotypes and brain-exposure requirements when developing anti-Toxoplasma leads (Ence et al., 2024).

The 2025 LOPAC screen by Uddin and colleagues is an ideal dataset for integrated secondary analysis, as it contains a large library of compounds that are pharmacologically annotated, as well as measurements of tachyzoite and bradyzoite growth, and host-cell cytotoxicity assays. The screen was used to test 1,280 compounds and identify 44 compounds that inhibited bradyzoites by more than 50% and subsequently prioritized compounds were concentration-response characterized. The open repository contains EC50 data for tachyzoites, two bradyzoite-induction conditions, HepG2 and differentiated THP-1 CC50 data for the leading panel (Uddin et al., 2025a, 2025b). This allows for a straightforward comparison of efficacy, safety and selectivity in the same experimental setting.

In this regard, the present study aimed to comparatively evaluate the anti-*T. gondii* activity of selected compounds using quantitative EC50 measurements in tachyzoite and bradyzoite stages, assess host-cell cytotoxicity and therapeutic selectivity by combining HepG2 and differentiated THP-1 CC50 with parasite EC50, and integrate potency, stage consistency, host-cell toxicity, and selectivity in a comparative prioritization framework to identify the most promising compounds for further anti-Toxoplasma investigation.

2. Literature Review

One of the most fruitful methods for discovering anti-Toxoplasma chemical matter has been phenotypic screening, which is the screening of whole cells without a preset target for the parasite. Dittmar et al. (2016) showed that this approach is possible on a large scale, and they found 94 compounds from a library of 1,120 compounds with IC50 values < 5 μ M. The study was significant not only due to the hit rate, but also because some active compounds (e.g. pimozone, tamoxifen) showed activity in the parasites without having their recognized mammalian targets. This is one of the main benefits of reusing drugs: "old" drugs can reveal new weaknesses in parasites. The same characteristic, however, also makes it difficult to create safe compounds for human pharmacology since compounds chosen for human pharmacology can have high activity on host pathways.

This was expanded upon by the open access screening collections. Spalenka et al. (2018) tested 400 compounds in the Pathogen Box and found eight compounds to be selective, with IC50 < 2 μ M and SI > 4, including MMV675968 (IC50 0.02 μ M and SI 275). Chemically diverse libraries were also found to be useful for identifying new anti-Toxoplasma agents as Adeyemi et al. (2018) used chemical libraries to find new anti-Toxoplasma agents. However, Subramanian et al. (2018) demonstrated that by screening across the apicomplexan systems, you can also identify mechanism relevant activity, further supporting the argument for comparative parasite biology rather than single endpoint screening.

Recent repurposing studies increasingly have used selectivity as a lead criterion. Cajazeiro et al. (2022) repurposed drugs in silico and validated their targets in cell culture, and found a number of nanomolar or micromolar anti-Toxoplasma agents including almitrine with an SI > 47. In the MMV COVID Box study, dos Santos et al. (2023a) have shown how a shortlist of the most potent compounds (EC50 between 0.04 and 0.92 μ M) can be further narrowed down to the compounds that are not toxic (CC50 between 2.48 and >50 μ M). A Pandemic Response Box investigation, related to this one, reported 42 selective compounds, with SI values reaching very high levels, and advanced the compound RWJ-67657 to a chronic mouse model (dos Santos et al., 2023b). These studies indicate that the estimation of potency, cytotoxicity and translational feasibility should be considered in combination.

It is also found in literature that the choice of host-cell has an impact on the literature. To conclude, cytotoxicity is not a unique number that is inherent to a compound, but depends on cell type, metabolic competence, the stage of cell differentiation, length of exposure, assay chemistry, and concentration range. HepG2 cells are frequently used as

metabolically active human hepatoma model while macrophage-like cells derived from THP-1 cells can contain a different biological sensitivity profile. Large differences between cell types thus yield information, rather than "noise. If the therapeutic window of a compound is narrow and weak, as it is if the compound is selective in one host cell and toxic in another, this compound will be less useful than a compound with a concordant selectivity in both host cells. Over the past few years, this idea was increasingly emerging in the field of antiparasitic discovery, where host-cell counter-screens are crucial to remove false-positive antiparasitic hits that are caused by generalized cytotoxicity (Deng et al., 2019).

