Kufa Journal of Pharmaceutical Sciences

© 2026 KJPS | Volume 3 | Issue 1 | Print ISSN : 3005-7833

In silico, design, synthesis and evaluation of new naphthyridine-dione analogues as potential candidates for carbonic anhydrase inhibitors

Akeel Abo Alard *[1], Atheer Mohammed Jasim[2], Ammar Azzam[3]

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

[2] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Kufa, Najaf, Iraq.

[3] Department of Pharmacology and Toxicology, College of Pharmacy, University of Babylon, Babil, Iraq.

ABSTRACT

Hypoxia in many kinds of solid tumours represent the main factor to induce Carbonic anhydrase IX (CAIX) and thus supports tumour pH homeostasis under low-oxygen condition. In mouse models, pharmacological CAIX inhibition has been shown to delay tumour growth, reduce metastatic spread, and restrain cancer stem cell expansion. Accordingly, targeting carbonic anhydrase overexpression—especially CAIX—represents a promising anticancer approach.

Based on this premise, a new series of naphthyridinedione derivatives (5a to 5c) was designed, synthesised, and evaluated in cell lines (HCT116 and MCF-7), with acetazolamide (ACZ) used as a control compound. To prioritise the most promising candidates, molecular docking (Schrödinger Maestro 2021-1 and PyMOL) was performed prior to synthesis to select compounds with the highest predicted binding. Antiproliferative effects were then measured using an MTT assay in both cell models. Relative to acetazolamide, compound 5c demonstrated substantially greater antiproliferative potency in HCT116 (IC_{50} = 1.08 μM) and MCF-7 cells (IC_{50} = 1.49 μM) compared with acetazolamide (IC_{50} = 1.79 μM and 2.55 μM, respectively). In contrast, compounds 5a and 5b exhibited cytotoxicity broadly comparable to acetazolamide. Collectively, these findings suggest that naphthyridinedione derivatives (5a–c) provide a useful starting scaffold for further anticancer drug development.

Docking analyses further indicated that 5c achieved more favourable S-scores than acetazolamide, consistent with stronger predicted binding to the CAIX active site. The methoxy substituent and the naphthyridinedione core may contribute to increased conformational flexibility and improved receptor interactions. Overall, these naphthyridinedione derivatives (5a–c) show notable CAIX-related inhibitory potential alongside measurable cytotoxicity, supporting continued evaluation as anticancer lead compounds.

Keywords: Naphthyridinedione; carbonic anhydrase; docking study; cytotoxicity.

I. INTRODUCTION

Generally, cancer remains a major universal health issue, causing widespread deaths and reversing improvements in life expectancy [1]. In case of breast cancer, many patients ultimately under therapeutic resistance although the availability of multiple treatment ways, resulting in failure in treatment and progression in most cases of disease [2]. Meeting this challenge will require continued research and innovative treatment

KJPS | E- mail: phar.kjps@uokufa.edu.iq | Received: 12 December 2025 | Accepted: 16 Jan 2026

*Corresponding author E-mail: akeela.aboalard@uokufa.edu.iq

approaches, including strategies that target carbonic anhydrase enzymes.

Carbonic anhydrases (CAs) catalyse the reversible conversion between CO_2 and bicarbonate and are widely conserved and essential in kind of bacteria, and some of eukaryotic [3]. Generally, the $CO_2/HCO_3^-/H^+$ equilibrium drives many physiologically and disease-related processing, including many of biological pathways [4]. Number of mammalian CA isoenzymes have been identified, including cytosolic (CA I- XIII), and one isoform CA (VI) [5]. These enzymes have attracted increasing interest as tumour candidates. Different α-CA isoforms express by humane that differ in catalytic activity, tissue localization, and biological function; twelve of these (hCA I–XIV) are enzymatically active [6]. Notably, HIF-1 regulate the CAIX and CAXII in tumours isoforms that are minimal in most normal tissue driving acidification of the tumour and promoting tumour survive and proliferation in concert with glycolysis [7]. Therefore, lowering the express of CA IX/XII may therefore enhance the efficacy of chemotherapy while offering tumour selectivity that could reduce systemic toxicity [8–10].

