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# Design and in Silico Profiling of Novel 1,2-Benzisothiazole-para Aminobenzoic Acid Conjugates as Multi-Target Anticancer Agents

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***Abstract**—The 1,2-benzisothiazole (BIT) is one of the extensively studied scaffolds for different sorts of biological activities including anticancer properties. Ten novel 1,2-benzisothiazole-para-aminobenzoic acid derivatives was designed and screened for potential multi-target anticancer activity. The SwissADME web server was exploited to anticipate the drug-likeness and ADMET attributes, it reveals propitious gastrointestinal absorption for the majority of the compounds, incapability of traversing the blood-brain barrier, and consent with Lipinski's rule of five. Molecular docking simulations was conducted for the designed ligands against four worthy target proteins namely, Carbonic Anhydrase IX (CA-9), CA-XII, Dihydrofolate Reductase (DHFR), and Matrix Metalloproteinase-13 (MMP-13), the vast majority of ligands exhibit convincing binding scores against all the tested receptors with binding scores exceeding that recorded with their native co-crystallized ligands. Molecular dynamics simulation for 50 nanoseconds has confirmed the validity of Molecular docking procedure and provide reliable results that signify conformational stability of both ligand and protein, limited fluctuation of the ligand which is well retained in the binding pocket, emphasizes types and strength of bonding interactions. The overall results suggest that the designed 1,2-benzisothiazole-PABA molecular hybrids are promising lead compounds which deserve further experimental synthesis and screening for anticancer action.*

***Keywords**— 1,2-Benzisothiazole; Molecular docking; Molecular dynamics; ADMET; Anticancer agents.*

## I. INTRODUCTION

The 1,2-benzisothiazole-3(2H)-one (BIT) scaffold constitutes a main structural part in a variety of organic compounds that have important sorts of biological activities, for example caspase-3 inhibition, which play an essential role in apoptosis [1-3]. In addition, derivatives of BIT have displayed substantial antimicrobial [4-7], antifungal [8], and antiviral properties [9]. Particularly, the potential role in cancer treatment as underlined by records of benzoisothiazolones which have the potential to act as species-selective enzyme inhibitors [10] and, furthermore, more directly, by a unique analogue that suppressed the growth of solid tumor in vivo by means of anti-angiogenesis and apoptosis actions [11]. The BIT nucleus's synthetic flexibility, highlighted in various methodological reviews [12, 13], permits planned derivatization, which facilitates the precise adjustment of physicochemical and pharmacological features to address particular diseases.

The multi-target ligand approach has become superior strategy in order to develop effective anticancer agents, to overcome poor efficacy and drug resistance which are the main limitations of single-target agents. One of main strategies to develop multi-target drugs is the Molecular hybridization, which encompasses a conjugation of two or more separate pharmacophores into a single system [14]. In this sense, *para*-aminobenzoic acid (PABA) offers a charming coupling companion. Although PABA is a basic metabolite, conjugating it into larger molecular skeletons have been extensively investigated for potential biological properties. 1,2-benzisothiazole part of the designed hybrid possess pro-apoptotic and potential anti-angiogenic effects, while PABA may affects cellular uptake mechanisms or interfere with folate -requiring pathways, hence designing one molecular entity that combine both nuclei could get synergistic effects [1, 11].

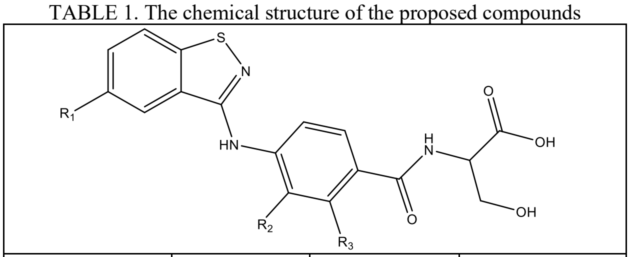
In silico techniques often incorporated in the early stages of drug discovery process which help the researcher concentrate on reasonable number of library compounds instead of dealing with the entire chemical library. ADME attributes predictions to assess drug-likeness followed by molecular docking studies to predict how ligands interact with the protein and how strong these bindings are, ultimately the molecular dynamics simulations to validate the docking results by confirming the binding poses in dynamic manner rather than static image derived from the molecular docking [15-17]. Moreover, molecular dynamics (MD) simulations grant profound details into the stability and morphological dynamics of the ligand-receptor complex, surpassing the static view adduced by the molecular docking [15-17]. This study is devoted to the computational design, ADME prediction, molecular docking, and molecular dynamics simulation of ten novel 1,2-benzisothiazole-PABA conjugates to estimate their possibility as multi-target anticancer agents

