Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Design, Synthesis and Cytotoxic Evaluation of New Quinolinedione Derivatives as Potential Candidates for the Treatment of Cancer

Akeel Abo Alard [1] *

[1] Pharmaceutical Chemistry Department, Faculty of Pharmacy, University of Kufa, Najaf, Iraq

***Corresponding Author:**
Akeel Abo Alard: akeela.aboalard@uokufa.edu.iq

Received: 28/07/2025
Accepted: 30/10/2025
Published: 31/12/2025

Keywords: Quinolinedione, Carbonic anhydrase, human isoform IX, Docking study.

DOI: 10.62472/kjps.v16.i27.196-209

Abstract

Background: Carbonic anhydrase IX (CAIX) affected by hypoxia and thus lead to upregulated in many types of tumors and thus helps in maintain tumor pH balance under these conditions. Pharmacologic inhibition of CAIX has been shown in mouse models to slow tumor growth, curb metastasis, and limit tumor stem cell expansion. Therefore, inhibiting the overexpression of CA, particularly CA IX, consider a promising anticancer therapy.

Objective: The primary objective of this study was to design and synthesis of a set of new quinolinedione derivatives (5a-c), study antiproliferative activity by MTT assay against HCT116 cell lines and MCF-7 cell lines and molecular docking studies to prioritize candidates' compounds with the highest predicted binding.

Methods: The synthesis of new quinolinedione derivatives (5a-c) through derivatives bearing methylsulfonyl aniline scaffolds, characterized via TLC, melting point, and spectral data acquisition (HNMR, and CNMR), and cytotoxic evaluated for the synthesised derivatives using antiproliferative activity by MTT assay against HCT116 cell lines and MCF-7 cell lines.

Results: Among the tested compounds, 7-methoxy-3,4-dihydroquinolin-2(1H)-one (compound 5c) exhibited markedly stronger antiproliferative activity in HCT116 cells (IC_{50} = 0.98 µM) and MCF-7 cell lines (IC_{50} = 1.39 µM) versus acetazolamide (IC_{50} = 1.69 µM, IC_{50} = 2.45 µM). While, both compounds 5a and 5b showed a close cytotoxic activity results to acetazolamide. These results position quinolinedione derivatives (5a-c) as a promising starting point for further development as an anticancer agent. Docking study indicated that 5c achieved good S-scores (-6.211) in compare with acetazolamide (-5.574), suggesting stronger affinity for the CA IX active site. The substituted methoxy group and quinolinedione scaffold appear to enhance flexibility and receptor interactions.

Conclusion: Overall, the quinolinedione derivatives (5a-c) were effectively synthesized and exhibit meaningful CA IX inhibition and cytotoxic activity, supporting their further evaluation as potential anticancer leads.

تصميم وتصنيع وتقييم السمية الخلوية لمشتقات الكينولينيديون الجديدة كمرشحات محتملة لعلاج السرطان

عقيل ابو العرد

الخلاصة

يتأثر أنزيم الكربونيك أنهيدراز IX (CAIX) بنقص الأكسجين، مما يؤدي إلى زيادة نشاطه في العديد من أنواع الأورام، وهذا بدوره يساعد في الحفاظ على توازن درجة حموضة الورم في ظل هذه الظروف. وقد ثبت أن التثبيط الدوائي لـ CAIX في نماذج الفئران يُبطئ نمو الورم، ويُكبح النقائل، ويُحد من توسع الخلايا الجذعية السرطانية. لذلك، يُعد تثبيط الإفراط في التعبير عنCA ، وخاصةCA IX ، علاجًا واعدًا للسرطان. واستنادًا إلى هذا الأساس المنطقي، صُممت ورُكّبت مجموعة من مشتقات الكينولين الجديدة (5a-c).علاوة على ذلك، اختُبرت المركبات المُصنعة ضد سلالات خلايا HCT116 وMCF-7، باستخدام دواء الأسيتازولاميد كمعيار لغرض المقارنة. ولإعطاء الأولوية للمركبات المرشحة ذات أعلى ارتباط متوقع، استُخدم الالتحام الجزيئي (إصدار برنامج شرودنجر مايسترو 2021-1 وبرنامج بايمول) قبل تصنيع المركبات (5a-c). بالإضافة إلى ذلك، تم تقييم النشاط المضاد للتكاثر في الخلايا السرطانية باستخدام اختبار MTT على سلالات خلايا HCT116 و .MCF-7 بالمقارنة مع دواء الأسيتازولاميد، وقد أظهر المركب 5c نشاطًا مضادًا للتكاثر في الخلايا السرطانية أقوى بشكل ملحوظ في خلايا HCT116 (IC_{50} = 0.98 μM) وخلايا MCF-7 (IC_{50} = 1.39 μM) مقارنةً بدواء الأسيتازولاميد (IC_{50} = 1.69 μM)، بينما أظهر المركبان 5a, 5b نشاطًا سامًا للخلايا السرطانية مشابهًا للأسيتازولاميد. هذه النتائج تضع مشتقات الكينولينيديون المصنعة كنقطة انطلاق واعدة لمزيد من التطوير كعامل مضاد للسرطان. أشارت دراسة الالتحام الجزيئي إلى أن 5c حقق درجات S جيدة مقارنةً بأسيتازولاميد، مما يشير إلى تقارب أقوى مع موقع الارتباط النشط في CA IX ويبدو أن مجموعة الميثوكسي المستبدلة وهيكل الكينولينيديون يعززان المرونة والتفاعلات مع المستقبلات. وبشكل عام، تُظهر مشتقات الكينولينيديون (5a-c) تثبيطًا فعالًا لـ CA IX ونشاطًا سامًا للخلايا السرطانية ، مما يدعم تقييمها الإضافي كمؤشرات محتملة لمكافحة السرطان.

