

Computationally guided inhibition of the HIF-1 α –CBP/p300 coactivator interface suppresses hypoxia signaling in Caki-1 renal carcinoma cells

Reem M. Gahtani¹

King Khalid University

Mesfer Mohammad Al Shahrani¹

King Khalid University

Gaffar Sarwar Zaman¹

drgszaman@gmail.com

King Khalid University

Article

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Abstract

Hypoxia-inducible factor 1 (HIF-1) drives adaptive transcription through recruitment of the homologous coactivators CBP and p300, but this interface remains difficult to modulate with small molecules. We combined virtual screening of approximately 250,000 ZINC natural-product-like compounds against the CBP CH1 domain (PDB 1L8C), molecular docking and a 100-ns molecular dynamics simulation to identify candidate interface binders. SIHC926 (ZINC000097971950) remained within the predicted pocket, and MM/PBSA analysis produced a negative total binding-free-energy estimate dominated by favourable van der Waals contributions. In a homogeneous time-resolved fluorescence assay using the p300 CH1 domain, SIHC926 inhibited HIF-1 α -coactivator association with an IC₅₀ of 1.88 μ M, compared with 4.72 μ M for YC-1. SIHC926 also inhibited hypoxia-response-element-dependent transcription (IC₅₀ 2.52 μ M), reduced Caki-1 cell viability under hypoxia (reported GI₅₀ 2.29 μ M), decreased vascular endothelial growth factor secretion, increased Annexin V-positive apoptosis and elevated intracellular reactive oxygen species. These findings nominate SIHC926 as a candidate scaffold for modulating HIF-1 coactivator recruitment. Direct intracellular target engagement, coactivator selectivity, complete compound characterization and validation in additional renal carcinoma models remain necessary.

Introduction

Renal cell carcinoma (RCC) is a molecularly heterogeneous kidney malignancy in which dysregulated oxygen sensing and angiogenic signalling are particularly prominent in clear cell RCC. Loss of von Hippel–Lindau function can stabilize hypoxia-inducible factors (HIFs), while regional hypoxia further reinforces transcriptional programmes that support angiogenesis, metabolic adaptation, survival and treatment resistance [1, 2]. HIF-1 is an attractive therapeutic node, although the pathway is difficult to inhibit selectively because it is regulated at multiple levels and participates in normal physiological responses.

Transcriptional activation by HIF-1 α requires recruitment of CREB-binding protein (CBP) and its close paralogue p300. The C-terminal transactivation domain of HIF-1 α binds the CH1 domains of CBP/p300, creating a protein–protein interface that couples oxygen sensing to transcription of genes such as vascular endothelial growth factor A (VEGFA) [3, 4]. CBP and p300 are highly homologous but are not interchangeable in every cellular context; evidence obtained with one coactivator should therefore not automatically be interpreted as direct engagement of the other.

Protein–protein interfaces were historically considered poorly druggable, but compact energetic hot spots and pre-formed or inducible pockets can sometimes support small-molecule binding [5]. Previous work has shown that the HIF-1 α coactivator-binding surface can be perturbed by designed helix mimetics, providing proof of principle that coactivator recruitment is pharmacologically tractable [6]. A small molecule that interferes with this interface could suppress several HIF-1-dependent outputs simultaneously, although biochemical inhibition must be separated from nonspecific cytotoxicity and confirmed by orthogonal target-engagement methods.

Here, structure-based virtual screening and molecular dynamics (MD) simulation were used to nominate SIHC926 (ZINC000097971950) as a candidate binder at the HIF-1 α -binding surface of CBP. We then evaluated inhibition of HIF-1 α association with the homologous p300 CH1 domain, HIF-1-dependent reporter activity, cell viability, VEGF secretion, apoptosis and reactive oxygen species in hypoxia-exposed Caki-1 renal carcinoma cells. The study was designed as an initial computational and in vitro assessment rather than a demonstration of therapeutic efficacy or CBP-selective intracellular binding.

Results

Virtual screening nominates SIHC926 at the HIF-1 α -binding surface of CBP

The crystal structure of the CBP CH1 domain in complex with the HIF-1 α C-terminal transactivation domain (PDB 1L8C) showed a predominantly helical interaction surface with a localized groove suitable for computational pocket mapping (Fig. 1a,b). Screening of an approximately 250,000-compound ZINC natural-product-like library against this region produced a ranked set of docking poses (Fig. 1c). SIHC926, corresponding to ZINC000097971950, was selected from the highest-ranked candidates on the basis of docking score, pose placement and predicted contact geometry.

The predicted pose placed SIHC926 within the CBP groove adjacent to the HIF-1 α -binding surface (Fig. 1d). The model suggested polar and hydrophobic contacts involving Lys21, Arg25, Ala28, Leu88, Ala91 and Asp100 (Fig. 1e,f). These calculations support a testable binding hypothesis but do not, by themselves, establish direct binding or inhibition of the native CBP–HIF-1 α complex.

A 100-ns MD trajectory supports retention of SIHC926 in the predicted pocket

In a 100-ns MD trajectory of the CBP–SIHC926 model, ligand root-mean-square deviation remained mainly within approximately 0.15–0.22 nm after the initial equilibration period (Fig. 2a). The trajectory contained recurrent hydrogen-bond contacts, most commonly zero or one contact at a given frame, with transient increases up to four contacts (Fig. 2b).

Comparison of the starting and 100-ns conformations indicated that SIHC926 remained associated with the modelled pocket while its local interaction network reorganized (Fig. 2c,d). Contacts observed during the trajectory included residues near Lys21, Cys22, Arg25, Glu26, Glu31, Val32 and Arg33. Because one production trajectory was analysed and no experimental structural measurement was performed, the MD analysis supports pose persistence rather than proving affinity or residence time.