Most classical CA inhibitors (CAIs) act as zinc binders, by coordinating the active-site Zn^{2+}, which adopts trigonal bipyramidal or tetrahedral coordination geometries upon inhibitor binding. Sulfonamides are the archetypal carbonic anhydrase inhibitors (CAIs) that act via this mechanism [10–13]. Sulfanilamide, first reported in 1940, was the earliest organic inhibitor of carbonic anhydrase [14]. Subsequent studies established that sulphonamides where as a primary aromatic or heterocyclic act broadly in inhibit CA isoforms, stimulating drug development initially for diuretic and antiglaucoma indications [15], with acetazolamide remaining the archetypal agent. Over time, sulfonamide CAIs have been adapted for additional therapeutic uses—including antiepileptic, anti-obesity, and anticancer applications—by targeting specific mammalian isoforms relevant to each indication [16]. Sulfonamides as aromatic or as heterocyclic having ureido or thioureido functionalities have been widely investigated as CA inhibitor [17], with early examples demonstrating selectivity for different isoform type of hCA I, II, or IV, and some compounds showing notable antitumour and antimetastatic effects in vivo [18].

Based on these advantage features, we report a few groups of incorporating an linker between the sulfonamid group and zinc-binding group and a tail molecule. Using a synthetic route, these analogues were assessed against different CA isoforms: I and II (cytosolice) and IX and XII [19]. Moreover, metal complexes based on sulfonamide CA inhibitors have been reported to display 10–100-fold greater potency than the parent sulfonamides [20]. This may reflect a dual mode of action: partial dissociation at low concentrations can generate sulfonamide anions that coordinate the active-site Zinic (II), while the liberated ions may disrupt the residues of proton-shuttle which is essential for catalysis [21]. Based on these vision, a new analogues were designed that incorporate these advantageous properties. Analysis docking studies supported their promise, giving a rationale to advance synthesis and experimental evaluation.

II. METHODS AND MATERIAL

Chemistry of synthesised naphthyridinedione analogues

Regards the starting chemicals, reagentts, and solventts, all were obtained from commercial suppliers, including Merck, abor, and Thermo Fisher. Starting materials such as methylsulfonylaniline, chloroacetyl chloride, thiourrea, and naphthyridinedione derivatives in addition to all solvents used in this study were collected and used directly and the reaction process was checked by TLC. All analogues structurally characterised by NMR ^1H and ^13C spectroscopy. Furthermore, NMR spectra were used on a Bruker spectrometer operating at 400 MHz (^1H) and 100 MHz (^13C). Chemical shifts (δ) are recorded in (ppm), and used as the standard. Mp were measured by using a Stuart mp with glass capillaries.

Synthesis of compound 2: (2-Chloro-[4-(methylsulfonyl)phenyl]acetamide)

A solution of methylsulfonylaniline (0.43 g, 2.5 mmol) in dichloromethane (20 mL) was treated with 2-chloroacetyl chloride (1 mL, 7.5 mmol) and TEA (1.7 mL, 12 mmol). The ice bath was used to cooled the mixture with continued stirred for 30 min and then left to warm to rt and continue stirred for 1.5 h. Reaction progress was monitored by TLC. Upon end of reaction, EtAc was added and the resulting mixture was washed with saturated NaHCO3 solution, water, and NaCl solution sequentially. The organic layer was dried over anhydrous MgSO4, then filtration, and then concentrated under reduced pressure. The crude final compound

purified through washing with Et2O to give the final analogue as a dark brown ppt (85%). Mp = 161–164 ^{0}C, ^{1}H NMR (ppm): δ 8.44 (s, 1H), 7.99 – 7.93 (m, 2H), 7.81 – 7.85 (m, 2H), 4.31 (s, 2H), 3.11 (s, 3H). TLC: R_f 0.45[a].

Synthesis of compound 3: (N-(4-methylsulfonylphenyl-thiazole-diamine)

A mixture of compound 2 (0.4 g, 1.5 mmol) and thiourea (0.11 g, 1.5 mmol) in anhydrous acetone (50 mL) was left under the refluxe for 10 h. The reaction process was checked through TLC. Upon finishing, evaporate the solvent and the resulting ppt was left into ice-cold water. The crude product was cleaned by recrystallisation from methanol. The resulting product was then washed sequentially with saturated NaHCO3 solution, and water, followed by filtration and drying. A final recrystallisation from ethanol/water afforded the final compound as a brown solid (76% yield). mp = 112–115 ^{0}C, ^{1}H NMR (ppm): δ 10.10 (s,1H), 8.35-7.30 (m, 4H, Ar-H and1H, s, CH of thiazole), 5.71 (s, 2H), 3.40 (s, 3H). TLC: R_f 0.57[a].