1. Introduction

Generally, cancer consider a leading global health burden, causing extensive mortality and undermining gains in life expectancy. WHO data indicate that around 13 million cancer deaths cases could be expected by 2030 (Alatorre etal., 1987). In breast cancer, many cases eventually develop long term resistance despite multiple therapeutic options, leading to treatment failure and disease progression (Aleksandrs et al., 2020). Overcoming this resistance will require continued research and innovative strategies. One of the promising strategies is targeting carbonic anhydrase enzyme. Carbonic anhydrase (CA) catalyzes the reversible interconversion of CO_2 and bicarbonate and is indispensable across eukaryotes and bacteria (Alterio et al., 2012). While, in humans, this kind of reaction linking CO_2, HCO_3^-, and H^+ underpins numerous physiological and pathological processes, including respiration, electrolyte secretion, and lipogenesis (Angeli et al., 2016). Sixteen mammalian CA isozymes are known: (CA I-XIII) in cytosolic, (CA IV, IX, XII, XIV) as membrane-bound, (CA VA, VB) in mitochondrial, and finally a secreted form (CA VI) (Cronk et al, 2001). Carbonic anhydrases (CAs) have emerged as attractive tumor targets. Humans express 15 α-CA isoforms (hCAs) with distinct catalytic efficiencies, tissue localization, and functions; twelve are catalytically active (hCA I-XIV) (Ghorab et al., 2021). Notably, hypoxia-inducible factor-1 (HIF-1) upregulates CA IX and XII in hypoxic tumors isoforms largely absent from normal tissues thereby acidifying the tumor microenvironment and supporting survival and proliferation in concert with anaerobic glycolysis (hen et al., 2011). Inhibiting these enzymes can enhance chemotherapy effectiveness while offering tumor selectivity that may reduce adverse effects (Kalinin et al., 2021, Kumar et al., 2022, Lehtonen et al., 2004). Classically, zinc binding inhibitors (CAIs) target the active site Zn^{2+}, which adopts trigonal bipyramidal or tetrahedral coordination during inhibition. Sulfonamides are prototypical CAIs that exploit this binding mode (Li et al., 2025, Linkuviene et al., 2018, Mboge et al., 2018). Sulfanilamide, identified in 1940, was the first organic CA inhibitor (Mehta et al., 2007). It was later shown that aromatic or heterocyclic sulfonamides molecules broadly act on inhibit CAs, spurring drug development first as diuretics compounds and antiglaucoma molecules (Patil et al., 2022); acetazolamide remains the archetype. Subsequent sulfonamide CA inhibitors (CAIs) have found use as antiepileptic, anti-obesity, and antitumor agents, with different mammalian isoforms targeted for distinct indications (Pawel et al., 2009). Aromatic/heterocyclic sulfonamides bearing ureido or thioureido groups have been extensively explored as CAIs (Rosen et al., 2019), with some of the earliest examples showing isoform selectivity for hCA I, II, or IV. Certain members of this class also demonstrated significant antitumor/antimetastatic activity in vivo (Sani et al., 2020). Building on this, we describe a small series of benzenesulfonamides featuring an extended linker between the sulfonamide and a semicarbazide “tail.” Prepared via an original route, these compounds were evaluated against four human isoforms (physiologically relevant isoform): cytosolic hCA I and II (antiglaucoma targets) and transmembrane hCA

IX and XII (anticancer targets) (Scozzafava et al., 2003). Moreover, metal complexes derived from sulfonamide CAIs can exhibit 10–100-fold stronger inhibition than the parent sulfonamides, (Supuran et al., 2008) likely via a dual mechanism in which low-concentration dissociation forms sulfonamide anions that bind Zn(II) in the active site while added metal ions disrupt proton-shuttle residues of the enzyme (Supuran et al., 2018). Guided by these insights, we designed new compounds incorporating these features. Computational studies support their potential, providing the rationale and confidence to advance their synthesis and evaluation.

2. Materials and Methods

2.1. General Chemistry

All chemicals, reagents and solvents were purchased from standard suppliers such as Fluorochem, Sigma Aldrich, Apollo scientific, abcr GMBH and Fisher Scientific. The starting materials 4-(Methylsulfonyl) aniline, bromacetyl bromide, thiourea and quinolinedione molecules, as well as the solvents used in this experiment were obtained commercially without further purification. The progress of reactions was monitored via TLC using silica gel 60 F254 with UV detection. All compounds were structurally identified by 1H NMR and 13 C NMR. Unless otherwise stated, 1H and 13C NMR spectra were recorded on Bruker at 400 MHz spectrometer. Melting points were determined using a Stuart melting point apparatus (SMP-30) with an open glass capillary tube.

2.2. Synthesis

2.2.1.Preparation of 2-Bromo-*N*-[4-(methylsulfonyl)phenyl]acetamide (2)

Add 2-bromoacetyl bromide (1.3 mL, 15 mmol) and triethylamine (3.5 mL, 25 mmol) to a solution of 4-(methylsulfonyl) aniline (0.85 g, 5 mmol) in dichloromethane (40 mL). Then, the mixture was cooled in an ice bath and stirred for 30 minute then for 1.5 hour at room temperature. The reaction was monitor for completion by TLC. After the reaction reached completion ethyl acetate (EtOAc) was added to the reaction, and the mixture was then washed with saturated solution of ammonium chloride, sodium bicarbonate, water and brine respectively. The organic layer was dried with magnesium sulphate, filtered, and then evaporated under vacuum. The resulting product was then purified by triturated with diethyl ether to afford the title compound as light brown solid (yield: 81%). mp = 167–170 ^{0}C, 1H NMR (400 MHz, DMSO-*d*6): δ 8.34 (s, 1H), 7.91 – 7.95 (m, 2H), 7.71 – 7.75 (m, 2H), 4.21 (s, 2H), 3.01 (s, 3H). TLC: R_f0.42[a].