Binding-energy and comparative dynamic analyses of the CBP–SIHC926 model

MM/PBSA component analysis yielded a negative total binding-free-energy estimate for the CBP–SIHC926 complex (Fig. 3a). Favourable gas-phase terms were driven mainly by van der Waals interactions, whereas polar solvation opposed binding. The estimate is interpreted qualitatively because a complete frame-selection, convergence and uncertainty report was not available in the supplied output.

Backbone RMSD analysis showed that both apo and ligand-bound CBP remained bounded over the trajectory, although the ligand-bound model sampled a higher RMSD plateau for much of the simulation before the profiles became more similar during the later interval (Fig. 3b). Residue-wise RMSF profiles were broadly comparable, with a shared major flexible region and several smaller local peaks (Fig. 3c). These patterns suggest altered conformational sampling rather than uniform rigidification of CBP by SIHC926.

SIHC926 inhibits HIF-1 α –coactivator association and HIF-1 reporter activity

An HTRF assay containing biotinylated HIF-1 α C-terminal transactivation-domain peptide and His-tagged p300 CH1 domain was used to test disruption of the coactivator interaction. SIHC926 produced concentration-dependent inhibition with an IC_{50} of 1.88 μ M, whereas YC-1 produced an IC_{50} of 4.72 μ M in the same assay (Fig. 4a). Because the biochemical assay contains p300 rather than CBP, it demonstrates inhibition of a homologous HIF-1 α –coactivator interface but does not directly prove CBP binding.

In Caki-1 cells exposed to 1% O₂, SIHC926 reduced hypoxia-response-element-driven firefly luciferase activity after normalization to Renilla luciferase. The fitted IC_{50} was 2.52 μ M for SIHC926 and 5.71 μ M for YC-1 (Fig. 4b). The similar concentration ranges for biochemical and reporter inhibition are consistent with the proposed mechanism, but orthogonal intracellular target-engagement experiments are required to exclude indirect pathway effects.

SIHC926 reduces Caki-1 cell viability and VEGF secretion under hypoxia

After 72 h of treatment under hypoxia, SIHC926 reduced ATP-dependent CellTiter-Glo signal in a concentration-dependent manner, with a reported GI_{50} of 2.29 μ M. YC-1 produced a reported GI_{50} of 4.94 μ M (Fig. 5a). The viability effect occurred within the same broad concentration range as reporter

inhibition; loss of viable cells may therefore contribute to some downstream readouts at higher concentrations.

Hypoxia increased secreted VEGF relative to normoxic cultures. SIHC926 reduced VEGF concentrations across the tested dose range, with the largest reductions at 3 and 10 μM (Fig. 5b). YC-1 showed a similar but less pronounced pattern in the supplied data. VEGF values were normalized to viable cell number, reducing—but not eliminating—the possibility that the effect was secondary to cytotoxicity.

SIHC926 increases apoptosis and intracellular reactive oxygen species under hypoxia

Annexin V–FITC/propidium iodide analysis showed a concentration-dependent increase in early and late apoptotic Caki-1 cell populations after SIHC926 treatment under hypoxia (Fig. 6a). The total apoptotic fraction was higher at 10 μM than at 3 μM . YC-1 at 10 μM also increased apoptosis, while staurosporine served as the positive control. Hypoxia alone was not significantly different from normoxia in the supplied analysis.

DCFDA fluorescence increased after treatment with SIHC926 under hypoxia, with a larger response at 10 μM than at 3 μM (Fig. 6b). YC-1 also increased the oxidation-sensitive signal. These data indicate increased intracellular oxidation-sensitive fluorescence but do not identify the reactive species or demonstrate that oxidative stress caused apoptosis.

Discussion

This study combines computational screening with biochemical and cellular assays to investigate the HIF-1 α coactivator interface as a drug-discovery target in renal carcinoma cells. SIHC926 was predicted to occupy a pocket at the CBP CH1 surface, remained associated with that model during a 100-ns MD trajectory, produced a favourable MM/PBSA estimate, inhibited HIF-1 α binding to the homologous p300 CH1 domain and reduced HIF-1 reporter activity, viability and VEGF secretion under hypoxia. Increased Annexin V-positive apoptosis and DCFDA fluorescence further indicated cellular stress. Together, the data nominate a candidate scaffold and support further evaluation of coactivator-interface modulation.

The structural model is biologically plausible because HIF-1 α recognition by CBP/p300 is mediated by a compact helical interface [3, 4]. Nevertheless, docking scores, one production trajectory and MM/PBSA estimates cannot establish affinity, binding kinetics or selectivity. The residue contacts and energy components in Figs. 1–3 should therefore guide mutagenesis or biophysical testing rather than be treated as experimentally confirmed interactions. Surface plasmon resonance, isothermal titration calorimetry or another direct-binding method, ideally combined with substitution of predicted contact residues, would strengthen the structural claim.

The biochemical and reporter potencies were in a similar low-micromolar range. This concordance is consistent with interference in coactivator recruitment, but the HTRF assay used p300 CH1 whereas the docking model used CBP CH1. High homology makes cross-reactivity plausible, yet a CBP-specific assay is required before concluding that SIHC926 directly disrupts CBP. Co-immunoprecipitation, proximity-based assays, NanoBRET or cellular thermal-shift approaches could test intracellular target engagement. Profiling against other CBP/p300-dependent transcription-factor interfaces would also be required to determine selectivity.

SIHC926 reduced VEGF secretion and cell viability more potently than YC-1 in the supplied experiments. YC-1 is a useful pathway comparator but is not a selective HIF-1 α -CBP/p300 protein-protein interaction inhibitor and has multiple reported cellular effects [11]. Relative potency against YC-1 therefore does not establish superior selectivity or therapeutic index. The overlap between reporter IC₅₀ and the reported viability GI₅₀ also means that cytotoxicity may influence transcriptional outputs at higher concentrations. Shorter exposures, matched viability controls, a constitutive reporter and measurement of additional HIF targets at RNA and protein levels would help distinguish primary transcriptional inhibition from secondary loss of cell fitness.

