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Novel Sulfonamide Derivatives: Proliferation and Migration in MCF-7 Cells under Normoxia and Hypoxia

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ABSTRACT

Hypoxia is a recognised characteristic of the tumour microenvironment and significantly contributes to therapeutic resistance and aggressive behaviour in breast cancer. Carbonic anhydrase IX (CA IX) is an enzyme induced by hypoxia and is important for maintaining pH homeostasis, thereby enabling cancer cell survival in low-oxygen environments. Sulfonamide-based compounds are known to act as selective CA IX inhibitors, offering therapeutic promise especially under hypoxic stress. The purpose of this study was to elucidate the effects of newly synthesised sulfonamide derivatives on the viability and migration of the breast cancer cell line MCF-7 under normoxic and chemically induced hypoxic conditions (150 µM cobalt chloride). The compounds were synthesised via standard sulfonamide coupling reactions, purified, and characterised using spectroscopic techniques. Cytotoxicity was assessed through the MTT assay, while the scratch assay was utilised to measure the compounds' effect on cell migration. Results showed that the sulfonamide derivatives (compounds 3b, 3d-g) demonstrated a significant dose-dependent reduction in cell viability in the MCF-7 cell line, with greater inhibition under hypoxic conditions. Moreover, migration was notably suppressed in hypoxia-treated cells, indicating impaired cellular motility upon CA IX inhibition. These findings suggest that the synthesised sulfonamide derivatives may act by targeting both proliferation and migration mechanisms under hypoxia. Their efficacy in the luminal breast cancer model positions them as promising candidates for further development. Additional molecular assays, including qPCR and protein expression studies, are recommended to further elucidate their mechanisms.

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INTRODUCTION

Breast cancer remains the most frequently diagnosed cancer among women worldwide and ranks as the second most common cancer overall. In 2022 alone, approximately 2.3 million new cases were reported globally, resulting in an estimated 670,000 deaths (WHO, 2025). However, the impact of breast cancer varies significantly across regions, with disparities in incidence, mortality, and access to care shaped by socioeconomic and healthcare factors. The disease is heterogeneous, comprising multiple subtypes that exhibit distinct histopathological and molecular characteristics, which affect prognosis and therapeutic response (Supuran, 2020). Oestrogen receptor-positive (ER+) breast cancer represents the predominant subtype and is commonly studied in vitro with the MCF-7 cell line (Comşa et al., 2017). MCF-7 cells, derived from a patient with metastatic breast cancer and pleural effusion, retain functional oestrogen receptors and exhibit hormone-dependent growth, making them a valuable model for exploring targeted therapies (Chiche et al., 2009).

One of the most critical hallmarks of the tumour microenvironment is hypoxia—a state of reduced oxygen availability resulting from an imbalance between oxygen supply and demand, caused by abnormal tumour vasculature (Pastorekova et al., 2021). Hypoxia triggers adaptive cellular mechanisms that promote tumour survival, angiogenesis, and resistance to therapy. The hypoxia-inducible factor-1 alpha (HIF-1α) primarily facilitates this process by functioning as a transcription factor that upregulates genes involved in glycolysis, angiogenesis, and pH regulation (Supuran, 2020). Among its downstream targets, carbonic anhydrase IX (CA IX) plays a pivotal role in regulating both intracellular and extracellular pH, thereby enabling cancer cells to maintain homeostasis in acidic microenvironments (Alterio et al., 2012; Winum et al., 2015).

The membrane-bound zinc metalloenzyme CA IX catalyses the reversible hydration of carbon dioxide to protons and bicarbonate (Sung et al., 2021). Its expression is typically restricted to certain gastrointestinal tissues but is strongly induced under hypoxic conditions in various solid tumours, including breast cancer (Perou et al., 2000). A worse prognosis, a higher risk of metastasis, and resistance to chemotherapy and radiation are all linked to overexpression of CA IX (Soule et al., 2000). Consequently, CA IX has emerged as an attractive therapeutic target for hypoxia-selective cancer treatment.