2.2.2.Preparation of N4-(4-(methylsulfonyl)phenyl)thiazole-2,4-diamine (3)

A mixture of 2-Bromo-*N*-[4-(methylsulfonyl)phenyl]acetamide (2) (0.73 g, 3 mmol) and thiourea (0.23g, 3 mmol) in absolute acetone (100 ml) was refluxed for 10 hr. After the reaction reached completion, as shown by TLC, the solvent was evaporated to obtain a solid precipitate. The later was poured through an ice-cold water, and the resultant precipitate was purified by recrystallization from methanol. The solid precipitate was then washed with saturated solution of ammonium chloride, sodium bicarbonate, and then water respectively.

Filtered, dried and purified through recrystallization from ethanol/water to to give the title compound as brown powder (yield: 71%). mp = 110–113 ^{0}C, 1H NMR (400 MHz, DMSO-*d*6): δ 10.20 (s,1H), 8.25-7.20 (m, 4H, Ar-H and1H, s, CH of thiazole), 5.61 (s, 2H), 3.30 (s, 3H). TLC: R_f0.52[a].

2.2.3.General procedure for the synthesis of quinoline Dione derivatives 5a-c

To a mixture of synthesised intermediate (3) (0.98 g, 4.5 mmol) in ethanol (20 mL), a selected quinolinedione molecule (4.5 mmol), and few drops of glacial acetic acid were added. The reaction mixture was refluxed for 10 hours. Then, it was cooled through poured into crushed ice and then filtered. Ethyl acetate was then added and the mixture was then washed with saturated ammonium chloride, sodium bicarbonate, water and brine respectively. The organic layer was dried with magnesium sulphate, then filtered, and then evaporated. The resulting product was then further purified by recrystallization from methanol to obtain the final compounds (Wang et al., 2024, Wong et al., 2017). The melting point and spectral data of target derivative (5a-c) for each compound are reported in synthesis section below.

2.2.4.Preparation of (E)-4-((4-((4-(Methylsulfonyl) Phenyl) Amino) Thiazol-2-Yl) Imino)-3,4-Dihydroquinolin-2(1H)-One (5a)

Following general procedure for the preparation of quinolinedione derivatives, intermediate (3) (0.98 g, 4.5 mmol) and 2,4(1*H*,3*H*)-Quinolinedione (0.72 gm, 4.5 mmol) were dissolved in EtOH and then glacial acetic acid (few drops) were added. The resulting product was then further purified by recrystallization from methanol to obtain the final compound **5a** as a light brown powder (yield: 79%). mp = 213–215 ^{0}C; 1H NMR (400 MHz, DMSO-*d*6): δ 10.23 (br s, 1H), 10.01 (br s, 1H), 8.25 – 7.70 (m, 7H), 7.08 – 6.95 (m, 2H), 3.3 (s, 3H), 2.70 (d, 2H). 13C NMR (DMSO-*d*6): δ 170.07, 166.49, 138.7, 137.9, 135.15, 135.32, 128.88, 128.02, 127.89, 124.67, 124.0, 122.4, 122.12, 117.55, 115.5, 52.65, 40.95, 30.9, 25.2.

2.2.5.Preparation Of (E)-7-Methyl-4-((4-((4-(Methylsulfonyl) Phenyl) Amino) Thiazol-2-Yl) Imino)-3,4-Dihydroquinolin-2(1H)-One (5b)

Following general procedure for the preparation of quinolinedione derivatives, intermediate (3) (0.98 g, 4.5 mmol) and 7-Methyl-2,4(1*H*,3*H*)-quinolinedione (0.78 gm, 4.5 mmol) were dissolved in EtOH and then glacial acetic acid (few drops) were added. The resulting product was then further purified by recrystallization from methanol to obtain the final compound **5b** as a beige powder (yield: 81%). mp = 233–235; 1H NMR (400 MHz, DMSO-*d*6): δ 11.01 (br s, 1H), 10.91 (br s, 1H), 8.05 – 7.85 (m, 5H), 7.72 – 7.56 (m, 2H), 7.18 – 6.86 (m, 1H), 3.45 (s, 3H), 3.23 (s, 3H), 2.97 (br d , 2H). 13C NMR (DMSO-*d*6): δ 171.05, 167.48, 137.9, 137.2, 136.10, 135.02, 128.08, 127.92, 127.09, 125.87, 124.09, 123.08, 122.22, 118.35, 115.91, 53.45, 42.75, 35.97, 33.19, 26.22.

2.2.6.Preparation of (E)-7-Methoxy-4-((4-((4-(Methylsulfonyl) Phenyl) Amino) Thiazol-2-Yl)Imino)-3,4-Dihydroquinolin-2(1H)-One (5c)

Following general procedure for the preparation of quinolinedione derivatives, intermediate (3) (0.98 g, 4.5 mmol) and 7-Methoxy-2,4(1*H*,3*H*)-quinolinedione (0.85 gm, 4.5 mmol) were dissolved in EtOH and few drops of glacial acetic acid were added. The resulting product was then further purified by recrystallization from methanol to obtain the final compound **5c** as a light brown powder (yield: 68%). mp = 252–254 ^{0}C; 1H NMR (400 MHz, DMSO-*d*6): δ 10.81 (m, 1H), 9.98 (br s, 1H), 8.25 – 7.85 (m, 3H), 7.62 – 7.46 (m, 2H), 7.33 (t, 1H), 7.23 – 7.01 (m, 2H), 3.77 (s, 3H), 3.23 (s, 3H), 2.55 (br d , 2H). 13C NMR (DMSO-*d*6): δ 174.15, 169.47, 139.19, 138.22, 136.87, 135.91, 129.18, 128.92, 127.89, 126.97, 124.89, 124.58, 123.22, 117.65, 114.98, 54.05, 43.65, 36.17, 34.14, 25.65.