Another important gap is the lack of stage-specificity. Traditional anti-Toxoplasma therapy is most effective against the proliferative tachyzoites, and fails to reliably reduce the number of tissue cysts. Montazeri et al. (2018) found that there was still a lack of evidence of the anti-cyst activity of the compound after decades of testing. Following Cerutti et al. (2020), the biological challenges of this problem are due to the changes in bradyzoite metabolism, replication, gene expression, and cyst-wall biology. Zhao and Ewald (2020) also stressed that persistent infection is a dynamic balance between differentiation of the parasite, tissue environment and host immunity. Therefore, a drug-development approach focusing on tachyzoite growth will make choices that will not necessarily take into account the chronic reservoir.

The LOPAC screen seeks to overcome this limitation head-on, compared to most previous repurposing studies. The activity against tachyzoites and bradyzoites was measured using luciferase reporters by Uddin et al. (2025a). Forty-four compounds showed more than 50% inhibition of bradyzoites in the primary screen and follow-up testing resulted in compounds being highly stage-dependent in their inhibitory activity. The sulfate form of sanguinarine was then found to be fast acting against in vitro bradyzoites and ex vivo cyst derived parasites. More importantly, the study also reported toxicity to the host cells (HepG2 and differentiated THP-1 cells), providing a dataset that allows the stage breadth and host-cell toxicity to be compared directly.

The same research program has advanced to the target oriented medicinal chemistry. Ence et al. (2024) reported bicyclic pyrrolidine inhibitors of *T. gondii* phenylalanyl-tRNA synthetase that exhibited in vitro activity and brain exposure, and Uddin et al. (2025c) later reported on the improvement of in vivo efficacy of these analogues. These studies illustrate the direction of translation after screening: An initial hit needs to be turned into a compound that is potent, selective, has good pharmacokinetics, has high tissue exposure and is in vivo effective. In this study, no medicinal-chemistry optimisation is attempted, but rather an earlier decision problem is tackled: how to prioritise repurposed hits when efficacy and toxicity data are conflicting. A common shortcoming of this literature is that a single potency value is used to describe a compound. Molecules can be stage-restricted or host-toxic and be overvalued by ranking based on lowest EC₅₀. When there is a large difference in the cytotoxicity of cell lines, ranking based on a single SI may also be unstable. It is not that the EC₅₀, CC₅₀ or SI data are not available, but that there is a methodological gap: there is no integrated interpretation that highlights robustness throughout the parasite's lifecycle and across a range of host-cell contexts. In the present secondary analysis, the open LOPAC dataset has been used to calculate stage-level geometric means, an across-stage potency summary, a conservative minimum SI across host-cell models and cross-model concordance measures.

3. Methodology

3.1 Research Design and Data Source

The design for the study was a quantitative secondary-analysis design. The main data source was the open dataset which has been made available on Mendeley Data (Uddin et al., 2025b) under a Creative Commons license (CC BY 4.0). A source study was performed using stage-specific luciferase reporter assays with the Library of

Pharmacologically Active Compounds (LOPAC) which contains 1280 drug-like and pharmacologically annotated compounds. Exported CSV files, Prism source files, source data for figures and replicate data for the principal potency/selectivity table are all contained within the repository. This analysis did not produce new biological data, but rather reanalyzed reported data to enable comparison of compounds using a common decision framework.

3.2 Analytical Sample and Variables

The analytes on the panel were the nine prioritized LOPAC hits reported with full follow-up values plus two benchmark compounds (atovaquone and BRD7929) that were added to the source table for contextual comparison. Four measures of parasite potency were extracted for each compound: Tachyzoite EC50 (Tg68Fluc EC50 and Me49Fluc EC50); and Bradyzoite EC50 under alkaline (Tg68nLuc EC50) and glutamine limited (Tg68nLuc EC50) conditions. The host cell safety variables were HepG2 CC50 and differentiated THP-1 CC50. The source study also presented SI values relative to EC50 of Tg68Fluc for the host cells. All concentrations were given in micromolar units and did not involve imputation of the source concentrations.

3.3 Derived Potency and Selectivity Metrics

The multiplicative summaries were preferred to the arithmetic means, since the EC50 values ranged over orders of magnitude. The geometric mean of the EC50s for Tg68Fluc and Me49Fluc were used to summarize the tachyzoite potency. The alkaline and glutamine-induced EC50 values were used to summarise Bradyzoite potency as the geometric mean. A geometric-mean EC50 was computed across-stage, for all four parasite conditions, to describe the general potency. The stage-ratio metric was calculated as bradyzoite geometric-mean EC50/tachyzoite geometric-mean EC50; activity greater than 1 is considered weaker against bradyzoites, and activity less than 1 is considered relatively stronger against bradyzoites. The lower value of HepG2 and THP-1 SI was conservatively set as the robust selectivity. The ratio of the larger/smaller CC50 values was defined as the host-cell toxicity discordance ratio and values near unity indicate concordant toxicity between cell models.

