International Journal of Biological and Pharmaceutical Sciences Archive

ISSN: 0799-6616 (Online)

Journal homepage: https://ijbpsa.com/

(RESEARCH ARTICLE)

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Virtual screening of phytochemicals for the identification of novel anti-*Leishmania donovani* Agents

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International Journal of Biological and Pharmaceutical Sciences Archive, 2025, 10(02), 186–195

Publication history: Received 11 November 2025; revised on 16 December 2025; accepted on 19 December 2025

Article DOI: https://doi.org/10.53771/ijbpsa.2025.10.2.0089

Abstract

Leishmania donovani is the causative agent of visceral leishmaniasis, a severe and potentially fatal neglected tropical disease prevalent in many developing regions. The parasite is transmitted through the bite of infected sandflies, and its ability to invade and replicate within host macrophages contributes to significant morbidity and mortality. Despite the availability of chemotherapeutic agents, treatment is often limited by toxicity, high cost, emerging resistance, and complex dosing regimens. These limitations highlight the need for new, safer, and more effective anti-leishmanial compounds. This study employed an in-silico drug discovery approach to evaluate the potential inhibitory effects of phytochemicals against a bromodomain-containing protein of L. donovani (PDB ID: 5C4Q). The receptor was prepared using Chimera, PyMOL, and AutoDock Tools, while 6,500 phytochemicals obtained from Phytochemical databases were screened using DataWarrior based on Lipinski's Rule of Five and predicted toxicity parameters. A total of 1,700 compounds passed preliminary filtering and were prepared for molecular docking using AutoDock Vina. Docking protocol validation was performed prior to docking, and post-docking visualization was carried out using PyMOL. The reference ligand, bromosporine (BMF), displayed a mean binding energy of -6.1 kcal/mol. In contrast, the top 20 phytochemicals exhibited significantly stronger binding affinities, ranging from -10.7 kcal/mol to -9.2 kcal/mol, indicating higher predicted interaction strength within the receptor's binding pocket. These findings suggest that several phytochemicals particularly Anastatin B (-10.7 kcal/mol), abyssinone I (-10.2 kcal/mol), abyssinoflavone V (-10 kcal/mol), and calopogonium isoflavone B (-9.9 kcal/mol) may serve as promising lead compounds for anti-leishmanial drug development.

Keywords: *Leishmania donovani*; Visceral leishmaniasis; Phytochemicals; Molecular Docking; Virtual Screening; Binding Affinity.

1. Introduction

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania* and transmitted to humans through the bite of infected female *Phlebotomus* or *Lutzomyia* sand flies. The disease remains endemic in 98 countries and territories, with an estimated 12 million people currently infected and 1.5–2 million new cases occurring annually [1,2]. The World Health Organization (WHO) classifies leishmaniasis among the most important neglected

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tropical diseases due to its substantial morbidity, mortality, and socioeconomic burden in low- and middle-income countries [2,3].

More than 53 *Leishmania* species have been described, of which 31 infect mammals and approximately 20 are pathogenic to humans [4]. Clinical manifestations vary widely and depend on the infecting species and host immune response. The three primary forms are: cutaneous leishmaniasis, affecting the skin; mucocutaneous leishmaniasis, involving the nasopharyngeal mucosa; and visceral leishmaniasis (VL), also known as kala-azar, which is the most severe form and is almost always fatal if left untreated [2, 5]. *Leishmania donovani* and *Leishmania infantum* are the principal etiological agents of VL, affecting vital organs such as the liver, spleen, and bone marrow [6]. The incidence of leishmaniasis has increased among immunocompromised individuals, particularly patients with human immunodeficiency virus (HIV), where VL is a recognized opportunistic infection [7, 8].

Despite the global burden, therapeutic options remain limited. First-line drugs—including pentavalent antimonials, amphotericin B, miltefosine, and paromomycin—are constrained by high toxicity, long treatment duration, variable efficacy, and emerging parasite resistance [9-12]. Moreover, the cost and logistical challenges of current therapeutics make them unsuitable for large-scale use in many endemic regions. These limitations underscore the need for novel, safe, and affordable antileishmanial agents.