2.3. Docking Study

In this docking study, protein and ligand structures were prepared using the Schrodinger Maestro software release 2016-1 and Pymol software. For ligand preparation, the three-dimensional structures were protonated, partial charges were assigned, and energy minimization was carried out. The crystal structure of carbonic anhydrase IX (PDB ID: 4Z0Q) was obtained from the Protein Data Bank (PDB) and processed by removing water molecules and other non-essential components to facilitate ligand interaction. The protein structure was further refined by determining its potential, repairing damaged bonds, and adding missing protons. The active site of carbonic anhydrase IX was then identified using Schrodinger Maestro software release 2021-1, allowing the recognition of the specific amino acid residues which was expected to be involved in the binding site.

2.4. Cytotoxic Cell Line Study

All the cell lines, human colon carcinomas cells (HCT-116), human breast adeno-carcinomas cells (MCF-7) and normal cells (MCF10A), were obtained from the National Cell Bank of Mashhad. Cells were cultured in RPMI-1640 or DMEM media which was supplemented with 10% fetal bovine serum (FBS), 100 µg/mL streptomycin, and 100 U/mL penicillin. Cultures were stores at 37 °C in a humidified atmosphere with 5% CO_2, and subculturing was performed using phosphate-buffered saline (PBS) and trypsin/EDTA. Three-dimensional colonies were established under the same media and conditions as the monolayer cultures. Using the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to assessed the cell viability. For monolayer experiments, cells were seeded at a density of 1.4×10^4 cells/well in 96-well plates with 200 µL of fresh medium after trypsinization and counting. Test compounds were applied at concentrations ranging from 100 to 6.25 µg/mL and incubated for 24 h at 37 °C with 5% CO_2, followed by monolayer formation. After treated, the MTT solution (0.5 mg/mL in PBS) was added and then subjected to incubate for 4 h. The supernatant was then discarded, and dimethyl sulfoxide (DMSO, 100 µL/well) was added to

dissolve the formazan crystals, with gentle shaking at 37 °C until complete solubilization. Cell viability was quantified by measuring absorbance at 570 nm using an ELISA plate reader. IC_{50} values, defined as the compound concentration required to inhibit 50% of cell viability, were calculated from the dose–response curves.

3. Results

3.1. Chemistry

Synthesis of the quinolinedione derivatives (5a-c) are outlined in Fig.1. Methyl Sulfonyl aniline (1) was reacted with bromoacetyl bromide in dichloromethane and triethylamine to produce the corresponding methyl Sulfonyl bromoacetyl compound 2. Furthermore, the reaction of compound 2 with thiourea gave thiazole derivative 3 in good yields. The quinolinedione derivatives (5a-c) were synthesised by reaction between the substituted aromatic quinolinedione with thiazole derivative 3. The quinolinedione used was incorporated 3-substituted, 3-methyl, and 3-methoxy, as these groups usually exert good binding affinity and promising binding with the enzyme active site. Generally, more purification by column was not required because all the intended compounds were obtained in good purity results after recrystallization. 1H NMR and 13C NMR were used to characterized all the synthesized quinolinedione derivatives (5a-c).

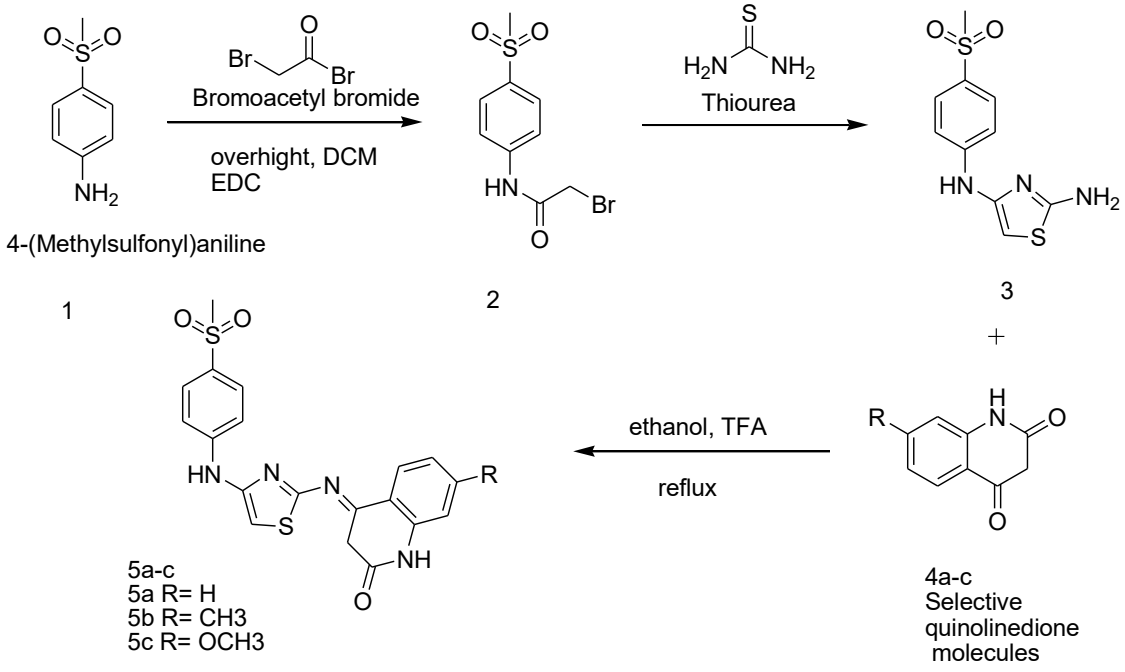


Figure1: Synthetic route for quinolinedione derivatives 5a–c and intermediates 2 and 3

