Journal of Exploratory Research in Pharmacology **2026** vol. 11(4) | e00003e
DOI: 10.14218/JERP.2025.00003e

Editorial

Solvent Matters: A Call for Rigorous Consideration of Organic Co-solvents in Drug-target Interaction Studies

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Received: May 11, 2026 | **Revised:** June 17, 2026 | **Accepted:** July 07, 2026 | **Published online:** July 27, 2026

We read with great interest the research paper by Li *et al.*[1] published in *Pharmacological Research*. In the original article on amyotrophic lateral sclerosis (ALS), the small molecule isoginkgetin (ISO) was shown to be an effective modulator of the glycogen synthase kinase-3 beta (GSK-3β)–transcription factor EB pathway, improving lysosomal biogenesis and function. We agree with Li *et al.*[1] that the reported study could preliminarily elucidate the mechanisms of action and serve as a proof-of-concept for ISO as a potential therapy for ALS.

In addition to this point, we would like to raise a less frequently discussed concern regarding the solvent used in this study, *i.e.*, dimethyl sulfoxide (DMSO). In our opinion, the introduction of DMSO may have implications for understanding drug-target interactions, as detailed below. It should be noted that we do not intend to overstate this concern, as similar scenarios can be widely found in current studies. Instead, this editorial aims to stimulate discussion in the field and draw broader attention to this issue.

Isothermal titration calorimetry (ITC) was employed to determine the interaction between ISO and GSK-3β (and its mutants), and according to the original article, both ISO and GSK-3β were dissolved in buffer containing 2% DMSO. It should be noted that the original study used matched DMSO concentrations (2%) in both the protein and ligand solutions, which is standard practice for ITC experiments. Our concern is not about mismatched buffers, but rather the potential for the co-solvent itself to alter the fundamental nature of the interaction being measured, even when matched. The presence of 2% DMSO might exert the following effects:

1) Deviation from the physiological environment. The interaction conditions should align with the actual situation as closely as possible so that ITC results can be reliable. However, biological fluids in the human body, where *in vivo* ISO-GSK-3β interactions take place, are aqueous systems without DMSO. Thus, the ITC profile measured in a solvent system containing DMSO cannot fully reflect the actual interactions in the physiological environment.

2) Alteration of the conformation of GSK-3β. We should bear in mind that GSK-3β is a "delicate" component in the system, since the conformational stability of proteins and peptides is vulnerable to the microenvironment. It is widely reported that the addition of organic solvents can lead to alterations in the conformation of macromolecules.[2] As a result, GSK-3β in 2% DMSO might not adopt its commonly understood conformation, which could influence drug-target interactions. Although direct experimental evidence for GSK-3β is lacking, it is well documented that organic solvents can induce structural rearrangements in proteins. Thus, DMSO at 2% could potentially alter the structural properties of GSK-3β under the ITC conditions used, although this possibility requires experimental verification.

3) Generation of artifact K_D data. Only "genuine" K_D data can be used for formal analysis. It should be recognized that the K_D values used for affinity comparisons are actually calculated from thermodynamic parameters fitted by ITC algorithms (ΔG, ΔH, and ΔS),[3] and different interaction modes would affect the values of these parameters. For example, hydrogen bonding, hydrophobic interactions, desolvation, and conformational changes can all contribute to the measured ΔH and ΔS values.[4] The presence of DMSO in the buffer will certainly affect hydrogen-bond and hydrophobic interactions among ISO-GSK-3β, ISO-solvent, and GSK-3β-solvent, ultimately causing changes in K_D values. Direct experimental evidence for such effects exists in the literature. For instance, Zhang *et al.*[5] systematically compared ITC profiles of hydrogen-bonded polymer complexes across multiple organic solvent systems (including DMSO, methanol, and ethanol) and observed markedly different thermodynamic signatures, demonstrating that solvent identity directly influences the measured ΔH and ΔS values. Furthermore, Usacheva *et al.*[6] quantified the complexation between L-phenylalanine and 18-crown-6 in water–DMSO mixtures and showed that the apparent

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How to cite this article: Guo M, Zhao Z, Wu C, Huang Z. Solvent Matters: A Call for Rigorous Consideration of Organic Co-solvents in Drug-target Interaction Studies. *J Explor Res Pharmacol* 2026;11(4):e00003e. DOI: https://doi.org/10.14218/JERP.2025.00003e.