3.4 Comparative and Statistical Analysis

They were compared descriptively on potency, host-cell CC50, reported SI, robust minimum SI, and stage-ratio. For the small sample size and non-normal distribution of the variables, exploratory measures of concordance were Spearman rank correlations. Three associations were studied: geometric-mean potency (GM) of tachyzoites vs bradyzoites, CC50 in HepG2 vs THP-1 and SI in HepG2 vs THP-1. No causal (or population) inferences were made; p values were used descriptively and no multiplicity adjusted hypothesis-testing framework was introduced. Profiles of candidates with low EC50 values, an SI comfortably greater than 1 and little or no reliance on a single model of host cell were more favourable.

3.5 Reproducibility, Ethics, and Reporting

All transformations were deterministic, and only used numerical values that were reported in the open source dataset and corresponding publication. This was a secondary analysis of public experimental data, and was not a new animal experiment or identifiable human record, so did not require separate institutional ethics approval. This analysis separates out measurements reported by the source from derived measurements. The word 'candidate' does not imply clinical suitability, it is used to mean that it is a compound that warrants further investigation. Conformatory assays, pharmacokinetics, tissue exposure, mechanism studies and in vivo safety and efficacy would be required to make final development decisions.

4. Results

4.1 Screening Context and Analytical Panel

These are the topics that will be covered in this section of the screening process: 1,280 pharmacologically active compounds were tested in the source LOPAC screen, and 44 compounds were found to inhibit bradyzoites by > 50% in the primary screen. Follow-up dose-response testing resulted in a smaller prioritized panel, and complete parasite stage and host-cell measurements. For the present analysis, only nine hits from the prioritized set and the two benchmark compounds (atovaquone and BRD7929) were fully characterised. This distinction is important because the comparative EC₅₀-CC₅₀-SI analysis does not include all 1280 compounds, it is the follow up panel of fully characterized compounds. The analytical flow of the full LOPAC screen to the multi-parameter comparison employed in this study is shown in Figure 1.

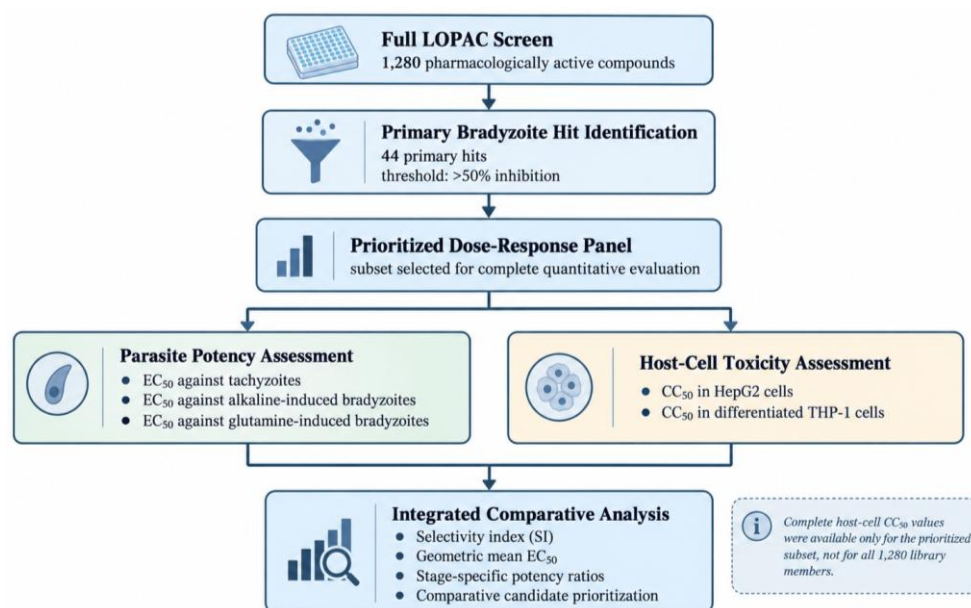


Figure 1. Analytical Flow from LOPAC Screening to Comparative Candidate Prioritization

The analytical sequence preserves the hierarchy of the source experiment while making clear that the final ranking problem depends on a subset with complete quantitative endpoints. This prevents the secondary analysis from implying that host-cell CC₅₀ values were available for every library member.