3.2. Cytotoxicity Evaluation

The antitumor activity of quinolinedione derivatives 5a-c were tested by using MTT proliferation assays after exposure to the derivatives for 72 hours. To estimate the concentration of molecules required to inhibit cell growth by 50%, inhibitory concentration (IC50) was applied. Two type of human cancer cell lines were employed to determine the cytotoxic effects on cancer; the cell lines used to estimate the effect of quinolinedione derivatives 5a-c on cell growth as cancer inhibiter were HCT-116 (human colon carcinomas cells) and MCF-7 (human breast adeno-carcinomas cells), while MCF10A normal cells were used to evaluate toxicity in non-cancerous cells. Both acetazolamide and newly synthesized quinolinedione derivatives 5a-c of carbonic anhydrase inhibitors were tested. Acetazolamide was chosen as a reference compound due to its established role as a carbonic anhydrase inhibitor, with extensive research supporting its potential in anticancer therapy. Previous studies (Supran et al., 2000) and ongoing clinical trials highlight the promise of carbonic anhydrase inhibitors in suppressing tumor progression by selectively targeting enzymes involved in cancer growth and metastasis (Thiry et al., 2007). Most synthesised derivatives showed potent effect on cancer cell in anti-proliferative activity. Among the tested derivatives, 5c showed significant differences in activity compared to acetazolamide in HCT116 cell lines, as well as notable variations in the MCF10A normal cell line. The IC50 of the synthesized quinolinedione derivatives 5a-c and the standard are presented in **Table1**. From Table1, compound 5c generally showed a good selectivity between cancer cells (HCT-116 and MCF-7) and normal cell line (MCF-10A) which is around 9 times. As shown in the table compounds 5b and 5c demonstrate a close anti-proliferative activity to acetazolamide. Although, these compounds (5b and 5c) show a close selectivity to acetazolamide, however, it represent a promising compounds for further development.

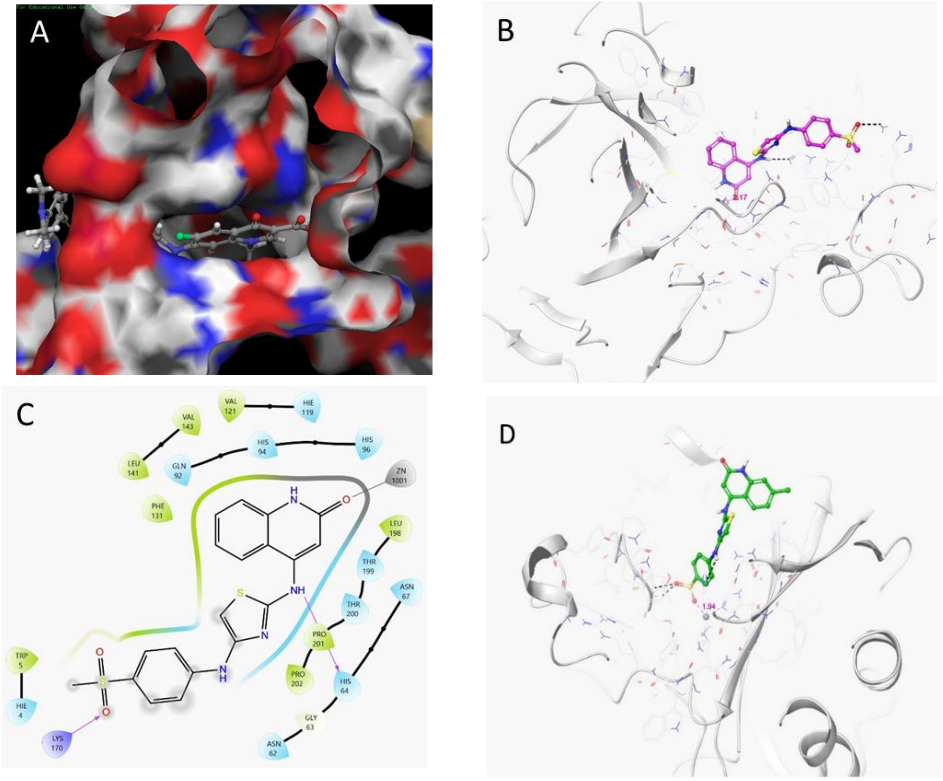
Table1: In Vitro Biological Evaluation of Quinolinedione Derivatives 5a–C Using the MTT Assay

			IC50(μM)	
Compounds	R	HCT-116	MCF-7	MCF-10A
Acetazolamide		1.69	2.45	16.53
5a	H	1.61	2.23	14.41
5b	CH_3	1.59	2.17	13.75
5c	OCH_3	0.98	1.39	9.94
MTT assays were performed after 72 h of exposure to quinolinedione derivatives 5a–c to evaluate their inhibitory effects on HCT-116 (colon cancer), MCF-7 (breast cancer), and MCF-10A (normal breast epithelial) cell lines.				

3.3. Docking Study

Examination the X-ray crystal structur of human carbonic anhydrase isoforms (hCA IX) (PDB 4Z0Q) immediately suggests a predicted binding site for quinolinedione derivatives 5a-c at the the catalytic site by connection with the Zn (II) ion, the negatively charged oxygen atom in the sulfonyl group improves binding

inside the catalytic site, as demonstrated by the compounds' deprotonated structural structures **Fig.2**. The quinolinedione derivatives binds in an extended conformation across the pockets, which would clearly stabilise the hCA IX complex. The methyl sulfonyl aniline rings reside in the hydrophobic pocket behind Thr199 residue. They form extensive hydrogen-bonding network with residues of the pockets that accommodate the sulfonyl aniline ring. The binding efficiency of the compounds was evaluated using S-score and RMSD (root mean square deviation) values. A lower S-score indicates stronger ligand–protein binding affinity, while RMSD reflects the positional deviation between the predicted and reference ligand conformations. Most of the synthesized molecules demonstrated stronger binding affinities compared with acetazolamide. Among them, compound 5c exhibited the lowest S-score, suggesting the most stable ligand–protein complex, whereas compound 5a showed the lowest RMSD value, indicating the closest alignment between both predicted and experimental poses. In contrast, acetazolamide displayed high S-score and RMSD values I compare to other tested compounds. Notably, the thiazole ring appears to play a key role in orienting the imine group and enhancing receptor interactions. The docking study highlights that compounds 5a-c, particularly 5c, establish favourable binding interactions within the CA IX active site.