The most extensively studied family of CA IX inhibitors is sulfonamide-based compounds, due to their strong affinity for the enzyme's active-site zinc ion (Levenson & Jordan, 1997). Classical sulfonamides, such as acetazolamide and ethoxzolamide, have demonstrated inhibitory effects against CA IX; however, their lack of selectivity and potential off-target effects limit their clinical translation (Semenza, 2012). Recent medicinal chemistry efforts have focused on synthesising novel sulfonamide derivatives with enhanced selectivity, potency, and pharmacokinetic profiles (Wykoff et al., 2000). Preclinical studies have demonstrated that such inhibitors can reduce tumour growth, inhibit metastatic spread, and enhance the efficacy of conventional chemotherapies under hypoxic conditions (Lou et al., 2011; McDonald et al., 2016).

The MCF-7 cell line provides an ideal model for assessing the hypoxia-selective activity of novel CA IX inhibitors, as these cells upregulate CA IX expression in response to hypoxic induction, such as cobalt chloride ($CoCl_2$) treatment (Janoniene et al., 2021). $CoCl_2$ acts as a hypoxia mimetic by stabilising HIF-1α, thereby triggering the transcriptional activation of hypoxia-responsive genes, including CA IX (Tan et al., 2009). This in vitro model enables the controlled investigation of drug efficacy under both normoxic and hypoxic conditions, allowing for a direct comparison of cytotoxic and anti-migratory effects.

This study evaluated six newly synthesised sulfonamide-based CA IX inhibitors for their cytotoxic and migration-inhibitory activities against MCF-7 cells under normoxia and hypoxia. Cytotoxicity was determined using the MTT assay, and cell migration was assessed using the scratch assay. The use of both assays provides complementary insights into the compounds' ability to inhibit not only cell survival but

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also metastatic potential. Our working hypothesis was that CA IX inhibition would preferentially impair cancer cell survival and migration under hypoxic conditions, where CA IX expression is highest.

The novelty of this research lies in the direct head-to-head comparison of multiple novel sulfonamide derivatives within the same experimental system, enabling the identification of lead candidates with superior hypoxia-selective activity. This study's findings aim to enhance the evidence for CA IX as a target in hypoxia-adapted breast cancer and to guide future preclinical development of sulfonamide-based therapeutics.

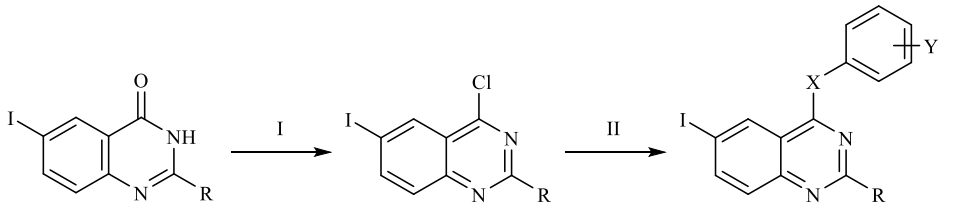
MATERIALS AND METHODS

Chemicals and reagents

Cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) has been purchased from Sigma-Aldrich (St. Louis, MO, USA) and used to induce hypoxia in vitro. The MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was sourced from Thermo Fisher Scientific (Waltham, MA, USA). Dimethyl sulfoxide (DMSO) and phosphate-buffered saline (PBS) were obtained from Merck (Darmstadt, Germany). Dulbecco's Modified Eagle Medium (DMEM) was utilised for cell culture, supplemented with 10% foetal bovine serum (FBS) and 1% penicillin–streptomycin.

Synthesis and characterisation of sulfonamide derivatives

Compounds 3b-h were synthesised *via* standard coupling reactions using substituted 4-chloroquinazoline and appropriate nucleophile (Alafeefy et al., 2016). The target quinazoline derivatives were prepared *via* a generalised route *via* standard coupling reaction (Alafeefy et al., 2016) as illustrated in Figure 1. The intermediates 4-chloroquinazolines (2a,b) were obtained by subjecting the corresponding quinazolines (1a–b) to reflux in phosphorus oxychloride, with N,N-dimethylaniline serving as the acid scavenger. Compounds 3b and 3d–g were subsequently produced through condensation of the appropriate nucleophiles with 4-chloroquinazolines (2a,b) in refluxing isopropanol.