The apoptosis and reactive-oxygen-species results provide additional evidence of cellular stress after SIHC926 exposure but should be interpreted cautiously. DCFDA is an indirect oxidation-sensitive probe, and increased fluorescence does not identify the reactive species or its subcellular source. Antioxidant-rescue experiments, mitochondrial measurements and caspase/PARP validation would be required to establish a causal pathway from oxidative stress to apoptosis.

The principal limitations are the use of one renal carcinoma cell line, absence of a non-malignant renal-cell comparator, lack of direct intracellular CBP/p300 target engagement, incomplete chemical characterization of SIHC926, and absence of pharmacokinetic, toxicological or in vivo data. Cell-line authentication, mycoplasma status, full computational parameter files, independently seeded MD replicas, numerical source data and exact statistical outputs should be documented for reproducibility. Subject to these validations, SIHC926 provides a starting point for structure-activity optimization and for testing whether selective modulation of HIF-1 coactivator recruitment can suppress hypoxia-adaptive programmes in RCC.

Methods

Structure preparation and pocket definition

The CBP CH1-HIF-1 α C-terminal transactivation-domain complex was obtained from the Protein Data Bank (PDB 1L8C) [4]. The HIF-1 α chain, crystallographic water molecules and non-protein heteroatoms were removed before docking. Hydrogens were added, bond orders were checked and side chains were inspected using BIOVIA Discovery Studio Visualizer. The putative pocket was defined at the HIF-1 α -contacting groove of CBP.

Virtual screening and molecular docking

An approximately 250,000-compound natural-product-like subset of ZINC was screened using the SiBioLEAD platform. ZINC is a publicly available database of purchasable compounds [7]. Compounds were prefiltered by physicochemical properties and Lipinski-type criteria. Docking was performed with AutoDock Vina [8], and candidates were ranked by predicted binding energy, pose placement at the coactivator interface and interaction geometry. SIHC926 (ZINC000097971950) was advanced for experimental testing.

Compound identity and preparation

SIHC926 was identified computationally as ZINC000097971950. A 10-mM stock was prepared in dimethyl sulfoxide (DMSO), and working solutions were diluted in assay buffer or culture medium. YC-1 was used as a pathway comparator. Final DMSO concentrations were matched between treatment and vehicle-control wells.

Molecular dynamics and MM/PBSA analyses

MD simulations were performed using GROMACS [9] through the SiBioLEAD platform. The CBP–SIHC926 complex and an apo CBP model were placed in solvated triclinic boxes containing SPC water and were parameterized with an OPLS-family force field. Sodium and chloride ions were added to neutralize the systems and achieve 0.15 M ionic strength. Energy minimization used the steepest-descent algorithm, followed by 300 ps of NPT equilibration. Production simulations were run for 100 ns, and ligand RMSD, protein backbone RMSD, residue-wise RMSF and protein–ligand hydrogen bonds were analysed. Structural snapshots were inspected at 0 and 100 ns.

Binding-energy components were estimated from the production trajectory by the molecular mechanics Poisson–Boltzmann surface-area method using the gmx_MMPBSA workflow [10]. The output included van der Waals, electrostatic, polar-solvation, nonpolar-solvation, gas-phase, solvation and total energy terms. The calculation is reported as an end-state computational estimate rather than an experimentally measured binding affinity.

Cell culture and hypoxic exposure

Caki-1 human renal carcinoma cells (ATCC HTB-46; RRID: CVCL_0234) were maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37°C in 5% CO₂. Hypoxic experiments were performed at 1% O₂, 5% CO₂ and 94% N₂ in a tri-gas incubator. Normoxic controls were maintained in parallel.

HTRF assay of HIF-1 α –p300 interaction

The ability of SIHC926 to inhibit HIF-1 α –coactivator association was measured with a commercial homogeneous time-resolved fluorescence kit (Cisbio, Cat. No. 64HIFPEA) containing biotinylated HIF-1 α C-terminal transactivation-domain peptide and His-tagged p300 CH1 domain. SIHC926 and YC-1 were

tested from 0.01 to 50 μM in 384-well plates (10 μL final volume; 1% DMSO). Vehicle, unlabeled HIF-1 α peptide and YC-1 at 30 μM were included as controls. Plates were incubated for 45 min at room temperature in the dark. Fluorescence was measured at 620 and 665 nm after 337-nm excitation on a FLUOstar Omega reader. HTRF ratios were calculated as $(665/620) \times 10,000$ and converted to percentage inhibition relative to vehicle and maximal-inhibition controls. IC_{50} values were estimated by four-parameter logistic regression.

HRE dual-luciferase reporter assay

Caki-1 cells were seeded in white 96-well plates at 1.2×10^4 cells per well and co-transfected with a 5 $\times$ hypoxia-response-element firefly luciferase reporter and a Renilla luciferase control vector using Lipofectamine 3000 in Opti-MEM. After 24 h, cells were treated with SIHC926 (0.1–30 μM), YC-1 or vehicle (0.1% DMSO) and incubated under normoxia or 1% O_2 for 18 h. Firefly and Renilla signals were measured with the Dual-Luciferase Reporter Assay System on a FLUOstar Omega reader. Firefly activity was divided by Renilla activity and expressed relative to the hypoxic vehicle control. IC_{50} values were estimated by four-parameter logistic regression.

Cell-viability assay

Caki-1 cells were seeded in white opaque 96-well plates at 8×10^3 cells per well and allowed to attach overnight. Cells were treated with SIHC926 (0.1–30 μM), YC-1 or 0.1% DMSO under 1% O_2 for 72 h. CellTiter-Glo reagent (Promega, Cat. No. G7570) was added according to the manufacturer's protocol. After 10 min at room temperature with orbital shaking, luminescence was measured on a FLUOstar Omega reader. Viability was expressed relative to hypoxic vehicle controls, and the fitted half-maximal concentration was reported as GI_{50} in the source analysis.