Preparation of naphthyridinedione derivatives 5a-c: General procedure

Selected naphthyridinedione molecule (2 mmol) with few drops of glacial acetic acid were mixed together with a mixture of compound 3 (0.45 g, 2 mmol) in ETOH (10 mL). The reaction mixture was refluxed for 10 h, then cooled and left onto crushed ice. The resulting ppt was filtrated and EtAc was added. The organic phase was washed with saturated NaHCO3 solution, water, and NaCl sequentially. The organic phase was dried by using MgSO4, filtered, and evaporated. The crude residue was further purified by recrystallisation from methanol to afford the final analogues (5a–c). Melting points and spectral data for each target derivative are provided in the synthesis section below [24,25].

Synthesis of compound 5a: (N-4-methylsulfonylphenyl amino thiazol-2-yl) imino)-3,4-dihydronaphthyridinedione

Follow up the general method for the synthesis of naphthyridinedione analogues, compound 3 (0.45 g, 2 mmol), few drops of glacial acetic acid and dihydroaphthyridinedione (0.35 gm, 2 mmol) were dissolved in EtOH. The crude product was additionally cleaned by recrystallisation from CH3OH to yield the final analogue as a white powder (69%). mp = 222–225 ^{0}C; ^{1}H NMR (ppm): δ 10.33 (br s, 1H), 10.11 (br s, 1H), 8.35 – 7.80 (m, 6H), 7.18 – 6.99 (m, 2H), 3.4 (s, 3H), 2.80 (d, 2H). ^{13}C NMR (ppm): δ 171.07, 167.50, 139.8, 138.1, 136.25, 136.42, 129.98, 128.12, 127.99, 125.77, 124.8, 123.4, 122.82, 118.65, 116.5, 53.75, 41.95, 31.8, 26.2.

Synthesis of compound 5b: 7-methyl-4-methylsulfonylphenyl aminothiazol-2-yl)imino)-3,4-dihydronaphthyridinedione

Follow up the general method for the synthesis of naphthyridinedione analogues, compound 3 (0.45 g, 2 mmol), few drops of glacial acetic acid and 7-Methyl-naphthyridinedione (0.35 gm, 2 mmol) were dissolved in EtOH. The crude product was additionally purified by recrystallisation from CH3OH to yield the final analogue as a light brown ppt (64%). mp = 222–224; ^{1}H NMR (ppm): δ 11.51 (br s, 1H), 9.81 (br s, 1H), 7.85 – 7.95 (d, 2H), 7.82 – 7.55 (d, 1H), 7.28 – 6.76 (m, 4H), 6.58 (s, 2H), 5.11 - 4.88 (br s , 6H). ^{13}C NMR (ppm): δ 168.57, 167.52,139.22, 137.34, 129.88, 128.25, 126.43, 119.77, 87.88, 50.45, 41.88.

Synthesis of compound 5c: 7-methoxy-4-(methylsulfonylphenyl aminothiazol-2-yl)imino)-3,4-dihydronaphthyridinedione

Follow up the general method for the synthesis of naphthyridinedione analogues, compound 3 (0.45 g, 2 mmol), few drops of glacial acetic acid and 7-Metoxy-naphthyridinedione (0.35 gm, 2 mmol) were dissolved in EtOH. The crude product was additionally cleaned by recrystallisation from CH3OH to yield the final analogue as a light beige ppt (81%). mp = 212–215 ^{0}C; ^{1}H NMR (ppm): δ 10.11 (br s, 1H), 9.99 (br s, 1H), 7.88 –7.57 (d, 4H), 7.32 – 6.99 (m, 4H), 6.77 (s, 2H), 5.11-4.88 (br s , 3H), 4.21-3.88 (m, 3H). ^{13}C NMR (ppm): δ 169.89, 168.78, 139.89, 138.21, 128.88, 128.52, 124.57, 118.43, 89.54, 52.65, 40.78.