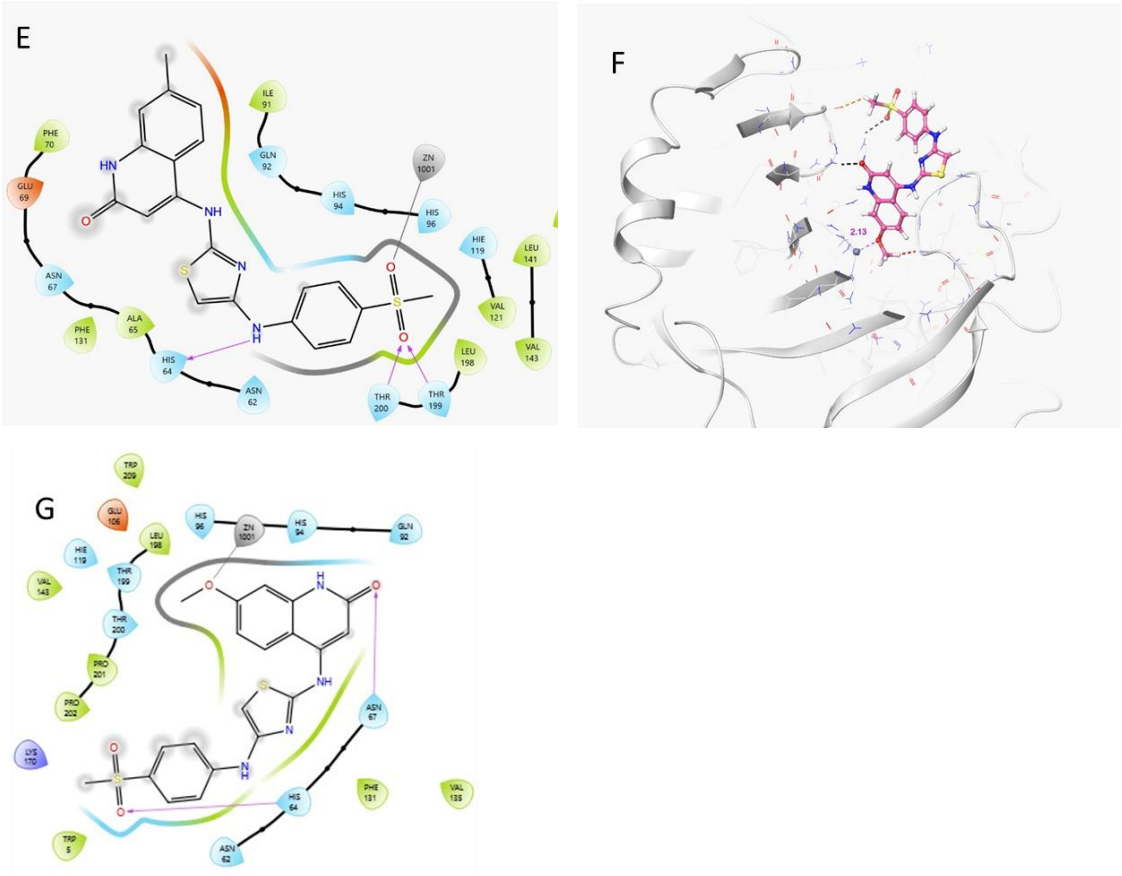


Figure2: Predicted Binding Conformation of Quinolinedione Derivatives 5a-C With The Carbonic Anhydrase Isoforms (Hca IX): (A) hCA IX hydrophobic pocket located at the interface; (B) binding mode of compound 5a in the active site of hCA IX; (C) representation of residues involved in the interaction with hCA IX, (D) binding of derivative 5b in the active site of hCA IX; (E) representation of residues involved in the interaction with hCA IX, (F) binding of derivative 5c in the active site of hCA IX; (G) representation of residues involved in the interaction with hCA IX, numbering according to hCA IX structure (PDB 4Z0Q); (The molecular docking studies were performed by using the Schrodinger Maestro software release 2021-1, to explore the binding ability of compound 5c to the hCA IX (PDB: 4Z0Q).