## II. COMPUTATIONAL METHOD

### *In silico drug-likeness and ADMET prediction*

The physicochemical and ADME properties and drug-likeness for the designed ligands were predicted ulitizing the SwissADME web server, http://www.swissadme.ch/index.php. [18,19]. A number of important descriptors were predicted to evaluate the compounds under study and ensure their eligibility for drug design and drug-likeness.

TABLE 1. The chemical structure of the proposed compounds



| Ligand ID | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 1 | H | H | H |
| 2 | H | H | $CH_3$ |
| 3 | H | H | $OCH_3$ |
| 4 | H | Cl | H |
| 5 | H | H | $CF_3$ |
| 6 | Cl | H | H |
| 7 | Cl | H | $CH_3$ |
| 8 | Cl | H | $OCH_3$ |
| 9 | Cl | Cl | H |
| 10 | Cl | H | $CF_3$ |

*Molecular Docking Studies*

Molecular docking simulations were executed to compute the binding affinity and manner of interaction between the ten designed ligands (**1-10**) and four distinct protein targets: Carbonic anhydrase-9 (CA-9, PDB: 5FL5), Carbonic anhydrase-12 (CA-12, PDB: 1JD0), Dihydrofolate Reductase (DHFR, PDB: 5SDB), Matrix Metalloproteinase-13 (MMP13, PDB: 5UWK). All simulations were implemented utilizing the Molecular Operating Environment (MOE) software suite, version 2024.06. The 3D chemical structures of the designed compounds (Table 1) were created within MOE. Each structure was energy-minimized using the MMFF94x forcefield. Protonation states were assigned at physiological pH (7.4) using the Protonate3D utility to ensure chemical accuracy prior to docking [18,19]. The protein crystal structures were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) at http://www.rcsb.org/pdb/home/home.do. All co-crystallized water molecules were cleared, except for those that take part in the ligand-protein binding interactions serving as water bridges. Using the MOE software in its default settings, the protein structure was protonated in three dimensions, and energy minimized. For the molecular docking process, the receptors and solvent atoms were processed, and polar hydrogens were added. During the docking procedure, the ligand atom was specified, and rescoring1 was configured to London dG while rescoring2 was set to GBVI/WSA dG, refinement set to induced fit explore the flexibility of both ligand and receptor, with the interaction between the ligand and the protein being evaluated. [18,19].

*Molecular dynamics simulations*

MD simulation was accomplished exploiting the Schrödinger 2021-2 suite to examine the stability of the complex and types of interaction involved between the ligand and the protein namely ligand4-MMP13. The simulation was carried out for 50 nanoseconds time period at 300 K, and NPT ensemble. Choice ligand-4 for Molecular Dynamic assessment based on the Molecular Docking results, then the system outlined using SPC water model in box with 10 Å dimension and OPLS4 force field was used. Neutralizing the system was accomplished with 0.15 NaCl at neutral PH [22,23].

## III. RESULTS AND DISCUSSION

*Evaluation of Drug-Likeness and ADMET Properties*

The compounds under study were computationally appraised for key physicochemical properties and drug-likeness to estimate their bioavailability after oral administration, and their drug likeness. Most of the compounds were expected to own superior gastrointestinal (GI) absorption, implying effective possibilities for oral bioavailability, with only three Compounds (3, 7, and 11) were anticipated to be of poor GI absorption which could be due to their higher polarity. All of the compounds are portended to have lack the ability to cross the blood-brain barrier (BBB) which is a desirable feature for drugs intended to target peripheral systems rather than the central nervous system (CNS) to avoid CNS side effects. Most of the are likely Pgp substrates which could be a potential risk for diminished intracellular concentration due to pumping out of the cells by this transporter which may entail higher dosing. Lipophilicity is a critical variable for a compound to penetrate biological membrane efficiently by passive diffusion. The predicted Log Po/w values for the ligands are within the optimal range for drug-like molecules which is preferably 0-3. This in turn means that almost all ligands own a balanced hydrophobicity/hydrophilicity which favor adequate passive diffusion extent through biological membranes. All compounds reveal outstanding drug-likeness properties, like Bioavailability Score (BS) of 0.56 signifying an elevated chance of being at least 10% orally bioavailable in rats or permeable through Caco-2 cells. All compounds obey Lipinski's rule of five, likewise Hydrogen Bonding (nHBA, nHBD) are in the normal range, while the Polarity (TPSA), is slightly upraised in ligands with higher number of hydrogen bond acceptors (nHBA= 7). all these charecteristics make most of them suitable for subsequent lead optimization table (2).