Docking study

For molecular docking, protein preparation and ligand preparation using Schrödinger Maestro (release 2021-1). Ligands were generated as three-dimensional models, protonated, assigned partial charges, and energy-minimised prior to docking. The X-ray crystal structure of CAIX (PDB ID: 4Z0Q) was achieved from the PDB and followed by reducing crystallographic H2O to enable ligand–protein interactions. The protein was subsequently refined by assigning appropriate potentials,

missing or damaged bonds, and added hydrogen atoms. The CAIX active site was then defined in Maestro and visualised in PyMOL, allowing identification of key amino acid residues comprising the binding pocket.

Cytotoxic study

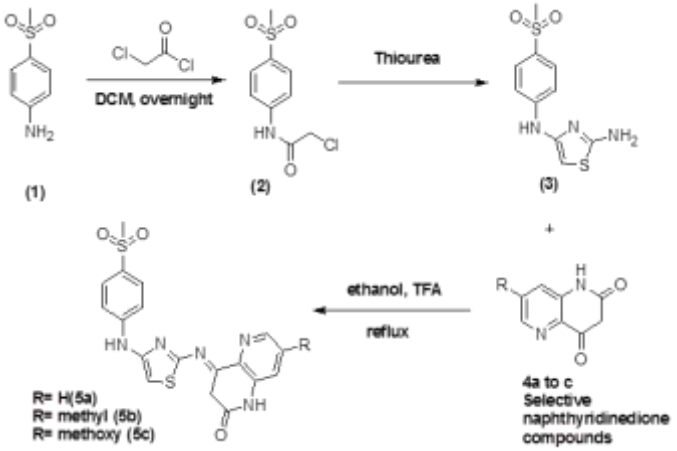
HCT-116, MCF-7, and MCF10A cells lines were achieved from the National Cell Bank. Cells were maintained in RPMI or DMEM fluid with 10% FBS, 100 μg/mL streptomycin, and 100 U/mL penicillin, and incubated at 37 °C in incubator containing 5% CO_2. Cells were routinely passaged using phosphate-buffered saline (PBS) and EDTA. TDC were generated using the same medium and culture of conditions as the corresponding monolayer cultures.

The MTT assay was used to assess Cell viability. Monolayer experiments, seeded the cells in 96-well plates at 1.4×10^4 cells per well in 200 μL of complete medium after trypsinisation and counting. Following monolayer formation, cells were treated with test compounds (100–6.25 μg/mL) and incubated for 24 h at 37 °C under 5% CO_2. MTT solution (0.5 mg/mL in PBS) was then added and plates were incubated for an additional 4 h. The supernatant was discarded and formazan crystals were solubilised with dimethyl sulfoxide (DMSO; 100 μL per well) with gentle shaking at 37 °C until fully dissolved. Absorbance was recorded at 570 nm using an ELISA plate reader, and IC_{50} values were derived from the curve of dose–response.

III.RESULTS AND DISCUSSION

General chemistry

The synthesis of naphthyridinedione analogues (5a to 5c) is summarised in scheme 1. Methylsulfonyl aniline compound used as starting point and was treated with chloroacetyl chloride in DCM with TEA to give methylsulfonyl chloroacetyl intermediate (2). Then, compound 2 react with thiourea to produce thiazole compound (3). Analogues (5a–c) were then obtained by coupling thiazole intermediate 3 with appropriately substituted aromatic naphthyridinedione. Naphthyridinedione analogues bearing 3-substituted, 3-methyl, or 3-methoxy groups were selected, as these substituents have been associated with favourable binding affinity and productive connection between the active site of such protein. Column chromatography was unnecessary, since all target final products were isolated at high purity (>95%) following recrystallisation. The structures of naphthyridinedione derivatives (5a to 5c) were confirmed by NMR both 1H and 13C spectroscopy.



Scheme 1: Synthetic route of naphthyridinedione derivatives 5a to 5c and compounds 2 and 3.

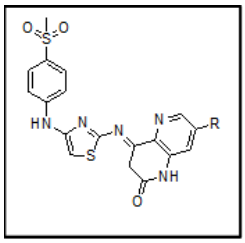
Evaluate of cytotoxicity

The antitumour action of naphthyridinedione analogues 5a to 5c was evaluated using an MTT proliferation assay following 72 h exposure. Half-maximal inhibitory concentrations (IC_{50}) were calculated to estimate the concentration of compound that used to lower 50% of cell viability. Cytotoxicity was assessed in two type of human cancer cell line which are HCT-116 colon carcinoma and MCF-7 (breastt cancer), while MCF-10A (normal breastt epithelial cells) were included to assessment the effects in non-malignant cells. Both acetazolamide and the newly synthesised naphthyridinedione analogues 5a to 5c were tested. Acetazolamide served as the control compound owing to its well-established inhibitory activity for CA, and the substantial body of work supporting the anticancer potential of this inhibitor class. Prior research studies and clinical investigations further as a strategy to suppress tumour progression by acting on enzymes implicated in cancer growing process and metastasis pathway [22, 23].