4. Discussion

The novel designed of quinolinedione derivatives (5a-c) were synthesised using a three-step synthetic procedure described before in Scheme 1. The Acylation of 4-(Methylsulfonyl)aniline which is nucleophiles with bromoacetyl bromide resulted in the formation of intermediate **2**, which was heated to reflux with thiourea to form the thiazole derivative **3**. This latter compound was the key intermediate for the preparation of final compounds **5a-c** via a Schiff base by the reaction with selected aromatic quinolinedione. The structures of the resulted compounds were established by different spectroscopic methods, such as 1H NMR, 13C NMR, and TLC. Generally, Protons peak belonging to the NH structure of aniline and quinolinedione were observed in all compounds (5a-c) as 1 hydrogen broad singlet between 11.6 and 10.13 ppm values. The quinolinedione used was incorporated 3-substituted, 3-methyl, and 3-methoxy, as these groups usually exert good binding affinity and promising binding with the enzyme active site. Based on the result of the activity studies, compound 5c was seen to have the highest activity among the synthesized compounds. To examine the binding modes of compound 5c with the enzyme active sites docking studies were carried out. The docking studies were performed, with the relevant enzyme active sites human carbonic anhydrase isoforms (hCA IX) (PDB 4Z0Q) which were retrieved form PDB data bank. Figures 1 were presented the obtained docking poses and the interaction between compound 5c and CA-IX isoenzyme. As can be seen in Figures 1, the methoxy moiety of quinolinedione group in compound 5c formed a single salt bridge with the zinc metal. A hydrogen bond is observed between the oxygen of amide group and the amine group of the Asn 67. The sulfone group forms a hydrogen bond with the amino group of the His 64. As mention before, the enzyme inhibitory activities of the synthesized compounds (5a-c) were investigated using MTT proliferation assays. Acetazolamide was used as the reference compound. Firstly, the % inhibition rates against cell growth were calculated after exposure to synthesis derivatives for 72 hours. Secondary, to determine the cytotoxic activity on cancer cell for the synthesised compounds, two type of human cancer cell lines were employed, HCT-116 (human colon carcinomas cells) and MCF-7 (human breast adeno-carcinomas cells), while MCF10A normal cells were used to evaluate toxicity in non-cancerous cells. Newly synthesized quinolinedione derivatives 5a-c and acetazolamide were tested for carbonic anhydrase inhibitory effect. All synthesised derivatives (5a-c) exhibited high effect on cancer cell in anti-proliferative activity, among these derivatives, compound 5c revealed a significant result in activity in compare with acetazolamide in both HCT116 and MCF-7 cell lines, as well as notable variations in the MCF10A normal cell line. IC50 of the compound 5c showed 0.98 μM and 1.39 μM in both HCT116 and MCF-7 cell lines respectively. This results one time better than the acetazolamide which exhibit 1.69 μM in HCT116 cell lines and 2.45 μM in MCF-7 cell lines. On the other hand, both compounds 5b and 5c showed a relatively similar results of anti-proliferative activity to

acetazolamide. Overall, all the synthesised compounds (5a-c) performed a good result which make them a promising compound for further development as a target for treatment of cancer.

5. Conclusion

In conclusion, the newly synthesized quinolinedione derivatives (5a–c), particularly compound 5c, demonstrated superior binding affinity toward CA IX and stronger antiproliferative activity against HCT116 and MCF-7 cell lines compared with the reference drug acetazolamide. These findings highlight quinolinedione scaffolds as promising and interesting lead compounds which can be used for the enhancing and development of new and novel CA IX-targeted anticancer compounds.

References

Alatorre, A. (1987). La 'Carta' de Sor Juana al P. Núñez (1682). *Nueva Revista de Filología Hispánica (NRFH)*, 35(2), pp.591–673. https://doi.org/10.24201/nrfh.v35i2.651.

Aleksandrs Pustenko, Alessio Nocentini, Gratteri, P., Bonardi, A., Igor Vozny, Raivis Žalubovskis and Supuran, C.T. (2020). The antibiotic furagin and its derivatives are isoform-selective human carbonic anhydrase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, [online] 35(1), pp.1011–1020. https://doi.org/10.1080/14756366.2020.1752201.

Alterio, V., Di Fiore, A., D'Ambrosio, K., Supuran, C.T. and De Simone, G. (2012). Multiple Binding Modes of Inhibitors to Carbonic Anhydrases: How to Design Specific Drugs Targeting 15 Different Isoforms? .*Chemical Reviews*, 112(8), pp.4421–4468. https://doi.org/10.1021/cr200176r

Angeli, A., Peat, T.S., Bartolucci, G., Nocentini, A., Supuran, C.T. and Carta, F. (2016). Intramolecular oxidative deselenization of acylselenoureas: a facile synthesis of benzoxazole amides and carbonic anhydrase inhibitors. .*Organic & Biomolecular Chemistry*, 14(48), pp.11353–11356 .https://doi.org/10.1039/c6ob02299e

Cronk, J.D., Endrizzi, J.A., Cronk, M.R., O'neill, J.W. and Zhang, K.Y.J. (2001). Crystal structure of E. coli β-carbonic anhydrase, an enzyme with an unusual pH-dependent activity. ,*Protein Science*, 10(5) .pp.911–922. https://doi.org/10.1110/ps.46301

Ghorab, M.M., Soliman, A.M., Bua, S. and Supuran, C.T. (2021). Biological evaluation, radiosensitizing activity and structural insights of novel halogenated quinazoline-sulfonamide conjugates as selective human carbonic anhydrases IX/XII inhibitors. ,*Bioorganic Chemistry*, 107(45) .p.104618. https://doi.org/10.1016/j.bioorg.2020.104618

Hen, N., Bialer, M., Yagen, B., Maresca, A., Aggarwal, M., Robbins, A.H., McKenna, R., Scozzafava, A. and Supuran, C.T. (2011). Anticonvulsant 4-Aminobenzenesulfonamide Derivatives with Branched-Alkylamide Moieties: X-ray Crystallography and Inhibition Studies of Human Carbonic Anhydrase Isoforms I, II, VII, and XIV. *Journal of Medicinal Chemistry*, 54(11), pp.3977–3981. https://doi.org/10.1021/jm200209n.

Kalinin, S., Malkova, A., Sharonova, T., Sharoyko, V., Bunev, A., Supuran, C.T. and Krasavin, M. (2021). Carbonic Anhydrase IX Inhibitors as Candidates for Combination Therapy of Solid Tumors. *International Journal of Molecular Sciences*, [online] 22(24), p.13405. https://doi.org/10.3390/ijms222413405.