1a,b **2a,b** **3b, d-h**

1,2[a]: R = CH_3, 1,2[b]: R = C_6H_5.

3b: R = CH_3, X = NH, Y = 3-aminosulfonyl. **3d: R = CH_3, X = S, Y = 4-hydroxycarbonyl.**

3e: R = C_6H_5, X = NH, Y = 4-aminosulfonyl. **3f: R = C_6H_5, X = NH, Y = 3-aminosulfonyl.**

3g: R = C_6H_5, X = O, Y = 4-hydroxycarbonyl. **3h: R = C_6H_5, X = S, Y = 4-hydroxycarbonyl.**

Reagents and conditions: I = $POCl_3$/ NN-Dimethylaniline/ Reflux 5 hours.
II = the suitable nucleophile/Isopropyl alcohol reflux/2 hrs.

Fig. 1. Chemical structures of six newly synthesised sulfonamide-based compounds. These compounds were designed to explore the structural variations that influence CA IX inhibition under hypoxic conditions.

Melting points (°C, uncorrected) were measured using open capillaries on a Gallenkamp apparatus (Sanyo Gallenkamp, Southborough, UK). Thin-layer chromatography (TLC) was performed on precoated silica gel plates (0.25 mm, 60F254; Merck, Germany) using a dichloromethane/methanol (9.5:0.5) solvent system for development. Spots were visualised under ultraviolet illumination and/or iodine vapour. Infrared

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spectra were obtained in KBr discs with a Shimadzu IR-470 spectrometer (Shimadzu, Tokyo, Japan). NMR spectra (300 MHz for ^{1}H and 75 MHz for ^{13}C) were recorded on a Bruker AC-300 Ultra Shield spectrometer (Bruker, Flawil, Switzerland), using TMS as the internal reference; signal multiplicities were denoted as s (singlet), d (doublet), t (triplet), and m (multiplet). Mass spectra were acquired by electron impact ionisation on a Shimadzu GC-MS QP5000 instrument (Shimadzu, Tokyo, Japan). Elemental analyses were conducted with a Carlo Erba 1108 analyser (Heraeus, Hanau, Germany) at the Micro-analytical Unit, Faculty of Science, Cairo University. Analytical TLC monitored reaction progress during synthesis on silica gel plates. All solvents and reagents were purified and dried following standard laboratory procedures.

Cell culture

MCF-7 human breast adenocarcinoma cells were sourced from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in DMEM with 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified environment with 5% CO_2. Culture media were changed every 2–3 days, and cells were subcultured when they reached 80–90% confluence using 0.25% trypsin–EDTA.

Hypoxia induction

Hypoxic conditions were chemically induced using 150 μM $CoCl_2$ for 24 hours before drug treatment. $CoCl_2$ acts as a hypoxia mimetic by stabilising HIF-1α, thereby upregulating hypoxia-responsive genes, including CA IX (Rana et al., 2019). Control cells were maintained under normoxic conditions without $CoCl_2$ treatment.

Anti-proliferative assay (MTT)

The anti-proliferative effects of the sulfonamide-based CA IX inhibitors were assessed using the MTT assay (Mosmann, 1983). MCF-7 cells were seeded at a density of 5×10^3 cells/well in 100 μL complete DMEM in 96-well plates and allowed to adhere overnight. Under normoxic or hypoxic conditions, cells were treated with varying concentrations (0, 1, 10, 100, and 1000 μM) of the test compounds and acetazolamide (AAZ), a positive control, for 24 hours. After treatment, 10 μL of MTT solution (5 mg/mL in PBS) was added to each well, and the plate was incubated at 37 °C for 4 hours. The resulting formazan crystals were solubilised with 100 μL of DMSO, and absorbance was subsequently recorded at 570 nm using a TECAN microplate reader (Switzerland). All assays were conducted in triplicate to ensure reproducibility.