Phytochemicals derived from medicinal plants have long served as a rich reservoir of bioactive compounds and continue to contribute significantly to anti-infective drug discovery [13-15]. Their structural diversity, evolutionary optimization, and wide biological activities make them promising candidates for antileishmanial drug development. Recent studies have demonstrated significant inhibitory effects of various plant secondary metabolites—including alkaloids, flavonoids, and terpenoids—against *Leishmania* species [16-18].

Advances in computational drug discovery have further accelerated the identification of potential therapeutic molecules. Structure-based virtual screening, molecular docking, and in-silico toxicity prediction provide efficient and cost-effective platforms for evaluating large compound libraries before laboratory testing [19-21]. These tools enable prediction of ligand binding affinities, interaction patterns, and drug-likeness properties, thereby improving lead optimization and reducing late-stage failures.

In this study, we employed a comprehensive *in-silico* workflow to evaluate the potential antileishmanial activity of phytochemicals against a high-resolution *L. donovani* target protein (PDB ID: 5C4Q). A dataset of approximately 6,500 phytochemicals sourced from the Phytochemical database was screened for drug-likeness using Lipinski's Rule of Five and further evaluated for toxicity. The filtered compounds were docked against the target receptor using AutoDock Vina, followed by comparative binding-affinity assessment and structural visualization of ligand–protein interactions.

The objective of this research is to identify phytochemical candidates with favorable binding affinities and predicted safety profiles that may serve as potential inhibitors of *L. donovani*. The findings provide a foundation for future in-vitro and in-vivo validation studies and contribute to ongoing efforts toward the development of new therapeutic options for visceral leishmaniasis.

2. Materials and methods

An in-silico approach was employed to evaluate the potential activity of selected phytochemicals against *Leishmania donovani*. The computational tools used in this study included PyMOL, UCSF Chimera, AutoDock Tools, AutoDock Vina, DataWarrior, Open Babel, and MGL Tools. Bioinformatics and chemoinformatics resources comprised the Protein Data Bank (PDB) and the Phytochemical databases like PhytoHub. All analyses were performed on systems running Ubuntu Linux and Windows 10 Pro.

Molecular docking and preparation files included:

- **Conf.text**, a configuration file used to store binding-site coordinates and grid box dimensions for consistency during docking.
- **binbash.sh**, a Bash script used to automate docking runs in AutoDock Vina on Ubuntu.

2.1. Bioinformatics mining – target site identification

A systematic search was conducted in the Protein Data Bank (PDB) to identify suitable protein targets associated with *Leishmania donovani* pathogenicity. Selection criteria included a resolution of less than 4 Å, the presence of an organic ligand within the structure, and biological relevance to the parasite. A bromodomain-containing receptor complexed with bromosporine (PDB ID: 5C4Q; resolution 1.93 Å) was identified and selected for further analysis.

2.2. Chemoinformatic mining

Approximately 6,500 phytochemicals were obtained from the Phytochemical databases in SDF format. These compounds were subjected to drug-likeness screening using DataWarrior based on Lipinski's Rule of Five, eliminating molecules with molecular weight >500 Da, more than 5 hydrogen bond donors, more than 10 hydrogen bond acceptors, or LogP >5. Toxicity filters assessing mutagenicity, tumorigenicity, reproductive effects, irritant properties, and other unfavorable chemical functions were also applied. Compounds that passed both drug-likeness and toxicity screening were retained for molecular docking.

2.3. Selection and preparation of target protein

Following identification of a suitable structure, the selected protein (PDB ID: 5C4Q) was downloaded in PDB format. Protein preparation was performed using UCSF Chimera and AutoDock Tools. In Chimera, redundant chains were removed, retaining only chain A, which was saved as "Deleted 5C4Q". All ligands except the reference ligand bromosporine (BMF) were removed and the resulting structure was saved as "Deleted 5C4Q + Ligand". The reference ligand was subsequently removed to generate the apo-protein, saved as "Filtered 5C4Q".

The apo-protein file was then imported into AutoDock Tools, where polar hydrogens and Kollman charges were added. The structure was converted to PDBQT format and saved for docking procedures.

2.4. Selection and preparation of target ligands

The reference ligand was selected based on two criteria: (1) it must be an organic molecule, and (2) it must occupy a defined binding pocket within the target protein.