KD value varies systematically as a function of the DMSO molar fraction, confirming that the solvent environment can fundamentally alter apparent binding affinity. These studies underscore the need for caution when interpreting ITC data obtained in the presence of organic solvents, even when the solvent concentration is low and matched between the sample and reference cells.

Given the potential for DMSO to influence the measured parameters, we suggest that ITC data obtained in 2% DMSO should be interpreted with appropriate caution. While such data provide valuable insights, complementary techniques or validation under conditions with minimized solvent interference would further strengthen the conclusions regarding the molecular mechanism of interaction. Therefore, there remains room for further discussion regarding the molecular mechanisms underlying ISO-GSK-3β interactions. In addition, the interpreted drug-target interactions may not necessarily be consistent with virtual screening and molecular docking results, as the latter may not account for the potential intervention of DMSO in drug-target interaction predictions.

However, we recognize that the ITC study was relatively downstream in the logical flow of this paper, and this partial discrepancy would not challenge the overall conclusion of Li *et al*.[1]

Although the above analyses are mostly reasonable, it must be acknowledged that the use of DMSO is a "practical compromise" because ISO is poorly water-soluble (with a solubility of around 0.1 mg/mL),[7] and an organic solvent is needed as a co-solvent to improve its solubility. Solubilizers such as Tween® 80 and polyethylene glycol have been used to improve aqueous solubility in some formulation contexts, although their suitability for ISO and their effects on ISO–GSK-3β interactions remain to be established.[8] Accordingly, the use of these solubilizers in ISO drug development would require formulation-specific evaluation. Nevertheless, solubilizers, as participants in hydrogen bonding and/or hydrophobic interactions, may also interfere with ISO–GSK-3β interactions. Hence, introducing solubilizers may not be a more advisable choice for drug–target interaction investigations.

Regarding the use of organic solvents in ITC studies of drug–target pairs, several more feasible approaches remain available:

1) To limit the maximum amount of organic solvents. Seemingly, there is no well-established restriction on the upper limit of organic solvent concentration in ITC tests; however, the lower, the better. When experimentally feasible, the DMSO concentration should be minimized; concentrations of 0.5–1% have been used successfully in some ITC studies, although the acceptable level must be established for each protein–ligand system.[9,10] Furthermore, Usacheva *et al*.[6] demonstrated that KD values can vary systematically with DMSO concentration, underscoring the importance of using the lowest feasible amount.

2) To explore the impact of different concentrations of organic solvents. A gradient of DMSO concentrations can be used to explore its specific influence. For example, Usacheva *et al*.[6] used a series of water–DMSO co-solvents to determine the interaction between L-phenylalanine and 18-crown-6, and confirmed that the K_D value was a function of the DMSO molar fraction. A practical validation approach would involve performing a series of ITC experiments with fixed concentrations of ISO and GSK-3β while varying the DMSO concentration, for example 0.25%, 0.5%, 1%, and 2%, with the original 2% condition retained as a comparator. Comparing the resulting K_D, ΔH, and ΔS values across this series would allow researchers to empirically quantify the solvent's impact on the binding parameters for their specific system. This "solvent titration" approach can reveal whether the observed binding is robust to solvent concentration or whether it is an artifact of the selected experimental conditions.

3) To choose different types of organic solvents. Organic solvents with various functional groups tend to exert different degrees of effects on drug–target interactions by interfering with hydrogen bonds and hydrophobic interactions. Zhang *et al*.[5] found that ITC profiles differed substantially across multiple organic solvent systems (DMSO, methanol, ethanol, *etc*.).

From this perspective, for investigations of ISO–GSK-3β interactions, it is recommended to: (1) employ the lowest feasible DMSO concentration (e.g., 1%); (2) test the effects of different DMSO concentrations, such as 0.25%, 0.5%, 1%, and 2%, with the original 2% condition retained as a comparator; and (3) analyze the influence of organic solvent type (e.g., DMSO, methanol, and ethanol). To facilitate informed decision-making, Table 1 summarizes the key characteristics of common solvent systems discussed herein, along with their respective advantages and limitations. Of course, practice is always more complicated than theory; therefore, detailed experimental conditions must be optimized through preliminary tests. By these means, the molecular mechanisms underlying ISO–GSK-3β interactions can be claimed with greater rigor.