Overall, most synthesised analogues displayed notable antiproliferative activity in the cancer cell models. Among the derivatives, 5c showed a marked difference in activity relative to acetazolamide in HCT-116 cells, and also exhibited distinct behaviour in the MCF-10A cell line. The IC_{50} values for all analogues and the reference standard are summarised in table 1. Notably, analogue 5c demonstrated approximately nine-fold selectivity for cancer HCT-116 and MCF-7 cells over the normal MCF-10A cell line. As explains in table 1, analogue 5b and 5c exhibited antiproliferative activity comparable to acetazolamide. Although their selectivity

was also broadly similar to that of acetazolamide, both compounds—particularly 5c—represent promising leads for further optimisation and development research.

Table 1: Pharmacological evaluation results of cytotoxic activity for naphthyridinedione analogues 5a to 5c.



		IC_{50} (µM)		
Analogues	**R group**	**-HCT-116-**	**-MCF-7-**	**-MCF-10-**
ACZ		**1.79**	**2.55**	**16.63**
Analogue 5a	**H**	**1.71**	**2.33**	**14.51**
Analogue 5b	**Methyl**	**1.69**	**2.27**	**13.85**
Analogue 5c	**Methoxy**	**1.08**	**1.49**	**9.98**

Docking for naphthyridinedione derivatives 5a–c:

Inspection of the PDB ID: 4Z0Q protein suggests that naphthyridinedione derivatives 5a–c are likely to bind within the catalytic pocket through coordination with the zinc (II) ion. In particular, the charged oxygen of the sulfonyll group is predicted to enhance binding activity within the pocket of active site, consistent with the deprotonated forms of these compounds (Figure 1). The naphthyridinedione derivatives adopt an extended conformation spanning the active-site pockets, which would be expected to stabilise the hCA IX–ligand complex.

The methylsulfonyl aniline moiety is positioned within the hydrophobic region adjacent to Thr199, and the ligands form an extensive hydrogen-bonding network with residues lining the pocket that accommodates this ring system. Binding performance was assessed using S-score and RMSD (root mean square deviation) metrics. In this context, more favourable (lower) S-scores indicate stronger predicted ligand–protein affinity, whereas RMSD values reflect the deviation of the predicted pose from a reference ligand conformation. Most of the synthesised derivatives showed stronger predicted binding than acetazolamide. Notably, compound 5c achieved the most favourable (lowest) S-score, consistent with formation of stable complex, while analogue 5a produced the low value of RMSD, indicating that this is closest agreement between suppose and reference binding poses. By contrast, control compound (acetazolamide) exhibited the least favourable elevate in S-score and value of RMSD.

This docking results further suggest that the thiazole ring contributes importantly to ligand positioning, helping to orient the imine group and strengthen interaction with the receptor. Overall, these study indicates that quinolinedione derivatives 5a to 5c—particularly 5c—form preferable interactions in hCAIX active site and are therefore promising candidates for further investigation.

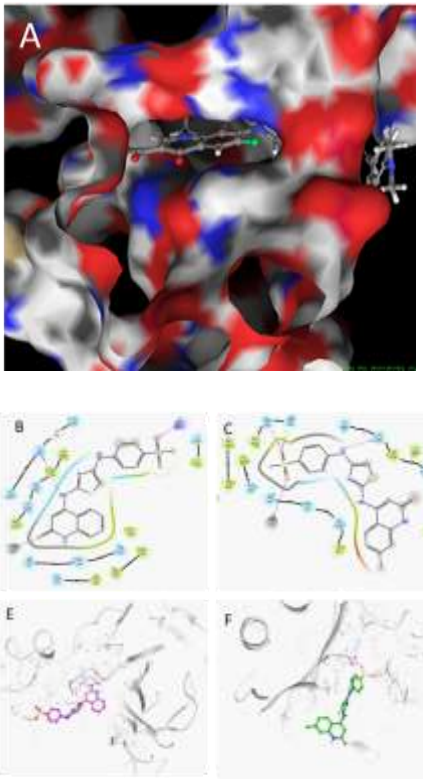


Figure 1: Naphthyridinedione predicted binding conformation with the CA isoforms (IX): (A) CAIX hydrophobic pocket; (B, C, D) representation of residues which could be involved in the interaction with CAIX for analogues 5a to 5c respectively; (E, F, G) analogues 5a to 5c binding mode; the a.a numbering was conducted based to CAIX (PDB 4Z0Q); (Schrodinger Maestro software release 2021-1).