4.2 Comparative Potency Across Parasite Stages

To determine whether the prioritized compounds maintained consistent antiparasitic activity across biologically distinct *Toxoplasma gondii* stages, potency was compared across tachyzoite and bradyzoite conditions. This comparison is important because strong activity against the rapidly replicating tachyzoite stage does not necessarily translate into equivalent activity against bradyzoite-associated conditions. Accordingly, the analysis considered the source-reported EC₅₀ values together with derived geometric-mean potency estimates and stage-specific potency ratios to characterize both absolute activity and stage-dependent shifts in compound performance. Table 1 compares the source EC₅₀ values and the derived stage-specific and across-stage potency summaries.

Table 1. Source EC50 values (μM) and derived potency metrics across tachyzoite and bradyzoite conditions. GM = geometric mean. Stage ratio = bradyzoite GM/tachyzoite GM.

Compound	Tg68 tach EC50	ME49 tach EC50	Alkaline brady EC50	Glutamine brady EC50	Tach GM	Brady GM	Across-stage GM	Stage ratio
Diphenyleneiodonium sulfate	0.01	0.01	0.27	0.06	0.010	0.127	0.036	12.73
Emetine dihydrochloride hydrate	0.03	0.03	2.50	1.08	0.030	1.643	0.222	54.77
Sanguinarine sulfate	0.30	0.10	4.00	1.25	0.173	2.236	0.622	12.91
Brefeldin A	0.06	0.05	0.40	0.16	0.055	0.253	0.118	4.62
Idarubicin hydrochloride	0.19	0.67	0.30	0.82	0.357	0.496	0.421	1.39
T0070907	0.75	0.87	0.64	0.60	0.808	0.620	0.708	0.77
MS012	2.46	2.68	0.85	2.23	2.568	1.377	1.880	0.54
Auranofin	2.75	1.12	0.68	0.38	1.755	0.508	0.945	0.29
JFD00244	3.89	3.39	2.88	4.48	3.631	3.592	3.612	0.99
Atovaquone	0.16	0.10	2.07	0.06	0.126	0.352	0.211	2.79
BRD7929	0.11	0.05	0.22	0.08	0.074	0.133	0.099	1.79

Diphenyleneiodonium sulfate had the lowest across-stage geometric-mean EC50 (0.036 μM), followed by BRD7929 (0.099 μM) and brefeldin A (0.118 μM). However, potency breadth differed markedly. Emetine was extremely potent against both tachyzoite strains (0.03 μM) but its bradyzoite geometric mean rose to 1.643 μM , producing a stage ratio of 54.77. Sanguinarine sulfate showed a similar, though less extreme, shift toward weaker bradyzoite activity. In contrast, T0070907, MS012, and auranofin had stage ratios below 1, indicating that their average EC50 values were lower under the two bradyzoite conditions than across the tachyzoite strains. The rank correlation between tachyzoite and bradyzoite geometric-mean potency was moderate (Spearman $\rho = 0.60$, $p = 0.051$), suggesting partial but incomplete preservation of compound ranking across developmental states. Figure 2 depicts the contrast between tachyzoite and bradyzoite geometric-mean EC50 values on a logarithmic scale.