Kumar, A., Kiran Siwach, Supuran, C.T. and Sharma, P.K. (2022). A decade of tail-approach based design of selective as well as potent tumor associated carbonic anhydrase inhibitors. ,(21)126 ,*43* .pp.105920–105920. https://doi.org/10.1016/j.bioorg.2022.105920

Lehtonen, J., Shen, B., Vihinen, M., Casini, A., Scozzafava, A., Supuran, C.T., Parkkila, A.-K., Saarnio, J., KiveläA.J., Waheed, A., Sly, W.S. and Parkkila, S. (2004). Characterization of CA XIII, a Novel Member of the Carbonic Anhydrase Isozyme Family *. *Journal of Biological Chemistry*, [online] 279(4), pp.2719–2727. https://doi.org/10.1074/jbc.M308984200.

Li, B., Liu, J., Cao, Y., Wang, Y., Wu, S., Hu, H., Xiao, X., Hu, J., Wang, Q., Wu, J., Luo, L., Liu, Y., Tang, Q., Xing, Y., Zhang, T., Zhou, J., Wang, L., Yang, J., Wang, J. and Wang, C. (2025). Multi-omics characterization of early chronic obstructive pulmonary disease. *Respiratory Research*, 26(1). https://doi.org/10.1186/s12931-025-03250-5.

Linkuvienė, V., Zubrienė, A., Manakova, E., Petrauskas, V., Baranauskienė, L., Zakšauskas, A., Smirnov, A., Gražulis, S., Ladbury, J.E. and Matulis, D. (2018). Thermodynamic, kinetic, and structural parameterization of human carbonic anhydrase interactions toward enhanced inhibitor design. .*Quarterly Reviews of Biophysics*, 51(53). https://doi.org/10.1017/s0033583518000082

Mboge, M., Mahon, B., McKenna, R. and Frost, S. (2018). Carbonic Anhydrases: Role in pH Control and Cancer. .*Metabolites*, 8(1), p.19. https://doi.org/10.3390/metabo8010019

Mehta, S., Pavana, R.K., Yogeeswari, P., Sriram, D. and Stables, J. (2007). Heteroaryl-substituted Semicarbazones: Synthesis and Anticonvulsant Activity of N-(3-Methylpyridin-2-yl)-substituted Semicarbazones. .*ChemInform*, 38(7). https://doi.org/10.1002/chin.200707220

Patil, J.V., Umar, S., Soni, R., Soman, S.S. and Balakrishnan, S. (2022). Design, synthesis and anticancer activity of amide derivatives of substituted 3-methyl-benzofuran-2-carboxylic acid. *Synthetic .Communications*, 53(3), pp.217–233. https://doi.org/10.1080/00397911.2022.2160648

Pawel Swietach, Shalini Patiar, Supuran, C.T., Harris, A.L. and Vaughan-Jones, R.D. (2009). The Role of Carbonic Anhydrase 9 in Regulating Extracellular and Intracellular pH in Three-dimensional Tumor Cell Growths. *Journal of Biological Chemistry*, 284(30), pp.20299–20310. https://doi.org/10.1074/jbc.m109.006478.

Rosen, I.M., Aurora, R.N., Kirsch, D.B., Carden, K.A., Malhotra, R.K., Ramar, K., Abbasi-Feinberg, F., Kristo, D.A., Martin, J.L., Olson, E.J., Rosen, C.L., Rowley, J.A. and Shelgikar, A.V. (2019). Chronic Opioid Therapy and Sleep: An American Academy of Sleep Medicine Position Statement. *Journal of Clinical Sleep Medicine*, 15(11), pp.1671–1673. https://doi.org/10.5664/jcsm.8062.

Sani, A., Dalhatu, M., Masa-Ibi, E. and Aliyu, A. (2020). Solutions by Optimization of the 2-Dimensional Heat Conductivity Problem on Grid Machines using C++ and OpenMP. *International Journal of Computer Applications*, 176(13), pp.1–11. https://doi.org/10.5120/ijca2020920007.

Scozzafava, A., Owa, T., Mastrolorenzo, A. and Supuran, C. (2003). Anticancer and Antiviral Sulfonamides. *Current Medicinal Chemistry*, 10(11), pp.925–953. https://doi.org/10.2174/0929867033457647.

Supuran, C. (2008). Carbonic Anhydrases An Overview. *Current Pharmaceutical Design*, 14(7), pp.603–614. https://doi.org/10.2174/138161208783877884.

Supuran, C.T., Alterio, V., Di Fiore, A., D' Ambrosio, K., Carta, F., Monti, S.M. and De Simone, G. (2018). Inhibition of carbonic anhydrase IX targets primary tumors, metastases, and cancer stem cells: Three for the price of one. .*Medicinal Research Reviews*, 38(6), pp.1799–1836 .https://doi.org/10.1002/med.21497

Supuran, C.T., Briganti, F., Scozzafava, A. and Ilies, M.A. (2000). Protease Inhibitors: Part 4. Synthesis of Weakly Basic Thrombin Inhibitors Incorporating Pyridinium-Sulfanilylaminoguanidine Moieties. .*Journal of Enzyme Inhibition*, 15(4), pp.335–356 .https://doi.org/10.1080/14756360009040692

Thiry, A., Dogne, J.-M., Supuran, C. and Masereel, B. (2007). Carbonic Anhydrase Inhibitors as Anticonvulsant Agents. *Current Topics in Medicinal Chemistry*, 7(9), pp.855–864. https://doi.org/10.2174/156802607780636726.

Wang, Y., Wu, S., Li, A., Huan Lv, Ji, X., Hu, Y. and Wang, S. (2024). Nanobody-based Food Allergen Surveillance: Current Status and Prospects. *Food quality and safety*, 35(12). https://doi.org/10.1093/fqsafe/fyae018.

Wong, M.C.S., Jiang, J.Y., Goggins, W.B., Liang, M., Fang, Y., Fung, F.D.H., Leung, C., Wang, H.H.X., Wong, G.L.H., Wong, V.W.S. and Chan, H.L.Y. (2017). International incidence and mortality trends of liver cancer: a global profile. .*Scientific Reports*, 7(1). https://doi.org/10.1038/srep45846