Visualization in PyMOL and Chimera confirmed that bromosporine (BMF) met these criteria and was therefore chosen as the reference ligand. BMF was downloaded in ideal SDF format.

Ligand preparation was performed using Open Babel and AutoDock Tools. Phytochemicals and the reference ligand were initially converted from SDF to MOL2 format in Ubuntu using the Open Babel command-line interface. These MOL2 files were transferred to Windows for final preparation in AutoDock Tools, where Gasteiger charges were computed and all torsional bonds were set to non-rotatable. Each ligand was saved in PDBQT format.

2.5. Validation of docking protocol

To validate the docking workflow, the binding site of the reference ligand BMF was identified by isolating it from the protein structure in Chimera and loading it alongside the processed protein in AutoDock Tools. A grid box was created to encompass the ligand-binding region, and its center coordinates and dimensions were recorded as follows:

Table 1 Centre and dimension of grid box

Grid Box	Center	Dimension
X	3.476	16
Y	24.763	14
Z	49.909	14

These parameters were used to generate the configuration file for molecular docking.

2.6. Molecular docking

Molecular docking simulations were performed using AutoDock Vina on the Ubuntu operating system. The PDBQT files for the protein (5C4Q), the reference ligand (BMF), and all screened phytochemicals were placed in a dedicated folder. This folder was accessed via the terminal, and docking was executed using the binbash.sh script, which automated batch processing of ligands against the prepared target receptor.

2.7. Post-docking analysis

Upon completion of the docking simulations, results from four docking runs per ligand were compiled into Microsoft Excel. The mean binding affinity and standard deviation for each ligand were calculated. Compounds exhibiting stronger binding affinity (more negative binding energy) than the reference ligand were identified as front-runner candidates.

Binding poses were visualized in PyMOL to confirm proper occupation of the binding pocket. Each ligand was color-coded for differentiation, and interactions with amino acid residues were examined to assess bonding patterns. Representative snapshots of ligand–protein interactions were generated and saved in PNG format for documentation and figure preparation.

3. Results

A total of approximately 6,500 phytochemicals were obtained from the Phytochemical database and subjected to drug-likeness and toxicity screening using DataWarrior. Compounds that failed Lipinski's criteria or showed mutagenicity, tumorigenicity, reproductive toxicity, irritant potential, or other undesirable features were removed. After filtering, roughly 1,700 phytochemicals were retained for docking.

All selected ligands were prepared and docked against the target protein 5C4Q, and their binding affinities were determined across four docking runs. The mean binding energy and standard deviation for each compound were calculated and compared with the reference ligand (BMF). Twenty phytochemicals demonstrated stronger binding affinity than the reference ligand and were identified as front-runner compounds.

The molecular descriptors of the top ligands are presented in Table 3. All front-runners satisfied Lipinski's Rule of Five, indicating good drug-likeness. Their toxicity profiles are shown in Table 4, and all compounds were predicted to be non-toxic across assessed parameters.

Binding energies for the top 20 ligands across four docking trials (P1-P4), along with their mean values and standard deviations, are shown in Table 2. The binding affinities ranged from -10.7 to -9.2 kcal/mol, significantly stronger than the reference ligand BMF, which produced a mean binding energy of -6.125 kcal/mol. Visualization of the ligand poses in the binding pocket is shown in Figure 1, while interaction maps of selected front-runner ligands are illustrated in Figure 2.

Table 2 The binding energies (p1-p4) of the phytochemicals when docked with 5c4q receptor.

S/N	MOLECULAR NAME	P1	P2	P3	P4	mean	SD
1	anastatin B	-10.7	-10.7	-10.7	-10.7	-10.7	0
2	abyssinone I	-10.2	-10.3	-10.2	-10.2	-10.2	0.1
3	abyssinoflavone V	-10	-10	-10	-10	-10	0
4	Calopogonium isoflavone B	-9.9	-9.9	-9.9	-9.9	-9.9	0
5	3'-O-methylorobol	-9.8	-9.8	-9.8	-9.8	-9.8	0
6	7-hydroxy-4'-methoxy-3'-prenylisoflavone	-9.8	-9.8	-9.8	-9.8	-9.8	0
7	abyssinone II	-9.8	-9.8	-9.8	-9.8	-9.8	0
8	5-deoxyabyssinin II	-9.7	-9.7	-9.7	-9.7	-9.7	0
9	1-O-acetyllycorine	-9.6	-9.5	-9.5	-9.5	-9.5	0.1