VEGF secretion assay

Caki-1 cells were seeded in 24-well plates at 1.5×10^5 cells per well. After attachment, cells were incubated for 12 h in medium containing 1% fetal bovine serum and then treated with SIHC926 (0.1–10 μM), YC-1 or vehicle under normoxia or 1% O_2 for 24 h. Conditioned medium was cleared at $1,000 \times g$ for 5 min and stored at -80°C . VEGF-A was quantified using a Quantikine human VEGF-A ELISA kit (R&D Systems, Cat. No. DVE00), with absorbance read at 450 nm and 570-nm correction. VEGF concentrations were derived from recombinant standards and normalized to viable cell number measured in parallel.

Annexin V–FITC/propidium iodide apoptosis assay

Caki-1 cells were seeded in six-well plates at 2.5×10^5 cells per well and treated under normoxia or hypoxia (1% O_2) with vehicle, SIHC926 (3 or 10 μM) or YC-1 (10 μM). Staurosporine (1 μM) served as a positive control. After 48 h, adherent and floating cells were collected, washed with phosphate-buffered saline and resuspended in binding buffer at 1×10^6 cells/mL. Aliquots were stained with Annexin V–FITC and propidium iodide for 15 min in the dark and analysed on a Guava easyCyte flow cytometer. At least 10,000 events were acquired per sample. Debris and doublets were excluded, compensation was

established with single-stained controls, and total apoptosis was calculated as the sum of early- and late-apoptotic cells. Three independent experiments were performed.

Intracellular reactive-oxygen-species assay

Caki-1 cells were seeded in black-walled 96-well plates at 1.0×10^4 cells per well and treated under normoxia or hypoxia with SIHC926 (3 or 10 μM), YC-1 (10 μM) or vehicle for 24 h. Cells were washed and incubated with 10 μM DCFDA in serum-free medium for 30 min at 37°C in the dark. Excess probe was removed, and fluorescence was measured at 485/535 nm on a FLUOstar Omega reader. Cell-free background was subtracted, and values were expressed relative to the hypoxic vehicle control. Three independent biological experiments with technical triplicates were performed.

Statistical analysis

Concentration–response data were fitted by four-parameter logistic nonlinear regression with variable slope in GraphPad Prism. Data are presented as mean $\pm$ standard deviation from three independent experiments unless otherwise stated; technical replicate wells were averaged within each biological experiment. Multiple-group comparisons were analysed by two-sided one-way analysis of variance with Dunnett's multiple-comparison procedure for prespecified comparisons with the hypoxic vehicle control. The family-wise alpha level was 0.05. Figure symbols denote $P < 0.05$ (*) and $P < 0.01$ (**). Exact P values, test statistics, degrees of freedom and complete curve-fitting outputs should be included in the numerical Source Data file submitted with the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to Gaffar Sarwar Zaman at drgszaman@gmail.com.

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Author Contribution

R.M.G. performed the experimental investigation, formal analysis, data curation, visualization, and writing of the original draft. M.M.A.S. contributed to conceptualization, methodology, supervision, funding acquisition, project administration, validation, and writing—review and editing. G.S.Z. contributed to conceptualization, standardization of experimental protocols, interpretation, critical revision, final manuscript preparation, and corresponding-author responsibilities. All authors reviewed and approved the final manuscript and agree to be accountable for their contributions.

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Data Availability

The datasets generated and/or analysed during the current study, including numerical source data underlying the figures and available docking and molecular-dynamics input/output files, are available from the corresponding author on reasonable request. PDB 1L8C is publicly available from the Protein Data Bank, and ZINC compound records are available through the ZINC database.

Ethics statement

This study used an established human cell line and did not involve human participants, identifiable human material, live vertebrate animals or higher invertebrates. Institutional human- or animal-research ethics approval was therefore not required.

Use of AI-assisted tools

ChatGPT (OpenAI) was used for language editing and organizational assistance during manuscript preparation. The authors reviewed and verified the complete manuscript and take responsibility for all scientific content. No AI-generated images or primary data were used.