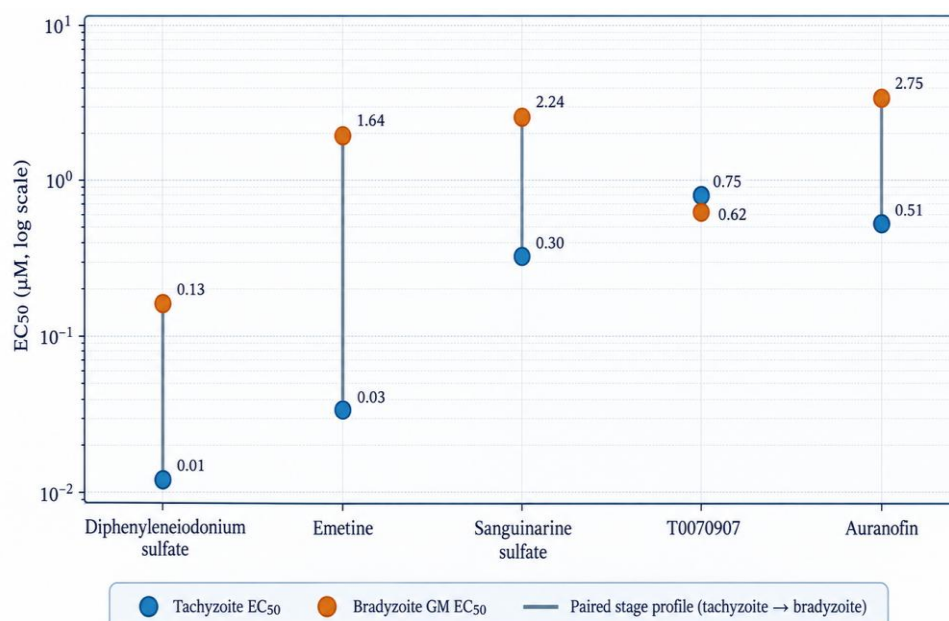


Figure 2. Tachyzoite versus Bradyzoite Geometric-Mean EC₅₀ Profiles of the Comparative Panel

The proposed figure makes stage-dependent shifts visible: emetine and sanguinarine move sharply toward weaker bradyzoite potency, whereas auranofin and T0070907 retain or improve relative activity under bradyzoite conditions. These patterns show why the lowest tachyzoite EC₅₀ alone is an insufficient criterion for chronic-stage-oriented prioritization.

4.3 Host-Cell Toxicity and Selectivity

The therapeutic relevance of the prioritized compounds was further assessed by examining whether their antiparasitic activity was accompanied by acceptable host-cell safety. Cytotoxicity was evaluated across HepG2 and differentiated THP-1 cell models to capture potential variation in compound tolerance between different mammalian cellular systems. Because a high selectivity index reflects a wider separation between antiparasitic efficacy and host-cell toxicity, selectivity was treated as a central criterion for comparative prioritization. To obtain a conservative estimate of safety robustness, the lower SI observed across the two host-cell models was retained as the minimum SI, while CC₅₀ discordance was used to identify compounds showing substantial variability in toxicity between cell types. Table 2 presents HepG2 and differentiated THP-1 CC₅₀ values, reported SI values, and the conservative minimum SI used to represent robustness across host-cell models.

Table 2. Host-cell toxicity and selectivity. CC₅₀ values are in μM. Minimum SI is the lower of the two source-reported selectivity indices; CC₅₀ discordance is the higher CC₅₀ divided by the lower CC₅₀.

Compound	HepG2 CC ₅₀	HepG2 SI	THP-1 CC ₅₀	THP-1 SI	Minimum SI	CC ₅₀ discordance
Diphenyleneiodonium sulfate	1.28	96	4.85	365	96	3.79

Emetine dihydrochloride hydrate	1.92	64	0.23	8	8	8.35
Sanguinarine sulfate	9.44	32	4.34	15	15	2.18
Brefeldin A	60.00	987	0.07	1	1	857.14
Idarubicin hydrochloride	0.14	1	0.59	3	1	4.21
T0070907	18.12	24	21.98	29	24	1.21
MS012	2.03	1	1.19	0.5	0.5	1.71
Auranofin	9.81	4	2.83	1	1	3.47
JFD00244	16.07	4	2.95	1	1	5.45
Atovaquone	9.67	62	14.26	91	62	1.47
BRD7929	6.08	54	2.87	25	25	2.12

The host-cell comparison materially changed the interpretation of several compounds. Diphenyleneiodonium sulfate retained a high minimum SI of 96 despite a 3.79-fold CC50 difference between host-cell models. T0070907 showed the most balanced combination of moderate-to-high CC50 values (18.12 and 21.98 μ M) and closely matched SI values (24 and 29), giving a minimum SI of 24. By contrast, brefeldin A appeared exceptionally selective in HepG2 cells (SI 987) because of a 60 μ M CC50, yet it was highly toxic to differentiated THP-1 cells (CC50 0.07 μ M; SI 1). Its CC50 discordance ratio exceeded 850-fold, demonstrating that a single-cell-line safety estimate would be misleading. Emetine also showed substantial cell-line dependence, with the THP-1 CC50 approximately eightfold lower than the HepG2 value. Across the panel, HepG2 and THP-1 CC50 ranks were weakly associated (Spearman $\rho = 0.17$, $p = 0.612$), while SI ranks showed only a moderate, non-significant association ($\rho = 0.50$, $p = 0.119$).