10	abyssinin I	-9.6	-9.6	-9.6	-9.6	-9.6	0
11	8-C-p-hydroxybenzylapigenin	-9.5	-8.4	-8.4	-8.5	-8.7	0.5
12	5,6-dihydrobicolorine	-9.4	-9.4	-9.4	-9.4	-9.4	0
13	Anhydrolycorine	-9.4	-9.4	-9.4	-9.4	-9.4	0
14	2(S),3',4'-dihydroxy-7-methoxyflavan	-9.3	-9.3	-9.3	-9.3	-9.3	0
15	3'-O-methylpratensein	-9.3	-9.3	-9.3	-9.3	-9.3	0
16	3'-prenylnaringenin	-9.3	-9.3	-9.4	-9.3	-9.3	0.1
17	abyssinin II	-9.3	-9.5	-9.5	-9.5	-9.5	0.1
18	4beta,8alpha-dihydroxy-6alpha-vanilloyloxydauc-9-ene	-9.2	-9.1	-9.2	-9.1	-9.2	0.1
19	6-bromo-N-propionylaplysinopsin	-9.2	-9.2	-9.2	-9.2	-9.2	0
20	7-O-methylluteolin	-9.2	-9.2	-9.2	-9.2	-9.2	0
611	bmf_reference ligand	-6.0	-6.2	-6.1	-6.2	-6.1	0.1

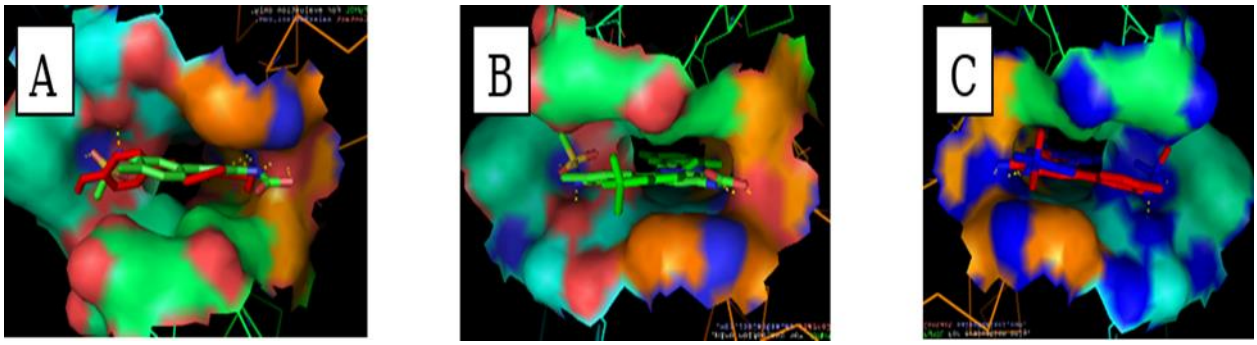

Figure 1 The top three ligands within the binding pocket of the selected protein; 5C4q. (A: Anastatin B, B: Abysinnone I, C: Abysinnoflavone B)

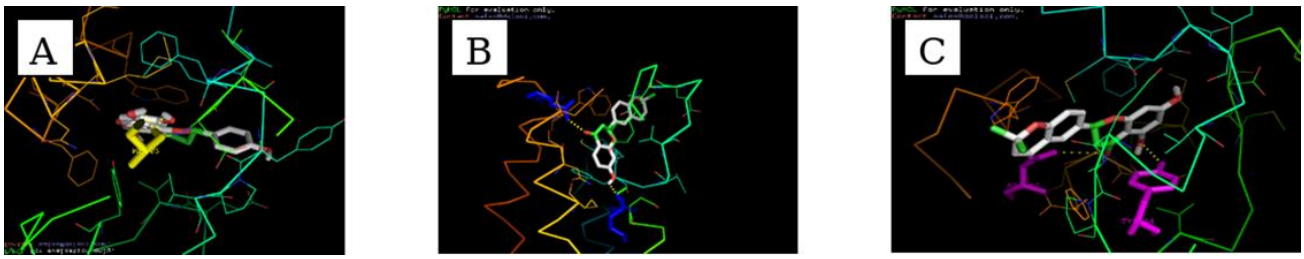

Figure 2 The interaction between the amino acid of 5C4Q and the phytochemical(A: Anastatin B, B: Abysinnone I, C: Abysinnoflavone B)