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Figures

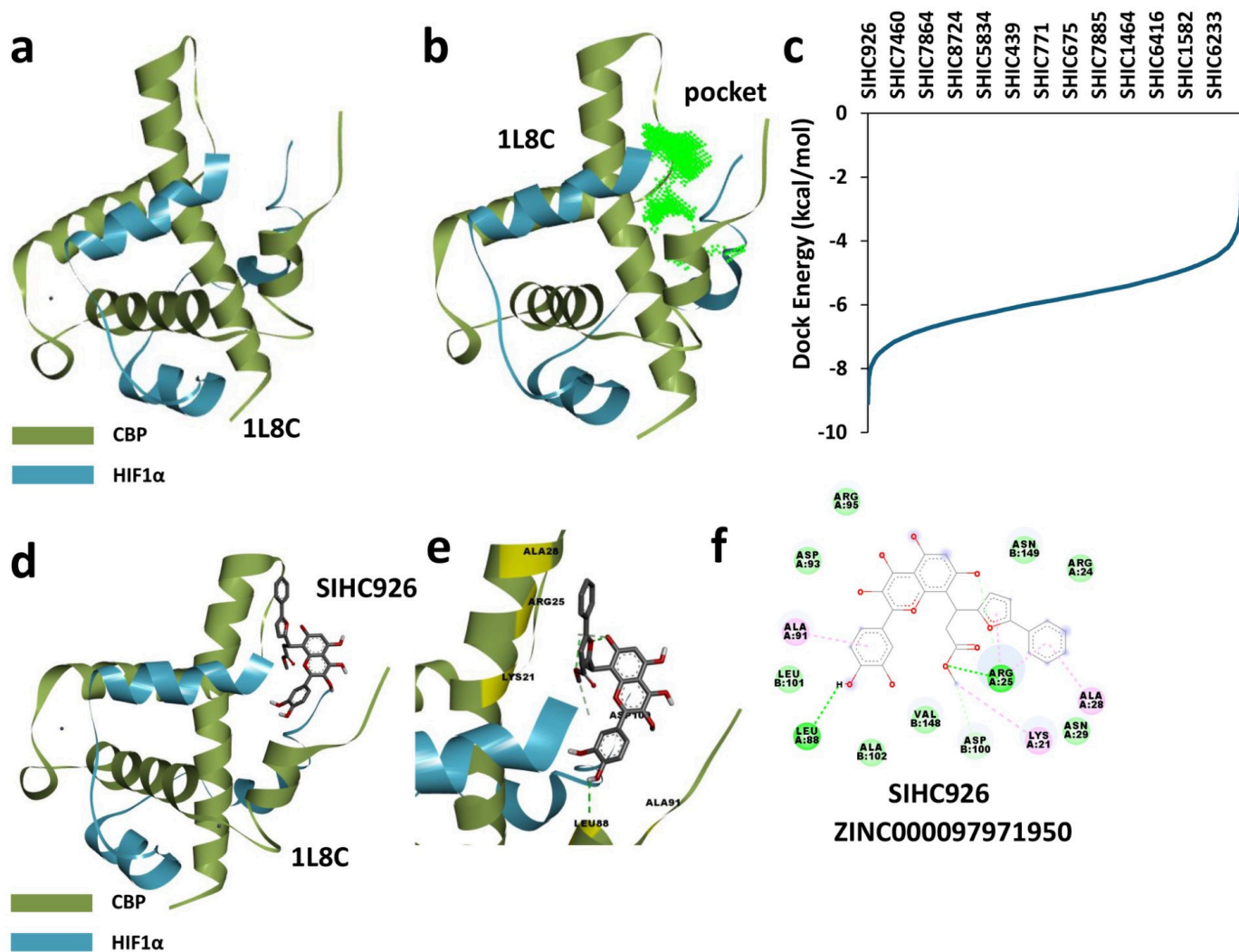


Figure 1

Structural model and virtual screening of the HIF-1α-binding surface of CBP. (a) Crystal structure of the CBP CH1 domain (green) bound to the HIF-1α C-terminal transactivation domain (blue; PDB 1L8C). (b) Predicted pocket at the coactivator interface. (c) Docking-energy ranking of selected virtual-screening hits. (d) Predicted placement of SIHC926 in the CBP groove. (e) Enlarged modelled contacts. (f) Two-dimensional interaction map for SIHC926 (ZINC000097971950). Docking interactions are computational predictions and have not been confirmed by structural or mutational experiments.