Migration assay (scratch assay)

Cell migration was assessed using the scratch wound healing assay (Liang et al., 2007). MCF-7 cells were seeded in 96-well plates at 3×10^4 cells/well in 100 μL complete DMEM and cultured until a uniform monolayer (~95–100% confluence) was achieved. A linear monolayer disruption was created using a sterile 200 μL pipette tip, and the wells were gently washed twice with PBS to remove dislodged cells. Fresh DMEM containing the test compounds at final concentrations of 0, 50, 100, 200, and 400 μM was added to each well. Cells were then incubated under normoxic or hypoxic conditions (150 μM $CoCl_2$ pre-treatment for 24 h before scratching). Images of the migration track were captured at 0 h and 24 h using an inverted phase-contrast microscope (Olympus, Japan). All assays were performed in triplicate. To quantify the migration effect, the scratch area was measured at each time point using ImageJ software.

The anti-migratory effect percentage was determined using the subsequent formula (1):

$$\text{Anti-Migratory Effect (\%)} = \frac{(A_{0h} - A_{24h})}{A_{0h}} \times 100 \quad (1)$$

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Statistical analysis

Experimental data are expressed as mean values ± standard deviation (SD) from three independent replicates. Statistical evaluation was performed using GraphPad Prism software, version 10.0 (GraphPad Software, San Diego, CA, USA). Comparisons between normoxic and hypoxic conditions were assessed with a paired t-test, and differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Six sulfonamide derivatives (compounds 3b, 3d-g) were synthesised via standard coupling reactions between substituted benzenesulfonyl chlorides and appropriate amines (Figure 1). This approach typically yields stable sulfonamide bonds and is widely used in medicinal chemistry due to the pharmacological relevance of the sulfonamide moiety (Wani et al., 2023). Recrystallisation yielded analytically pure compounds with moderate to high yields (62–85%). This efficiency is consistent with previous reports highlighting the reliability of sulfonyl chloride–amine coupling in generating diverse sulfonamide libraries (Chavan et al., 2023).

Structural verification of the synthesised derivatives was performed using infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry. The IR spectra of compounds 3b, 3e, and 3f displayed characteristic absorption bands for the SO_2 group at 1385 and 1197 cm^{-1}, along with signals attributable to NH_2 and NH functionalities in the 3350–2360 cm^{-1} region. In the ^1H NMR spectra, D_2O-exchangeable resonances corresponding to NH and NH_2 protons were observed at δ 11.2–12.3 and δ 7.40–7.60, respectively.

Compounds 3d, 3g, and 3h showed an absorption band at 3550-3300 cm-1, in addition to two bands at 1382 and 1195 cm-1 for SO_2. The carbonyl group appeared at 1670 cm^{-1} in their IR spectra. The ^1H NMR spectra presented a D_2O exchangeable signal for the OH group at 10.50–10.55. The ^{13}C spectra showed a peak at 86 -89 due to the common iodine-carrying atom C-6. The methyl carbon (3b and 3d) at ~ 21 – 22. Compounds 3d, 3g, and 3h showed a carbonyl carbon at ~170. Mass spectrometric analysis confirmed the presence of molecular ion peaks in each case, thereby supporting the proposed molecular structures of the synthesised compounds.

Next, all six sulfonamide derivatives (compounds 3b, 3d-g) were evaluated for their cytotoxic and anti-migratory effects on MCF-7 cells under normoxic and hypoxic conditions. Increasing concentrations of each compound resulted in a measurable reduction in cell viability, as determined by the MTT assay. Statistical evaluation demonstrated significant differences between normoxic and hypoxic conditions for each compound ($p < 0.05$); hypoxia produced a more pronounced inhibitory effect (Tables 1 and 2).