4.4 Integrated Candidate Profiles

The final comparative step integrated antiparasitic potency, stage breadth, and host-cell selectivity to provide a more balanced interpretation of the repurposing candidates. This approach was necessary because no single metric adequately captures the overall suitability of a compound for further investigation. Compounds with strong tachyzoite potency may show weaker activity under bradyzoite conditions, while highly potent candidates may also exhibit limited selectivity toward mammalian host cells. The integrated assessment therefore considered across-stage activity together with the consistency of the therapeutic window across host-cell models, allowing the main candidates to be compared on multiple complementary dimensions rather than on potency alone. Table 3 summarizes the integrated interpretation of potency, stage breadth, and robust selectivity for the main repurposing hits.

Table 3. Integrated descriptive prioritization of the nine main repurposing hits. Priority labels are descriptive summaries of the observed dataset and do not represent clinical recommendations.

Compound	Across-stage GM EC50	Minimum SI	Integrated priority	Interpretation
Diphenyleneiodonium sulfate	0.036	96	Very high	Strong overall potency; bradyzoite potency lower than tachyzoite potency; favorable minimum SI
T0070907	0.708	24	High	Moderate potency; balanced activity across stages; concordant host-cell safety
Sanguinarine sulfate	0.622	15	Moderate-high	Moderate broad potency; weaker under bradyzoite conditions; retains multi-cell selectivity
Emetine dihydrochloride hydrate	0.222	8	Moderate	Excellent tachyzoite potency but pronounced bradyzoite loss and THP-1 toxicity
Brefeldin A	0.118	1	Low	High apparent potency but extreme host-cell-model discordance undermines therapeutic window
Idarubicin hydrochloride	0.421	1	Low	Submicromolar average potency but cytotoxicity overlaps parasite activity
Auranofin	0.945	1	Low	Relatively stronger bradyzoite profile but narrow minimum selectivity in this dataset
MS012	1.880	0.5	Very low	Limited potency and no robust separation from host-cell toxicity
JFD00244	3.612	1	Low	Weakest broad potency with narrow minimum selectivity

On an integrated basis, diphenyleneiodonium sulfate emerged as the clearest potency-selectivity profile in the main hit set because its across-stage geometric-mean EC50 was substantially below 0.1 μ M and its minimum SI remained 96. T0070907 represented a different but potentially useful profile: it was less potent, yet its stage ratio was near unity and host-cell CC50 values were closely concordant, indicating greater consistency across biological contexts. Sanguinarine sulfate retained a favorable minimum SI of 15

but showed reduced bradyzoite potency, consistent with the need for additional cyst-directed confirmation. Emetine illustrates why potency-first ranking can be misleading: its tachyzoite EC₅₀ was 0.03 μ M, but its bradyzoite geometric mean was 1.643 μ M and its minimum SI fell to 8 because of THP-1 toxicity. Several other compounds lost priority because their host-cell toxicity approached their anti-parasite concentrations. Figure 3 provides the proposed potency-selectivity decision map.