IV.CONCLUSION

In summary, the synthesised naphthyridinedione analogues (5a to 5c), with analogue 5c as the most active candidate—showed enhanced predicted binding to CAIX and greater cytotoxic effects in HCT116 and MCF-7 cells compared with the control inhibitor acetazolamide. Collectively, these results identify the naphthyridinedione scaffold as a promising starting

point for developing new CA IX-targeted anticancer agents.

V. REFERENCES

1. Zhang YN, Xia KR, Li CY, Wei BL, Zhang B. Review of Breast Cancer Pathologigcal Image Processing. *Biomed Res Int*. 2021;2021:1994764. Published 2021 Sep 20. doi:10.1155/2021/1994764
2. Maughan KL, Lutterbie MA, Ham PS. Treatment of breast cancer. *Am Fam Physician*. 2010;81(11):1339-1346.
3. Mallia A, Brocca L, Papaianni GG, Banfi C. Carbonic anhydrases inhibition in the management of cardiovascular and cardiometabolic disorders. *Biomed Pharmacother*. 2025;190:118396. doi:10.1016/j.biopha.2025.118396
4. Capasso C. Carbonic Anhydrases: A Superfamily of Ubiquitous Enzymes. *Int J Mol Sci*. 2023;24(8):7014. Published 2023 Apr 10. doi:10.3390/ijms24087014 A., et al. (2020).
5. Supuran CT, Capasso C. Overview on fungal and protozoan carbonic anhydrases. *Enzymes*. 2025;57:1-20. doi:10.1016/bs.enz.2025.05.001
6. D'Ambrosio K, Di Fiore A, Alterio V, et al. Multiple Binding Modes of Inhibitors to Human Carbonic Anhydrases: An Update on the Design of Isoform-Specific Modulators of Activity. Chem Rev. 2025;125(1):150-222. doi:10.1021/acs.chemrev.4c00278
7. Menendez GB, Giovannuzzi S, Bonardi A, Nocentini A, Gratteri P, Supuran CT. Exploration of Aromatic Hydrazides as Inhibitors of Human Carbonic Anhydrases. *Arch Pharm (Weinheim)*. 2025;358(4):e202400963. doi:10.1002/ardp.202400963
8. Fuentes-Aguilar A, Merino-Montiel P, Montiel-Smith S, et al. 2-Aminobenzoxazole-appended coumarins as potent and selective inhibitors of tumour-associated carbonic anhydrases. *J Enzyme Inhib Med Chem*. 2022;37(1):168-177. doi:10.1080/14756366.2021.1998026
9. Supuran CT. Carbonic anhydrase inhibitors as emerging agents for the treatment and imaging of hypoxic tumors. Expert Opin Investig Drugs. 2018;27(12):963-970. doi:10.1080/13543784.2018.1548608
10. Parkkinen J, Bhowmik R, Tolvanen M, et al. Mycobacterial β-carbonic anhydrases: Molecular biology, role in the pathogenesis of tuberculosis and inhibition studies. *Enzymes*. 2024;55:343-381. doi:10.1016/bs.enz.2024.05.012
11. Čapkauskaitė E, Zubrienė A, Paketurytė V, Timm DD, Tumkevičius S, Matulis D. Thiazole-substituted benzenesulfonamides as inhibitors of 12 human carbonic anhydrases. *Bioorg Chem*. 2018;77:534-541. doi:10.1016/j.bioorg.2018.02.004
12. Vaškevičienė I, Paketurytė V, Pajanok N, et al. Pyrrolidinone-bearing methylated and halogenated benzenesulfonamides as inhibitors of carbonic anhydrases. Bioorg Med Chem. 2019;27(2):322-337. doi:10.1016/j.bmc.2018.12.011
13. Vaškevičienė I, Paketurytė V, Pajanok N, et al. Pyrrolidinone-bearing methylated and halogenated benzenesulfonamides as inhibitors of carbonic anhydrases. *Bioorg Med Chem*. 2019;27(2):322-337. doi:10.1016/j.bmc.2018.12.011