Compounds 3f (in normoxia) and 3h (in hypoxia) exhibited the most potent cytotoxicity against MCF-7 cells. Under normoxia, compound 3f had an IC_{50} value of 158.5 ± 9.2 μM, while under hypoxia, compound 3h achieved an IC_{50} of 129.0 ± 0.5 μM (Table 1). Interestingly, compound 3h had the lowest IC50 value among the others under hypoxic conditions and the second-lowest under normoxic conditions. The dual profile indicates that 3h is both hypoxia-responsive (enhanced potency when CA IX is upregulated) and baseline-active (still effective under normoxia). This makes it a promising candidate because it can act in the tumour microenvironment, where hypoxia is prevalent, and retain activity in regions with normal oxygen levels. Hence, the present study demonstrates that newly synthesised sulfonamide derivatives exhibit potent anti-proliferative activity against MCF-7 cells, particularly under hypoxic conditions. These findings align with the growing evidence that hypoxia-induced CA IX is critical in tumour survival, therapeutic resistance, and metastatic progression (Rankin & Giaccia, 2016; Mboge et al., 2018). The sulfonamide derivatives may disrupt pH homeostasis, potentially through the inhibition of CA IX, leading to reduced cell viability and impaired migration, two key hallmarks of cancer aggressiveness, demonstrated in Tables 1 and 2 (Supuran, 2020). By using computational design and

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structural optimisation, tethering classic zinc-binding sulfonamides to quinoline, quinazoline, or thiazole scaffolds, intracellular oncogenic signalling and extracellular pH regulation can be blocked simultaneously, cutting off tumour vascularisation and hypoxia adaptation (Charissopoulos & Pontiki, 2026).

Table 1. The IC_{50} (μM) values of the synthesised sulfonamides and acetazolamide in normoxia and hypoxia against MCF-7 cells (mean ± SD, n=3).

Compound	IC_{50} (μM)		paired t-test
	Normoxia	Hypoxia	Hypoxia vs Normoxia
Compound 3b	225.5 ± 29.0	143.0 ± 8.5	** ($p < 0.01$)
Compound 3d	290.0 ± 14.1	228.0 ± 7.2	** ($p < 0.01$)
Compound 3e	183.1 ± 3.0	160.0 ± 6.0	** ($p < 0.01$)
Compound 3f	158.5 ± 9.2	146.3 ± 3.5	** ($p < 0.01$)
Compound 3g	209.1 ± 0.7	212.2 ± 1.4	* ($p < 0.05$)
Compound 3h	163.5 ± 4.8	129.0 ± 0.5	** ($p < 0.01$)
AAZ	68.2 ± 3.4	51.4 ± 8.4	** ($p < 0.01$)

Data are expressed as mean ± SD (3 independent experiments). Statistical significance between normoxia and hypoxia conditions was assessed using a paired t-test. Statistical significance is denoted by ** for $p < 0.01$ and * for $p < 0.05$.

Table 2. Percentage inhibition of MCF-7 cell migration by synthesised sulfonamides and acetazolamide at 100 μM after 24 hours under hypoxic conditions. Data reflect anti-migratory effects as measured in a wound-healing assay.

Compound	% anti-migratory at 100 μM at 24 h		paired t-test
	Normoxia	Hypoxia	Hypoxia vs Normoxia
Compound 3b	71.2 ± 2.1	96.7 ± 1.5	** ($p < 0.01$)
Compound 3d	75.0 ± 1.8	97.5 ± 1.2	** ($p < 0.01$)
Compound 3e	80.0 ± 2.0	98.0 ± 1.3	** ($p < 0.01$)
Compound 3f	79.0 ± 2.5	97.2 ± 1.7	** ($p < 0.01$)
Compound 3g	99.0 ± 0.5	99.8 ± 0.0	ns (not significant)
Compound 3h	80.0 ± 1.9	97.0 ± 1.4	* ($p < 0.05$)
AAZ	77.5 ± 1.7	98.5 ± 1.1	** ($p < 0.01$)

Data are expressed as mean ± SD (3 independent experiments). Statistical significance between normoxia and hypoxia conditions was assessed using a paired t-test. Statistical significance is denoted by ** for $p < 0.01$ and * for $p < 0.05$.