*Molecular Docking Studies*

Predicated on the induced-fit docking outcomes, the compound library of 1,2-benzisothiazole-PABA conjugates demonstrate diverse binding affinity and selectivity against various target proteins. All designed ligands reveal significant capacity as inhibitors for the carbonic anhydrase isoforms CA9 and CA12, with binding scores surpassing that of the co-crystallized ligands. Although all compounds are less potent than the co-crystallized ligand of MMP13, show reasonably strong affinity for this receptor. Conversely, all docked ligands exhibit weaker binding scores to DHFR than its native ligand. Almost all poses are of low RMSD values which suggest honest pose predictions, it was found that certain substituents like chloro- and methoxy-substitutions present in ligands 3, 7, 8 improve receptor binding, placing these compounds as propitious leads for carbonic anhydrases targeting table (3).

TABLE 2 The physicochemical properties, drug-likeness, and drug-likeness scores of the designed compounds

| ID | GI | BBB | Pgp | BS | MWT | nHBA | nHBD | TPSA(Å) | iLOGP | Log $P_{o/w}$ | nLV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | High | No | No | 0.56 | 373.43 | 6 | 4 | 136.32 | 2.58 | 0.77 | 0 |
| 2 | High | No | No | 0.56 | 387.45 | 6 | 4 | 136.32 | 2.32 | 1.00 | 0 |
| 3 | Low | No | No | 0.56 | 403.45 | 7 | 4 | 145.55 | 1.68 | 0.54 | 0 |
| 4 | High | No | Yes | 0.56 | 407.87 | 6 | 4 | 136.32 | 2.29 | 1.19 | 0 |
| 5 | High | No | Yes | 0.56 | 452.32 | 6 | 4 | 136.32 | 1.87 | 1.06 | 0 |
| 6 | High | No | Yes | 0.56 | 466.35 | 6 | 4 | 136.32 | 1.85 | 1.34 | 0 |
| 7 | Low | No | Yes | 0.56 | 482.35 | 7 | 4 | 145.55 | 2.82 | 1.20 | 0 |
| 8 | High | No | Yes | 0.56 | 486.77 | 6 | 4 | 136.32 | 2.40 | 1.65 | 0 |
| 9 | High | No | No | 0.56 | 407.87 | 6 | 4 | 136.32 | 1.68 | 0.94 | 0 |
| 10 | High | No | Yes | 0.56 | 421.90 | 6 | 4 | 136.32 | 2.19 | 1.33 | 0 |
| 11 | Low | No | Yes | 0.56 | 437.90 | 7 | 4 | 145.55 | 2.71 | 1.10 | 0 |
| 12 | High | No | Yes | 0.56 | 442.32 | 6 | 4 | 136.32 | 2.18 | 1.53 | 0 |

TABLE 3: The binding scores (kcal/mol) of the designed ligands with potential targets.

| Proteins | | Ligands | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | CL |
| CA 9 (5FL5) | score | -9.69 | -9.96 | -11.33 | -9.86 | -9.94 | -10.98 | -11.00 | -11.34 | -9.74 | -11.04 | -7.47 |
| | RMSD | 1.18 | 1.54 | 1.45 | 1.60 | 1.09 | 1.43 | 1.92 | 1.57 | 1.72 | 1.89 | 1.50 |
| CA 12 (1JD0) | score | -10.38 | -10.14 | -10.33 | -10.20 | -10.15 | -9.94 | -10.00 | -10.32 | -10.31 | -10.37 | -7.53 |
| | RMSD | 1.99 | 1.48 | 1.45 | 1.99 | 1.03 | 1.36 | 1.61 | 0.83 | 1.42 | 1.82 | 1.34 |
| 5SDB (DHFR) | score | -8.28 | -8.37 | -8.47 | -8.25 | -8.14 | -8.42 | -8.66 | -8.99 | -8.49 | -8.68 | -10.42 |
| | RMSD | 1.64 | 1.14 | 1.34 | 1.66 | 1.70 | 1.44 | 1.98 | 1.27 | 1.92 | 1.40 | 1.05 |
| 5UWK (MMP13) | score | -12.78 | -12.87 | -12.20 | -12.20 | -11.74 | -11.82 | -12.40 | -12.10 | -12.26 | -11.35 | -16.82 |
| | RMSD | 1.53 | 1.92 | 1.44 | 1.69 | 1.69 | 1.66 | 1.12 | 1.65 | 1.39 | 1.58 | 1.68 |