Table 3 The molecular descriptors of the front liners

S/n	names of ligands	Chemical formula	MW	ClogP	HBA	HBD
1	anastatin B	C21H14O7	378.335	3.5785	7	4
2	abyssinone I	C20H18O4	322.359	3.8738	4	1
3	abyssinoflavone V	C20H18O5	338.358	3.5281	5	2
4	Calopogonium isoflavone B	C21H16O5	348.353	3.8024	5	0
5	3'-O-methylorobol	C16H12O6	300.265	1.5572	6	3
6	7-hydroxy-4'-methoxy-3'-prenylisoflavone	C21H20O4	336.386	4.2711	4	1
7	abyssinone II	C20H20O4	324.375	4.5239	4	2
8	5-deoxyabyssinin II	C21H22O5	354.401	4.4539	5	2
9	1-O-acetyllycorine	C18H19NO5	329.351	1.6924	6	1
10	abyssinin I	C21H20O6	368.384	3.4581	6	2
11	8-C-p-hydroxybenzylapigenin	C22H16O6	376.363	3.8059	6	4
12	5,6-dihydrobicolorine	C15H13NO2	239.273	2.7385	3	0
13	Anhydrolycorine	C16H13NO2	251.284	2.9845	3	0
14	2(S),3',4'-dihydroxy-7-methoxyflavan	C16H16O4	272.299	2.9822	4	2
15	3'-O-methylpratensein	C17H14O6	314.292	1.8329	6	2
16	3'-prenylnaringenin	C20H18O5	338.358	4.3582	5	3
17	abyssinin II	C21H22O6	370.4	4.1082	6	3
18	4beta,8alpha-dihydroxy-6alpha-vanilloyloxydauc-9-ene	C24H30O5	398.497	3.7483	5	1
19	6-bromo-N-propionylaplysinopsin	C17H17BrN4O2	389.252	2.3561	6	1
20	7-O-methylluteolin	C16H12O6	300.265	2.2657	6	3

Table 4 The toxicity profile of the front liners

S/n	names of ligands	Mutagenicity	Tumorigenicity	Effect on reproduction	Irritant effect	Nasty functions
1	anastatin B	None	None	None	None	None
2	abyssinone I	None	None	None	None	None
3	abyssinoflavone V	None	None	None	None	None
4	Calopogonium isoflavone B	None	None	None	None	None
5	3'-O-methylorobol	None	None	None	None	None
6	7-hydroxy-4'-methoxy-3'-prenylisoflavone	None	None	None	None	None
7	abyssinone II	None	None	None	None	None
8	5-deoxyabyssinin II	None	None	None	None	None
9	1-O-acetyllycorine	None	None	None	None	None

10	abyssinin I	None	None	None	None	None
11	8-C-p-hydroxybenzylapigenin	None	None	None	None	None
12	5,6-dihydrobicolorine	None	None	None	None	None
13	Anhydrolycorine	None	None	None	None	None
14	2(S),3',4'-dihydroxy-7-methoxyflavan	None	None	None	None	None
15	3'-O-methylpratensein	None	None	None	None	None
16	3'-prenylnaringenin	None	None	None	None	None
17	abyssinin II	None	None	None	None	None
18	4beta,8alpha-dihydroxy-6alphavanilloyloxydauc-9-ene	None	None	None	None	None
19	6-bromo-N-propionylaplysinopsin	None	None	None	None	None
20	7-O-methylluteolin	None	None	None	None	None

4. Discussion

Understanding ligand–protein binding affinity is fundamental in predicting the biological activity of candidate molecules. Binding affinity approximates the Gibbs free energy of interaction, where more negative values indicate stronger, spontaneous, and thermodynamically favorable binding [22, 23]. Docking scoring functions estimate this free energy to rapidly identify ligands with high potential for target engagement. A more negative ΔG generally corresponds to tighter binding, whereas less negative values reflect weaker interactions [24, 25].