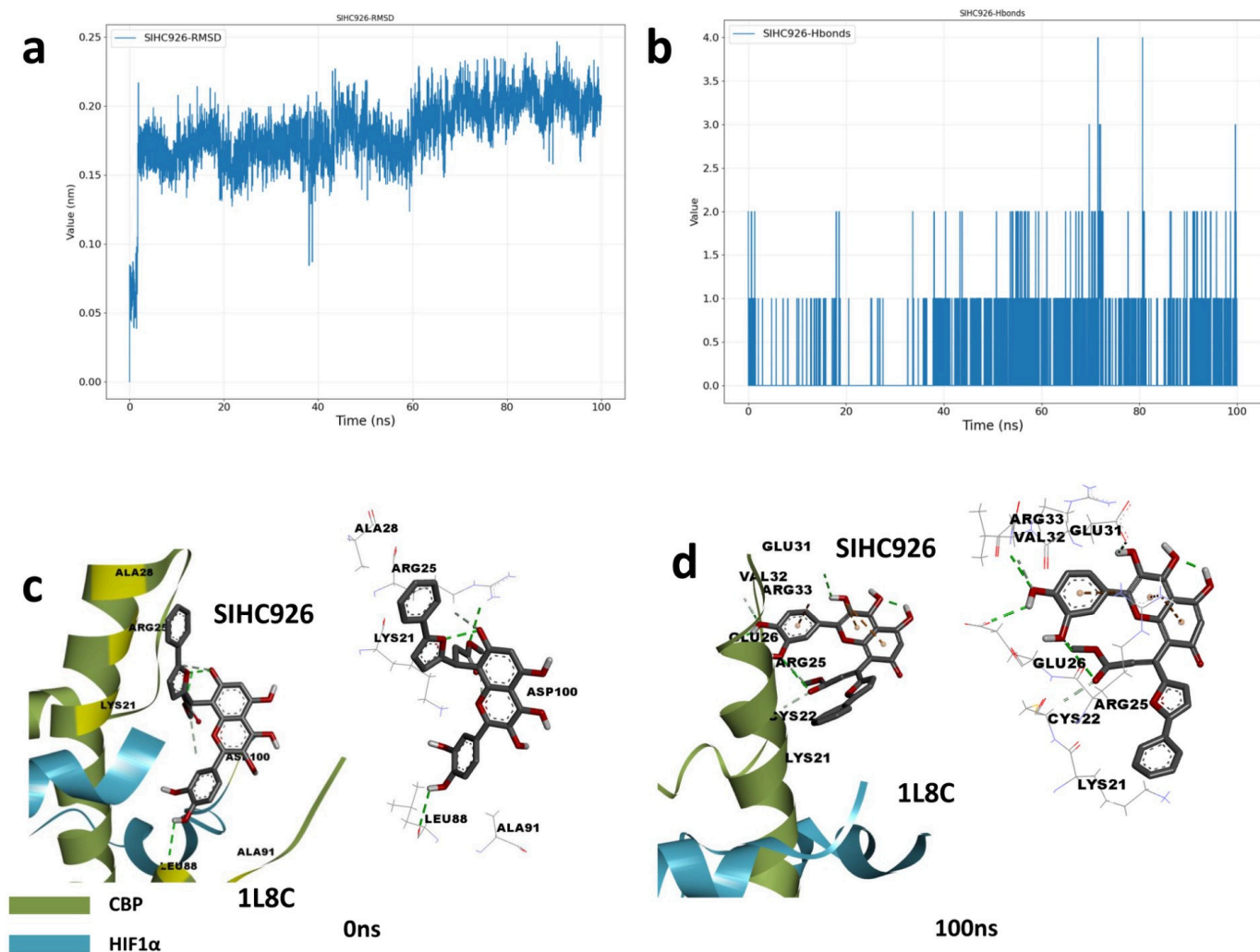


Figure 2

Molecular dynamics analysis of the CBP-SIHC926 model. (a) Ligand root-mean-square deviation during a 100-ns production trajectory. (b) Number of predicted protein-ligand hydrogen bonds over time. (c,d) Representative interaction views at 0 and 100 ns. One production trajectory was analysed; independent replicate simulations were not available.

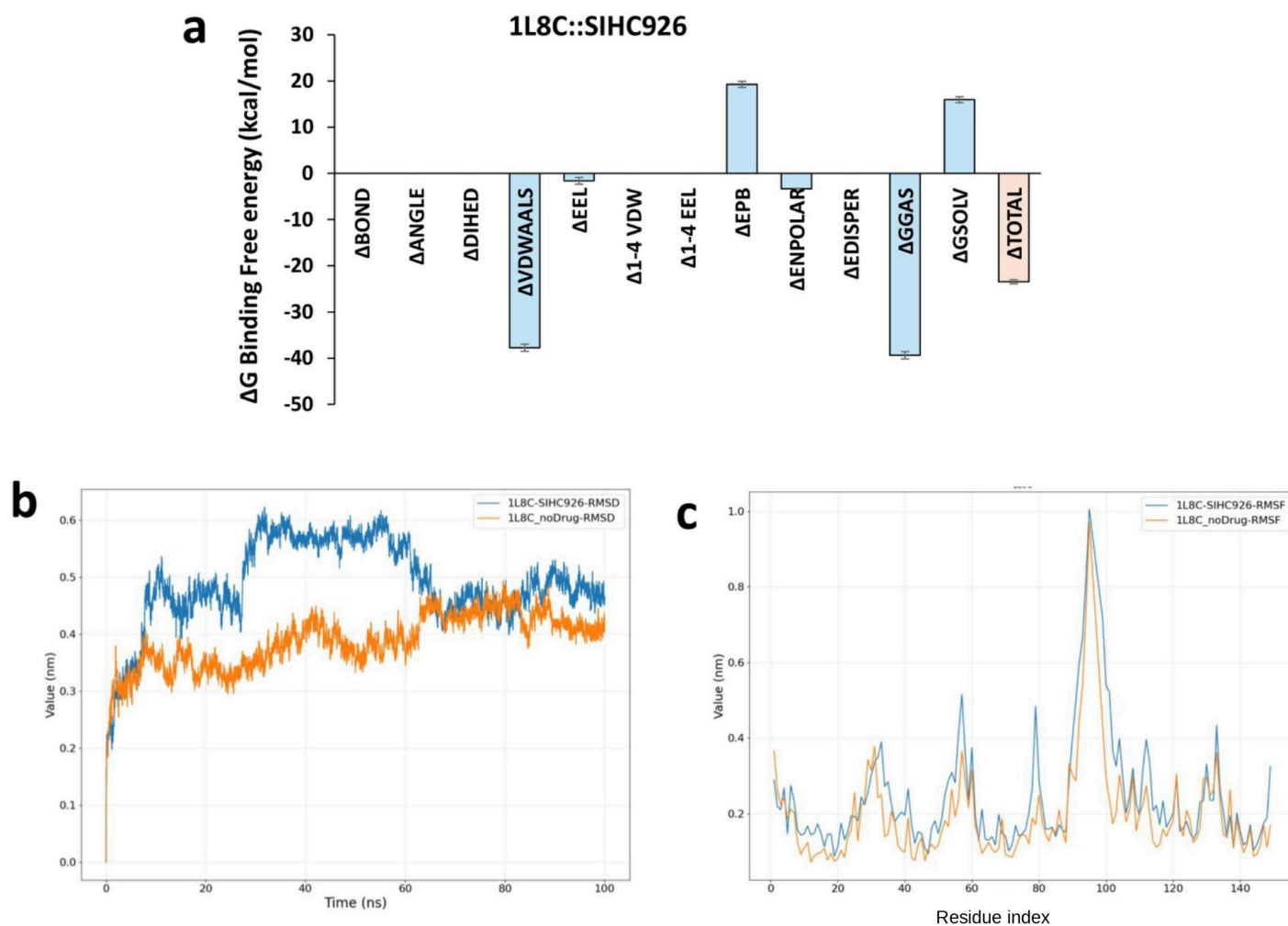


Figure 3

MM/PBSA and comparative dynamic analyses of the CBP–SIHC926 model. (a) Decomposition of the end-state MM/PBSA binding-energy estimate into van der Waals, electrostatic, polar-solvation, nonpolar-solvation, gas-phase, solvation and total terms. Negative components favour the calculated complex, whereas positive components oppose it. (b) Backbone RMSD profiles of ligand-bound and apo CBP during the 100-ns simulations. (c) Residue-wise RMSF profiles of ligand-bound and apo CBP. MM/PBSA values are computational estimates and should not be interpreted as experimentally measured affinity.