The scratch assay demonstrated that all tested compounds exerted anti-migratory activity at 100 μM after 24 hours. Markedly, compounds 3b, 3d–f, 3h, and AAZ exhibited significantly greater inhibition of cell migration under hypoxic conditions compared to normoxia, indicating that the anti-migratory effect was more pronounced in hypoxia. Compounds 3b, 3d-f, and AAZ demonstrated statistically significant increases in anti-migratory activity under hypoxia ($p < 0.01$), while compound 3h showed moderate significance ($p < 0.05$). Compound 3g maintained near-maximal inhibition under both conditions, with no significant difference, suggesting oxygen-independent efficacy. Compounds 3e and 3f exhibited high baseline activity (>79%) with further enhancement under hypoxia, indicating potential synergy with hypoxia-responsive pathways. Compound 3h, while effective, showed a smaller differential (80.0% to 97.0%, $p < 0.05$), suggesting moderate sensitivity to hypoxic modulation. The significant increase in AAZ's anti-migratory effect under hypoxia (from 77.5% to 98.5%) supports its role as a CAIX-targeted agent, consistent with previous findings (Matsue et al., 2022).

On that note, compound 3g displayed IC_{50} values under normoxia (209.1 ± 0.7 μM) and hypoxia (212.2 ± 1.4 μM) in MCF-7 cells and showed very high anti-migratory effects. It suggests that the compound lacks hypoxia-selective cytotoxicity but significantly interferes with migration-related pathways. This may be due to limited interaction with hypoxia-inducible targets, such as CA IX, or insufficient structural features to enhance cellular uptake and target engagement. However, sulfonamide compounds have been shown to exert strong anti-migratory effects in MCF-7 breast cancer cells, largely by interfering with carbonic anhydrase activity, EMT signalling, and cytoskeletal remodelling (Nguyen et al., 2021). Sulfonamides with strong anti-migratory effects typically possess electron-withdrawing groups and heterocyclic moieties that

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improve lipophilicity and binding affinity (Meena et al., 2023). The observed uniformity in activity across oxygen conditions indicates that the compound's mechanism is likely oxygen-independent and may require further structural optimisation to enhance therapeutic relevance in hypoxic tumour microenvironments.

The enhanced cytotoxicity observed under chemically induced hypoxia (via $CoCl_2$) underscores the oxygen-dependent expression and functional relevance of CA IX. Hypoxia stabilises HIF-1α, which transcriptionally upregulates CA IX, facilitating extracellular acidification and intracellular alkalisation, conditions that favour cancer cell survival and invasion (Lou et al., 2011). The sulfonamide derivatives tested in this study may have acted through zinc coordination, a mechanism well-characterised in previous inhibitor studies (Alafeefy et al., 2016; Supuran, 2020). However, direct binding studies are still required to confirm this mode of action.

AAZ was selected as a positive control due to its dual relevance in targeted cancer research in CA IX. As a classical sulfonamide, AAZ exhibits well-documented inhibitory activity against CA IX, a hypoxia-inducible isoform implicated in tumour cell migration, extracellular acidification, and survival under low-oxygen conditions. Its inclusion in the migration assay provides a mechanistic benchmark for evaluating anti-migratory efficacy, particularly under hypoxic conditions where CA IX expression is upregulated (McDonald et al., 2012). Additionally, AAZ has been widely employed in cytotoxicity assays, such as the MTT assay, to validate compound potency and assay integrity, given its consistent anti-proliferative effects across CA IX-expressing cancer cell lines (Mussie et al., 2022; Supuran, 2020). The inhibition observed with compound 3h in migration and viability assays underscores its possible mechanistic alignment with CA IX blockade and reinforces its translational promise in hypoxia-adapted cancer therapy.