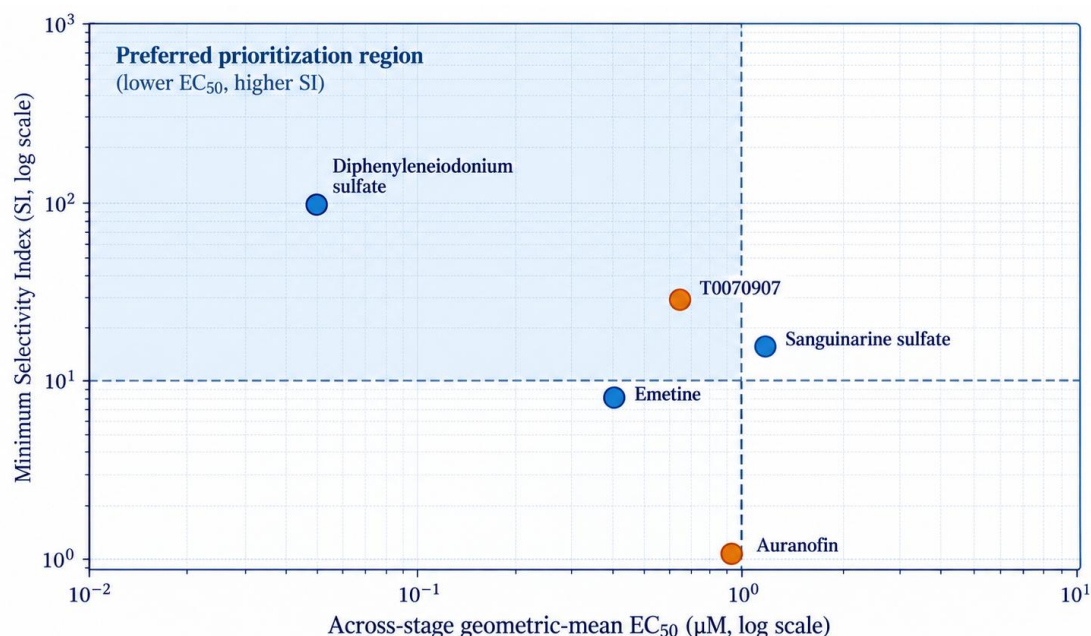


Figure 3. Integrated Potency-Selectivity Map for Prioritized Repurposing Candidates

The decision map highlights that the desirable candidates are in the low EC₅₀/high SI quadrant. It also helps to distinguish compounds which are appealing based on their potency from those that have a repeatable therapeutic window in both host cell models.

5. Discussion

This secondary analysis shows that the anti-Toxoplasma repurposing candidates can shift in position significantly when taking into account the potency, the stage of the parasite and the toxicity toward the host cells. The present analysis tackles a different question – which fully characterized compounds have a favourable profile after the most conservative part of host-cell and stage-specific information is included in the LOPAC library? This is not a straight-line ranking based on EC₅₀. Diphenyleneiodonium sulfate continues to be a strong candidate due to its high potency and relatively high minimum SI, while brefeldin A has a high potency with parasite inhibition, but has a very low minimum SI due to THP-1 toxicity.

The importance of diphenyleneiodonium sulfate is in line with the rationale of phenotypic repurposing, which is the ability of compounds to take advantage of parasite vulnerabilities, in addition to their known mammalian pharmacology. It had the lowest across-stage geometric-mean EC₅₀ of 0.036 μ M and a minimum SI of 96 (the more conservative of the two host-cell models) which were the lowest in the panel. The 12.7-fold ratio of bradyzoites to tachyzoites indicates that the activity of this strain is not consistent in different developmental conditions. The relevance of this qualification is that efficacy in the chronic stage of disease is the key unmet need in the treatment of toxoplasmosis and not just potency in the acute stage (Alday & Doggett, 2017; Cerutti et al., 2020). A compound may thus be very promising for further investigations even though it is not termed stage-invariant.

T0070907 is a free example. The overall potency was relatively weak, in comparison with the nanomolar compounds, but the geometric means of the tachyzoite and bradyzoite potency were comparable and the CC50 for the host cells showed a difference of approximately 21%. Both HepG2 and THP-1 models showed a maintenance of the minimum SI of 24. This consistency can be useful in early discovery as large variations between the different host-cell backgrounds may be indicative of context-dependent toxicity. The lack of correlation between HepG2 and THP-1 CC50 values for this small panel justifies the use of more than one counter-screen, and not assuming that cytotoxicity is a characteristic of a compound.

In particular, the difference between brefeldin A and T0070907 is interesting. Brefeldin A had an across stage geometric-mean EC50 of 0.118 μ M and an impressive HepG2 SI of 987, which would rank it towards the top of a single cell line analysis. Yet its THP-1 CC50 was only 0.07 μ M and its THP-1 SI was 1. The apparent selectivity of CC50 is very context dependent, as it is more than 850-fold different between the two host-cell models. Multi-cell counter screening is intended to find just this type of result. The same can be said for emetine, which has good tachyzoite potency, but a low minimum SI due to its toxicity to THP-1 cells.

The stage comparisons also show why the stage of chronic drug discovery can't be confined to the screening of tachyzoites. Emetine and sanguinarine sulfate were significantly less active in bradyzoite assays than in tachyzoite assays, while auranofin and MS012 had lower average EC50 values in bradyzoite assays as compared to tachyzoite assays. This last fact does not necessarily mean that they are more effective development candidates, since their minimum SI values were low, but it does show that the efficacy landscape changes as the developmental state of the parasites changes. Previous systematic studies also indicated that anti-cyst activity is not widely found and needs to be confirmed by experimental studies (Montazeri et al., 2018). Therefore, the use of different alkaline and glutamine cultures/conditions in the source study is a significant strength as it minimizes reliance on any one in vitro method for inducing bradyzoite formation.