CL =co-crystallized ligand

### *Molecular Dynamics Simulation*

### *Root Mean Square Deviation (RMSD) Analysis*

The root-mean-square deviation (RMSD) analysis validates the structural integrity of the protein-ligand complex in the course of the 50 ns simulation time. The protein backbone RMSD reach equilibrium after the first ~10 ns period, fixing in the range of 2.5–2.8 Å, which signify the stability of the entire fold alongside the ligand and lack of significant conformational realignments or unfolding. further, the ligand RMSD revealed robust interaction stability, fall in the middle of 1.2–1.5 Å after the early equilibration. This relatively downgrade ligand RMSD betoken that the ligand stays bound to the active site residues constantly with only confined conformational realignments observed. Overall, Both the protein and ligand RMSDs achieve equilibrium soon and maintain their stability throughout the 50 ns simulation time figure (1).

### *Root Mean Square Fluctuation (RMSF) Analysis*

The RMSF inspection designates that both the protein and ligand conserve overall structural stability with local flexible regions which play crucial role in function and interaction. The core and secondary structural regions of the protein elicit limited fluctuations at 0.3–1.0 Å, whereas loop regions (residues 65–85 and 130–150) and the C-terminal (>160 residues, RMSF >4.5 Å) display greater degree of flexibility, proposing dynamic motion crucial for ligand adaption. Amio acid residues close to the binding site unveil negligible fluctuation, indicating the stability state upon ligand interaction. Likewise, the ligand rigid core (atoms 1–13) settled in the protein site elicit modest whole fluctuations (0.6–1.4 Å), whilst middle region (atoms 14–23) which is exposed to the solvent is relatively flexible. This matching flexibility fashion between the protein and ligand endorses a stable yet adjustable binding interaction, assisting both structural incorruptibility and functional dynamics within the complex figure (1).

### *Ligand-Protein Contacts*

it was depicted from the figure (3) that the ligand forms a stable chemical bonding with the crucial active site residues. The carboxylic acid group make the main contribution by forming bidentate coordinated bonds with the $Zn^{2+}$ ion and hydrogen bond with GLU223 residue. Hydrogen bonds with PRO242 and π-π stacking with PHE252 and TYR244 help further stabilize the complex. The summation of these binding interactions help sequester the ligand within the protein binding site figure (1).

### *Protein-Ligand Contacts*

The range of total contacts is from 4 to 14 throughout the simulation time which in turns means the ligand is firmly sequestered within the protein active site. GLU223 and PRO242 form Hydrogen bonds, while HIS222, HIS226, and HIS232 create ionic interactions near the catalytic $Zn^{2+}$ ion. While Hydrophobic contacts mainly formed by LEU184, LEU218, LEU239, TYR244, and PHE252 residues. HIS222, GLU223, HIS226, and HIS232 form persistant interactions overall the simulation. GLY183, PRO242, TYR244, and PHE252 form intermittent bonding making them of secondary importance in complex stabilization. PRO181, GLY183, LEU185, ALA186, GLU223, PRO242, TYR244, and THR245 make little contribution to the complex mediated by water-bridge interactions figure (1).