Binding affinity can also be classified into categories: weak, moderate, strong, and very strong-based on established computational benchmarks [26]. Such stratification has been used across multiple receptor systems, including nuclear receptors and protozoan enzymes, supporting its relevance in early drug discovery.

In this study, approximately 6,500 phytochemicals were screened from the phytochemical database, with 1,700 compounds satisfying Lipinski and toxicity filters. These compounds, along with the reference ligand bromosporine (BMF), were docked against the bromodomain-containing receptor 5C4Q using AutoDock Vina. Bromodomains are epigenetic "reader" modules involved in chromatin remodeling, and their inhibition has emerged as a therapeutic strategy in parasitic protozoa, including Leishmania species [27-29]. The reference ligand demonstrated a mean binding energy of -6.1 kcal/mol. In contrast, the top 20 phytochemicals presented considerably more negative binding energies (-10.7 to -9.2 kcal/mol), indicating stronger predicted affinity toward the target receptor.

Anastatin B exhibited the strongest interaction at -10.7 kcal/mol, followed by abyssinone I (-10.2 kcal/mol), abyssinoflavone V (-10 kcal/mol), and calopogonium isoflavone B (-9.9 kcal/mol). Structural visualization confirmed stable interactions between these compounds and key residues in the binding pocket, such as MET-52, ASN-87, and TYR-44. Hydrogen bonding and hydrophobic interactions are critical determinants of molecular recognition and receptor specificity, and their presence supports the validity of these docking predictions [30, 31]. The docking poses illustrated in Figures A-C further demonstrate the compatibility of these phytochemicals with the structural architecture of the 5C4Q active site.

Evaluation of physicochemical properties using Lipinski's Rule of Five is essential in predicting oral drug-likeness [32, 33]. All top-ranking compounds complied with the rule's requirements, with acceptable molecular weight, hydrogen bond donor/acceptor counts, and cLogP values. Compliance with these criteria increases the likelihood of favorable absorption and permeation in vivo. While additional parameters such as polar surface area and molecular flexibility also influence bioavailability, Lipinski's criteria remain a useful first-line filter in computational screening [34].

Toxicological assessment provides another layer of filtering in early-phase drug discovery. Modern in silico toxicity models predict structural alerts, enabling rapid elimination of compounds with adverse profiles before experimental validation [35]. All front-runner phytochemicals in this study demonstrated clean toxicity profiles, with no alerts for mutagenicity, tumorigenicity, reproductive toxicity, or irritancy. This absence of predicted toxicity highlights their potential suitability as lead scaffolds for anti-leishmanial drug development.

Overall, the results indicate that several phytochemicals exhibit superior predicted binding to the 5C4Q bromodomain receptor compared with the reference ligand. Anastatin B demonstrated the highest affinity, while other candidates showed strong binding energy, favorable drug-likeness, and no predicted toxicity. These computational findings identify promising scaffolds for further evaluation. Nonetheless, docking predictions must be validated through in vitro biochemical assays, in vivo efficacy testing, and comprehensive ADMET profiling to confirm therapeutic potential.

5. Conclusion

In conclusion, the phytochemicals identified in this study particularly anastatin B, abyssinone I, and abyssinoflavone V, demonstrated markedly higher binding affinities to the 5C4Q bromodomain receptor compared with the reference ligand. Their favorable physicochemical profiles, compliance with Lipinski's criteria, and absence of predicted toxicity further strengthen their potential as promising anti-leishmanial candidates. The integration of computer-aided drug design, molecular docking, and in silico screening tools has greatly enhanced the efficiency of early drug discovery, enabling rapid identification of lead compounds with desirable pharmacological features.

Future research should focus on validating these computational predictions through in vitro enzyme inhibition assays, in vivo anti-leishmanial studies, and comprehensive ADMET evaluations. Such experimental confirmation will be essential for advancing these phytochemicals toward preclinical development.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this work.

Data availability

No new experimental data were generated. All computational data used in this study are available upon reasonable request.

Statement of ethical approval

This study did not involve human participants or animal experimentation. All analyses were conducted entirely using computational tools and publicly available datasets.

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