Despite promising findings, this study has several limitations. It relied solely on phenotypic assays without molecular confirmation of CA IX suppression or downstream signalling, leaving the mechanism of action unresolved. Moreover, direct evidence of CA IX targeting through enzyme activity assays, binding affinity measurements, or selectivity testing against other carbonic anhydrase isoforms is still required. The observed cytotoxicity could equally result from alternative pathways such as DNA damage, mitochondrial dysfunction, or cell cycle arrest, rather than CA IX inhibition. Reliance on cobalt chloride as a chemical hypoxia mimetic raises methodological concerns, as this model does not fully replicate the complexity of physiological tumour hypoxia. Real tumour hypoxia involves dynamic oxygen gradients, metabolic reprogramming, and multiple signalling cascades beyond HIF-1α stabilisation, and chemical induction fails to capture hypoxia–reoxygenation cycles common in vivo. Moreover, hydralazine (a well-known vasodilator/antihypertensive drug) has been shown to be a safer, low-toxicity chemical alternative to cobalt chloride for inducing and modelling cellular hypoxia in human MCF-7 breast cancer cell lines (Al-Mashhadani & Arif, 2026). Using a single cell line (MCF-7) limits generalisability across breast cancer subtypes, and other subtypes, such as triple-negative and HER2-positive breast cancers, may respond differently.

However, the study was designed to compare hypoxic and normoxic conditions in cancer cells, as hypoxia represents a key feature of the tumour microenvironment. While comparisons with normal cells would inform selectivity, the primary objective was to determine whether the compounds exhibit oxygen-dependent activity. Additionally, the specific mode of cell death was not delineated, leaving uncertainty about whether the compounds induced apoptosis, necrosis, or other pathways such as autophagy or ferroptosis. Future research should incorporate molecular assays (e.g., qPCR, Western blot) to validate CA IX inhibition and apoptotic pathways, expand testing across diverse cancer and non-cancerous cell lines, and adopt advanced 3D and in vivo models to simulate tumour architecture better and assess therapeutic efficacy. Exploring synergistic combinations with chemotherapeutics or phytochemicals may enhance anti-hypoxic activity and overcome resistance (Chaachouay, 2025; Zhang et al., 2019).

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CONCLUSION

This study highlights the therapeutic potential of newly synthesised sulfonamide-based carbonic anhydrase IX (CA IX) inhibitors in targeting hypoxia-adapted breast cancer cells. The compounds demonstrated significant cytotoxic and anti-migratory effects against MCF-7 cells, with enhanced efficacy under chemically induced hypoxic conditions. These findings reinforce the role of CA IX as a viable target in hypoxia-driven tumour progression and underscore the value of sulfonamide scaffolds in the design of selective inhibitors. By impairing both proliferation and motility, the tested compounds demonstrate dual-action effects, positioning them as promising candidates for further development in the treatment of luminal breast cancer. Future studies should incorporate molecular assays to elucidate apoptotic pathways, validate CA IX suppression, and explore synergistic potential with existing chemotherapeutics. This work contributes to the growing field of hypoxia-targeted cancer therapeutics and supports the rational design of CA IX inhibitors for clinical translation.

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CONFLICT OF INTEREST STATEMENT

The authors affirm that there were no financial or commercial conflicts of interest or self-benefit associated with this research, and they also state that they had no competing interests with the funder.

AUTHORS CONTRIBUTION

Mohd Nabil Afifi Mohd Zaki conducted the experiments, performed statistical analyses and data interpretation, drafted the manuscript, and designed the figures. Mizaton Hazizul Hasan, Ahmed Mahmoud Ahmed Alafeefy, Aisyah Hasyila Jahidin, and Maziana Mahamood established the theoretical framework and structured the study. Mizaton Hazizul Hasan headed the research process, structured the entire article and its revisions, and authorised the submission. Ahmed Mahmoud Ahmed Alafeefy, Aisyah Hasyila Jahidin, and Maziana Mahamood contributed to the interpretation of the findings. Each author contributed insightful feedback, shaping the study, analysis, and publication.

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