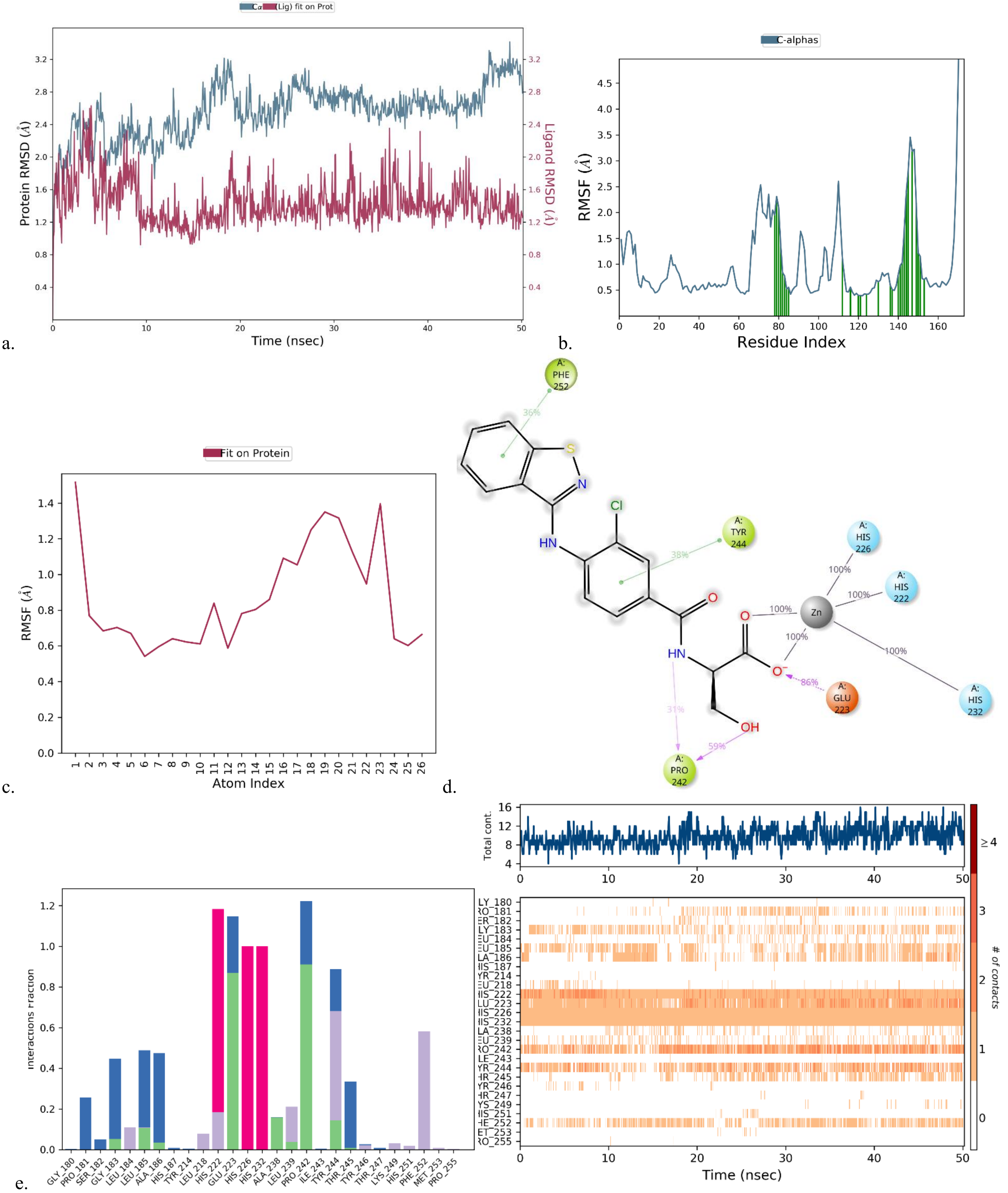


Figure 1: Molecular dynamics simulation results, a. ligand and protein RMSD, b. protein RMSF and c. ligand RMSF, d. Ligand-Protein Contacts, e. Protein-Ligand Contacts.

## IV. CONCLUSION

This computational study evaluated ten novel 1,2-benzisothiazole-PABA ligands for their possible actions on worthy target proteins that play significant role in cancer treatment. Almost all of these compounds possess the desirable drug-like properties, promising ADMET properties, good predicted oral bioavailability, and minimal blood brain barrier penetration. The ligands have good docking scores for MMP-13, CA 9, and CA 12, and moderate binding affinity for DHFR enzyme. Molecular dynamics simulation was used to validate the docking results, most importantly the stability of the ligand-protein complex, and types of bonding interactions. The combine results confirm the prospect that the designed compounds could have multi-target activity, and some of them are possible candidates for lead optimization.

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