The concerns raised here extend well beyond the specific case of ISO–GSK-3β binding. Organic solvents are ubiquitous tools in drug discovery and are employed to solubilize the growing proportion of poorly water-soluble candidates emerging from modern medicinal chemistry pipelines. However, their potential to introduce systematic biases into biophysical characterization—particularly in label-free techniques such as ITC, surface plasmon resonance, and microscale thermophoresis—remains insufficiently acknowledged in routine practice.

The solvent should not always be treated as an inert medium, because it may influence protein conformation, ligand solvation, and the thermodynamic contributions to molecular recognition. Consequently, parameters measured under a given solvent condition may not fully predict binding behavior in more complex biological environments.

We therefore call for more systematic integration of solvent effect assessments into the standard workflow of drug–target interaction studies. This includes: (1) explicit reporting of solvent type, concentration, and matching procedures in all publications; (2) solvent titration experiments to empirically evaluate the robustness of binding data; (3) cross-validation using orthogonal techniques with different sources of experimental bias; and (4) development of community-wide guidelines for acceptable solvent thresholds in biophysical assays, analogous to existing recommendations for DMSO in cellular assays.

By adopting these practices, the pharmacological community can enhance the reproducibility and translational relevance of early-stage binding data, ultimately bridging the gap between *in vitro* biophysics and *in vivo* efficacy.

Reliable characterization of drug–target interactions is

Table 1. Comparison of common organic solvent systems for ITC-based drug–target interaction studies

Solvent systems	Typical advantages	Potential disadvantages for ITC studies
DMSO	(1) Excellent solubilizing capacity for a wide range of hydrophobic compounds (2) Widely used and well-characterized in literature (3) Compatible with most proteins at low concentrations (<2%)	(1) High dilution enthalpy requires rigorous solvent matching (2) Can alter protein hydration shell and conformational dynamics even at sub-denaturing concentrations (3) May interfere with hydrogen bond and hydrophobic interactions, affecting Δ*H*, Δ*S*, and K_D values (4) K_D values can be a function of DMSO molar fraction
Methanol	(1) Lower viscosity than DMSO (2) Less interference with certain hydrogen bonding networks compared to DMSO (3) Volatile, facilitating removal if needed	(1) May denature proteins at higher concentrations (2) Strong hydrogen bond donor/acceptor, potentially competing with ligand–target interactions (3) Different ITC profiles compared to DMSO
Ethanol	(1) Generally well-tolerated by many proteins at low concentrations (<5%) (2) Biocompatible and FDA-approved for some pharmaceutical formulations	(1) Can stabilize or destabilize protein conformations depending on concentration (2) Exothermic dilution heat may complicate baseline subtraction (3) May promote protein aggregation at higher concentrations

DMSO, dimethyl sulfoxide; FDA, the U.S. Food and Drug Administration; ITC, isothermal titration calorimetry; K_D, dissociation constant; ΔH, enthalpy change; ΔS, entropy change.

important for pharmacological research and therapeutic development.[11] In this context, reliable methodologies for drug–target interaction studies should be emphasized. When considering future pharmacological studies involving water-insoluble compounds, we may reach a cautious consensus with the authors that the addition of DMSO or other organic solvents should be carefully scrutinized. We believe that this suggestion will enhance the credibility of related research. Our ultimate goal is to remind the journal's broad audience of the potentially neglected importance of organic solvents in biomedical studies.

Notwithstanding the above comments, we highly appreciate the efforts of Li *et al*.[1] in establishing a proof-of-concept study and look forward to further preclinical and clinical evaluation of ISO or related compounds as potential therapeutic candidates for ALS.

Acknowledgments

We thank the authors of the original article for making a valuable contribution to this area.

Funding

None.

Conflict of interest

Prof. Zhengwei Huang is an editorial board member of *Journal of Exploratory Research in Pharmacology*. The authors have no conflicts of interest to declare.

Author contributions

Conceptualization (MG, ZZ), writing – original draft (MG, ZH), writing – review and editing (ZZ, CW, ZH), supervision (ZH), project administration (ZH). All authors have approved the final version and publication of the manuscript.

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