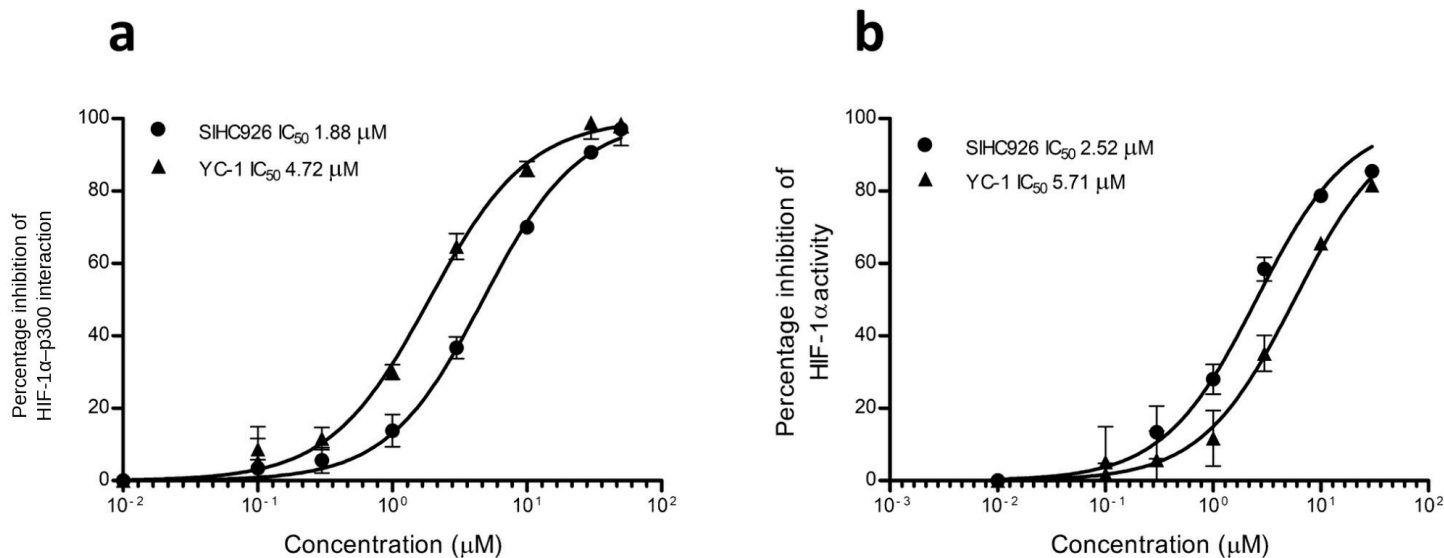


Figure 4

Inhibition of HIF-1α-coactivator association and HIF-1 transcriptional activity by SIHC926. (a) HTRF assay using the HIF-1α C-terminal transactivation-domain peptide and p300 CH1 domain. Fitted IC_{50} values were 1.88 μM for SIHC926 and 4.72 μM for YC-1. (b) HRE dual-luciferase assay in hypoxia-exposed Caki-1 cells. Fitted IC_{50} values were 2.52 μM for SIHC926 and 5.71 μM for YC-1. Points show mean $\pm$ SD from three independent experiments performed with technical triplicates. Curves were fitted by four-parameter logistic regression.