14. Smirnovienė J, Smirnov A, Zakšauskas A, et al. Switching the Inhibitor-Enzyme Recognition Profile via Chimeric Carbonic Anhydrase XII. ChemistryOpen. 2021;10(5):567-580. doi:10.1002/open.202100042
15. Ibrahim DA, Lasheen DS, Zaky MY, et al. Design and synthesis of benzothiazole-6-sulfonamides acting as highly potent inhibitors of carbonic anhydrase isoforms I, II, IX and XII. Bioorg Med Chem. 2015;23(15):4989-4999. doi:10.1016/j.bmc.2015.05.019
16. Alizadeh SR, Hashemi SM. Development and therapeutic potential of 2-aminothiazole derivatives in anticancer drug discovery. Med Chem Res. 2021;30(4):771-806. doi:10.1007/s00044-020-02686-2
17. Leitans J, Sprudza A, Tanc M, et al. 5-Substituted-(1,2,3-triazol-4-yl)thiophene-2-sulfonamides strongly inhibit human carbonic anhydrases I, II, IX and XII: solution and X-ray crystallographic studies. *Bioorg Med Chem*. 2013;21(17):5130-5138. doi:10.1016/j.bmc.2013.06.041
18. Saluja AK, Tiwari M, Vullo D, Supuran CT. Substituted benzene sulfonamides incorporating 1,3,5-triazinyl moieties potently inhibit human carbonic anhydrases II, IX and XII. *Bioorg Med Chem Lett*. 2014;24(5):1310-1314. doi:10.1016/j.bmcl.2014.01.048
19. Garaj V, Puccetti L, Fasolis G, et al. Carbonic anhydrase inhibitors: novel sulfonamides incorporating 1,3,5-triazine moieties as inhibitors of the cytosolic and tumour-associated carbonic anhydrase isozymes I, II and IX. *Bioorg Med Chem Lett*. 2005;15(12):3102-3108. doi:10.1016/j.bmcl.2005.04.056
20. Puccetti L, Fasolis G, Cecchi A, et al. Carbonic anhydrase inhibitors: synthesis and inhibition of cytosolic/tumor-associated carbonic anhydrase isozymes I, II, and IX with sulfonamides incorporating thioureido-sulfanilyl scaffolds. Bioorg Med Chem Lett. 2005;15(9):2359-2364. doi:10.1016/j.bmcl.2005.02.087
21. Winum JY, Rami M, Scozzafava A, Montero JL, Supuran C. Carbonic anhydrase IX: a new druggable target for the design of antitumor agents. *Med Res Rev*. 2008;28(3):445-463. doi:10.1002/med.20112
22. Guler OO, De Simone G, Supuran CT. Drug design studies of the novel antitumor targets carbonic anhydrase IX and XII. *Curr Med Chem*. 2010;17(15):1516-1526. doi:10.2174/092986710790979999
23. Akocak S, Güzel-Akdemir Ö, Kishore Kumar Sanku R, et al. Pyridinium derivatives of 3-aminobenzenesulfonamide are nanomolar-potent inhibitors of tumor-expressed carbonic anhydrase isozymes CA IX and CA XII. *Bioorg Chem*. 2020;103:104204. doi:10.1016/j.bioorg.2020.104204
24. Güzel O, Maresca A, Scozzafava A, Salman A, Balaban AT, Supuran CT. Carbonic anhydrase inhibitors. Synthesis of 2,4,6-trimethylpyridinium derivatives of 2-(hydrazinocarbonyl)-3-aryl-1H-indole-5-sulfonamides acting as potent inhibitors of the tumor-associated isoform IX and XII. *Bioorg Med Chem Lett*. 2009;19(11):2931-2934. doi:10.1016/j.bmcl.2009.04.068
25. Sarikaya B, Ceruso M, Carta F, Supuran CT. Inhibition of carbonic anhydrase isoforms I, II, IX and XII with novel

Schiff bases: identification of selective inhibitors for the tumor-associated isoforms over the cytosolic ones. *Bioorg Med Chem*. 2014;22(21):5883-5890. doi:10.1016/j.bmc.2014.09.021