The position of sanguinarine sulfate in the current analysis is in the middle. It was found to have an across-stage geometric-mean EC50 of 0.622 μ M and a minimum SI of 15. The source study conducted additional testing with good in vitro bradyzoite activity and ex vivo cyst-derived parasites, further supporting its biological interest beyond the summary data (Uddin et al., 2025a). Its stage ratio (about 12.9) however, suggests that the two bradyzoite EC50s were significantly higher than the tachyzoite EC50s. This combination is what makes it important to look at the results of different experimental readouts together: A compound could be more potent and/or more effective at a concentration-response in one assay but still be biologically active killing in another.

Auranofin also exemplifies the difference between an antiparasitic rationale and prioritizing the datasets. The redox pharmacology of Auranofin makes it a good candidate for repurposing in infectious and noninfectious diseases (Roder & Thomson, 2015; Yamashita, 2021). It also displays antiparasitic effects on various organisms, which have also been documented (Feng et al., 2020). The geometric mean of its bradyzoites was lower than its tachyzoites in the present dataset but its minimum SI was 1 due to the proximity of the tachyzoite cell culture 50 to the THP-1 CC50. Therefore, the analysis does not exclude auranofin as a research compound, but indicates that it would not be considered a high priority compound for this assay system and comparator set, given the lack of sufficient host-cell separation.

The study also goes into a methodological discussion of selectivity indices. SI is often considered a scalar, but its value will depend on the parasite assay employed in the denominator and host-cell model employed in the numerator. The source SIs were derived relative to Tg68Fluc tachyzoites, and are not directly measuring the selectivity against each bradyzoite condition. The current minimal SI metric is thus a measure of host-cell robustness, rather than a measure of therapeutic index. To improve future

datasets, SI values for each stage should be reported for each parasite condition and confidence intervals should be provided around the estimates of EC50 and CC50. This type of reporting would enable the propagation of uncertainty into the candidate-ranking models.

There are a number of caveats. First, the quantitative follow-up panel is small, and was chosen from a larger primary panel, therefore it is not representative for the chemical diversity of all 1280 LOPAC compounds. Second, reported summary values were used instead of refitting all of the raw concentration-response curves, so uncertainty in the estimation of these summary values could not be propagated through to the derived geometric means. Third, HepG2 and THP-1 cells are only two aspects of mammalian toxicity and do not represent organ-specific toxicity or primary human cells, pharmacokinetic exposure or in vivo tolerability. Fourth, bradyzoite induction in vitro is not a true representation of chronic-stage biology, but rather a model. Lastly, the priority categories integrated are descriptive, and are not meant to aid clinical decisions. Even with these constraints, the analysis is useful in practice since it applies a clear and transparent rule for selecting the leads, and thus does not suffer from the usual pitfalls of lead selection. It prefers compounds with multiple activity against different types of parasites and which are selective against more than one type of host cell in a series of assays and does not require a single exceptionally good assay. This is in line with the current approach of the anti-Toxoplasma discovery programs, which are increasingly merging phenotypic screening, cytotoxicity counter-screens, mechanistic studies, medicinal chemistry, tissue exposure, and in vivo validation (Deng et al., 2019; Ence et al., 2024; Uddin et al., 2025c). Confirmatory dose-response testing of the most promising profiles with other human cell types, direct cyst evaluation and in vivo pharmacokinetic evaluation prior to in vivo efficacy studies should be the next experimental steps.

6. Conclusion

This study reassesses a previously published open LOPAC anti-Toxoplasma dataset, aiming to unravel the interplay between compound potency, stage specificity, toxicity to host cells and selectivity in the repurposing-based prioritization of compounds. The results of this analysis demonstrated that development potential is not necessarily well reflected by potency alone. Diphenyleneiodonium sulfate was the most potent from the across-stage perspective but it also had the highest minimum selectivity index, whereas T0070907 was less potent but more balanced across the parasite stages and host-cell models. The potency of the bradyzoite remained moderate to high, while the selectivity of Sanguinarine sulfate was retained. Brefeldin A, on the other hand, showed how important it is to use multiple cytotoxicity assays to prove that an apparent exceptional SI for HepG2 was offset by a significant toxicity to THP-1. The results justify a conservative prioritisation method, which is based on several parasite states and on several counter-screening parasites of mammalian cells. Due to the small number of follow-up subjects selected from the population and the summary experimental values used in the analysis, the findings should be used to inform further studies, and should not be interpreted as a judgement on clinical appropriateness. Future studies should re-analyse raw dose-response data with uncertainty estimates, include other human cell systems in toxicity studies and assess the best candidates in direct tissue-cyst, pharmacokinetic and in vivo efficacy models.

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