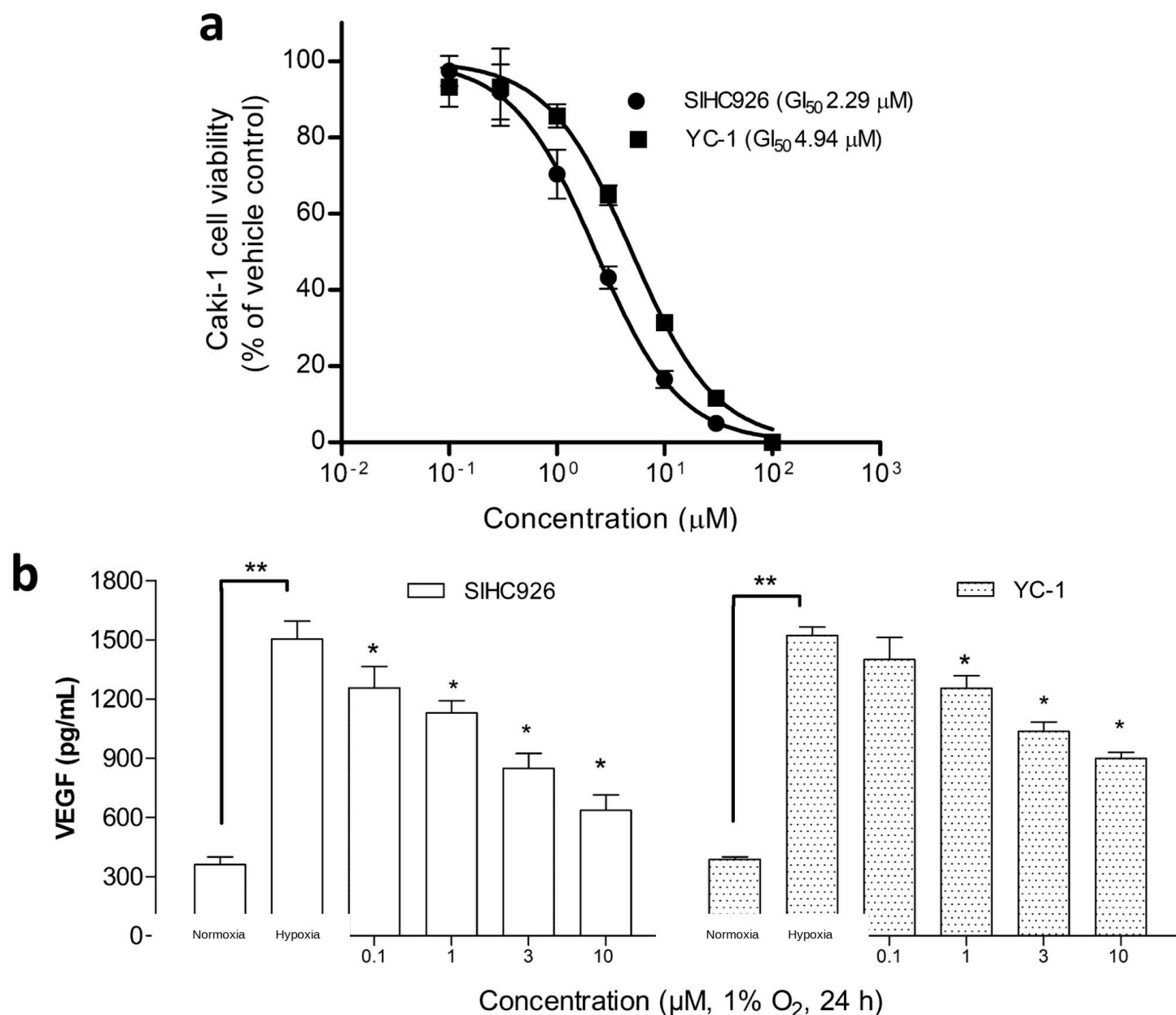


Figure 5

Effects of SIHC926 on Caki-1 cell viability and VEGF secretion under hypoxia. (a) CellTiter-Glo viability after 72 h at 1% O_2 . Reported fitted GI_{50} values were 2.29 μM for SIHC926 and 4.94 μM for YC-1. (b) Secreted VEGF-A after 24 h at 1% O_2 , normalized to viable cell number. Bars show mean $\pm$ SD from three independent experiments performed with technical triplicates. Symbols denote $P < 0.05$ (*) and $P < 0.01$ (**) for the comparisons indicated in the graph; exact P values and full statistical outputs should be supplied as Source Data.

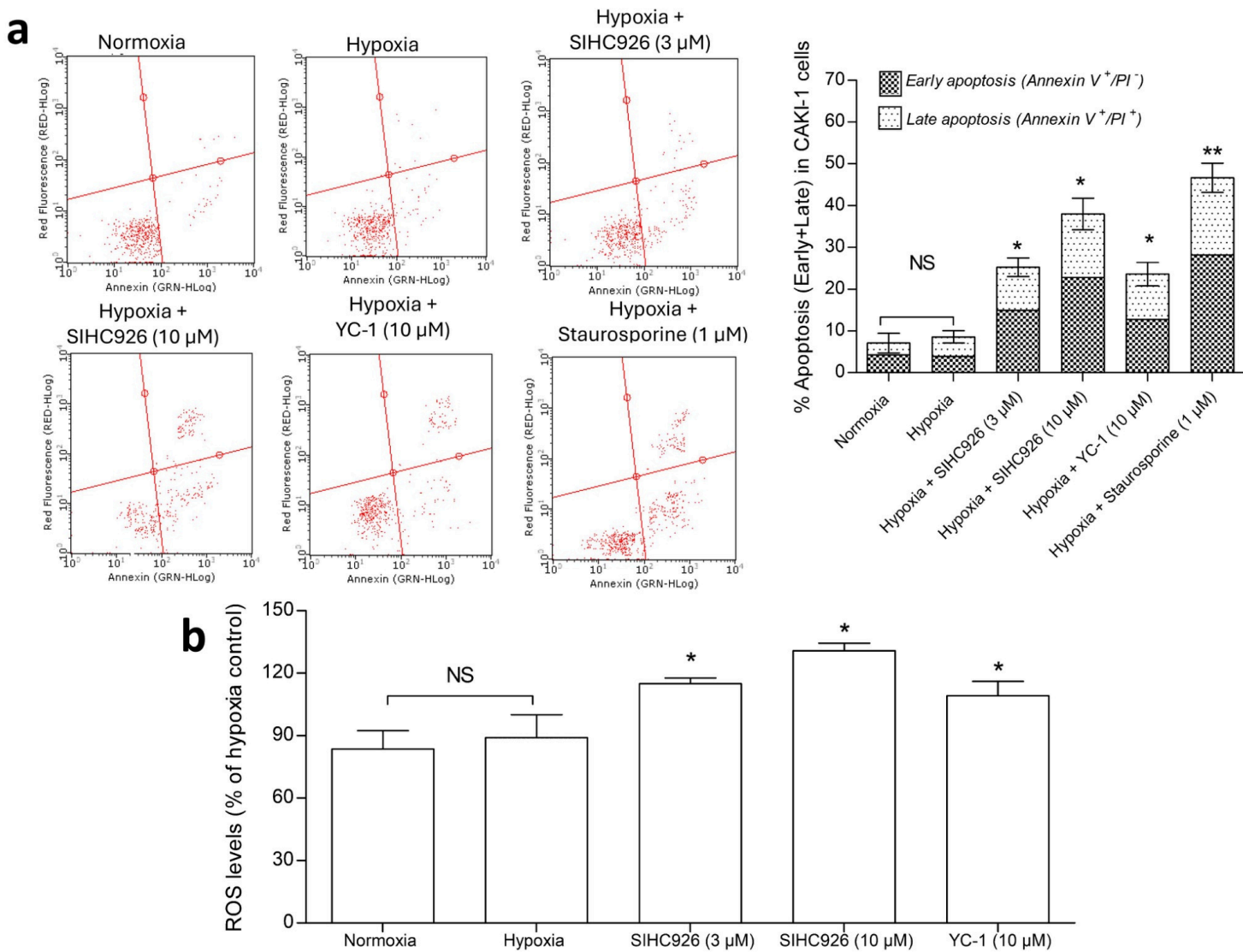


Figure 6

Effects of SIHC926 on apoptosis and intracellular oxidation-sensitive fluorescence in hypoxic Caki-1 cells. (a) Representative Annexin V–FITC/propidium iodide flow-cytometry plots and quantification of early and late apoptosis after 48 h treatment. Staurosporine was the positive control. (b) DCFDA fluorescence after 24 h treatment, expressed relative to the hypoxic vehicle control. Data are mean $\pm$ SD from three independent experiments; the DCFDA assay included technical triplicates. NS, not significant; * $P < 0.05$; ** $P < 0.01$. Exact P values, gating/source files and complete statistical outputs should accompany the submission.