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Original Article

Design, synthesis, and molecular docking studies of *N*-substituted sulfonamides as potential anticancer therapeutics

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الملخص

أهداف البحث: كان الهدف من هذه الدراسة هو تصميم وإتاحة السلفوناميدات المضادة للسرطان عن طريق اقتران الأمينات وكلوريد الدانسيل مع البدائل الإستراتيجية. وباستخدام تقنيات الرنين المغناطيسي النووي ومطياف الكتلة، تم تشخيص الهياكل المركبة. أيضا، تم استخدام تحليل الالتحام الجزيئي لمعرفة مدى ارتباط السلفوناميدات المصنوعة حديثا بـ 5-أسيتاميدو-4،3،1-ثيادايازول-2-سلفوناميد، وهو هدف دوائي محتمل. استلزم هذا التقييم مقارنة ارتباطاتها الملزمة بتلك الموجودة في عقار الأسيتازولاميد المعروف.

طريقة البحث: تم تصنيع السلفوناميدات عن طريق اقتران الأمينات وكلوريد الدانسيل في ظل ظروف مواتية للغاية. أدرجت السلفوناميدات المصممة بدائل ذات موقع استراتيجي لنقل خصائص بيولوجية متنوعة. تم التحقق من صحة الهياكل المركبة باستخدام الرنين المغناطيسي النووي وأطياف الكتلة. تم إجراء تحليل الالتحام الجزيئي لتقييم الارتباطات الملزمة للسلفوناميدات المركبة مع الهدف الدوائي المحتمل 5-أسيتاميدو-4،3،1-ثيادايازول-2-سلفوناميد.

النتائج: تم بنجاح تصنيع السلفوناميدات من خلال اقتران الأمينات وكلوريد الدانسيل. أكد التحقق من صحة الهياكل المركبة باستخدام الرنين المغناطيسي النووي وأطياف الكتلة هوياتها الكيميائية. كشف تحليل الالتحام الجزيئي أن السلفوناميدات المُصنّعة أظهرت ارتباطات ربط تتراوح من -6.8 إلى -8.2 كيلو كالوري / مول مع هدف الدواء المحتمل 5-أسيتاميدو-4،3،1-ثيادايازول-2-سلفوناميد. الأهم من ذلك، أن جميع المشتقات أظهرت ارتباطات ربط متفوقة مقارنة بالأسيتازولاميد (-5.25 سعرة حرارية/مول).

الاستنتاجات: أتاح اقتران الأمينات وكلوريد الدانسيل التوليف الفعال والمباشر للسلفوناميدات. إن التصميم الاستراتيجي للسلفوناميدات مع بدائل محددة سيوفر خصائص بيولوجية متنوعة، بما في ذلك إمكانية النشاط المضاد للسرطان. إن توضيح المركبات المحضرة باستخدام الرنين المغناطيسي النووي وأطياف الكتلة أكد بنيتها. أظهر تحليل الالتحام الجزيئي أن السلفوناميدات المصنعة أظهرت ارتباطات مواتية مع هدف الدواء المحتمل 5-أسيتاميدو-4،3،1-ثيادايازول-2-سلفوناميد. والجدير بالذكر أن جميع المشتقات أظهرت ارتباطات ربط أعلى تتراوح من -6.8 إلى -8.2 كيلو كالوري/مول من عقار الأسيتازولاميد الموصى به (-5.25 كيلو كالوري/مول)، مما يشير إلى إمكاناتها كنظائر فعالة للغاية لمزيد من التحقق من الصحة في علاج السرطان.

الكلمات المفتاحية: أسيتازولاميد؛ سرطان؛ الأنهيدراز الكربوني؛ الالتحام الجزيئي؛ السلفوناميد

Abstract

Objectives: The goal of this study was to design and enable development of anticancer sulfonamides by coupling amines and dansyl chloride with strategically selected substituents. The synthesized structures were characterized by NMR and mass spectrometry. In addition, molecular docking analysis was used to determine the binding ability of sulfonamides toward 1AZM, a possible drug target, as compared with that of the well-known drug acetazolamide.

Methods: Sulfonamides were synthesized by coupling amines and dansyl chloride under highly favorable conditions. The designed sulfonamides incorporated strategically positioned substituents to impart diverse biological properties. The synthesized structures were validated with NMR and mass spectra. Molecular docking analysis was performed to evaluate the binding affinities of the synthesized sulfonamides with the potential drug target 1AZM.

Results: The synthesis of sulfonamides through the coupling of amines and dansyl chloride was successfully achieved. The validation of the synthesized structures with NMR and mass spectra confirmed their chemical identities. Molecular docking analysis revealed that the synthesized sulfonamides displayed binding affinities ranging from −6.8 to −8.2 kcal/mol toward the potential drug

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target 1AZM. Importantly, all derivatives exhibited superior binding affinities to acetazolamide (−5.25 kcal/mol).

Conclusions: The coupling of amines and dansyl chloride enabled efficient, straightforward sulfonamide synthesis. The strategic design of sulfonamides with specific substituents endows diverse biological properties, including potential anti-cancer activity. The elucidation of the synthesized compounds with NMR and mass spectra confirmed their structures. Molecular docking analysis demonstrated that the synthesized sulfonamides exhibited favorable binding affinities toward the potential drug target 1AZM. Notably, all derivatives displayed higher binding affinities, ranging from −6.8 to −8.2 kcal/mol, than the recommended drug acetazolamide (−5.25 kcal/mol), thus suggesting their potential as highly effective analogues for further validation in cancer therapy.

Keywords: Acetazolamide; Cancer; Carbonic anhydrase; Docking; Sulfonamide

Introduction

Cancer is a devastating disease that has plagued humanity for centuries, and caused immeasurable suffering and loss. Cancer has been a major focus of medical research and treatment throughout history, and although progress has been made, remains a major challenge.[1] Globally, cancer poses a substantial risk that claims the lives of more than 7 million individuals annually. This figure is expected to rise to 19.3 million people by 2025.[2] Increased exposure to natural and synthetic cancer-causing agents has played a major role in cancer deaths.[3] Unfortunately, no drugs or medical procedures offer complete control over cancer therapies.[3–5] A major source of concern is the harmful effects of drugs used to treat cancer.[6–9] These treatments can effectively destroy cancer cells but often have substantial adverse effects, such as nausea, hair loss, and weakened immune system function.[5,10–12] Compelling arguments for developing novel cancer treatments include genotoxicity and cancer drug resistance.[9,13,14] Among other negative consequences, the cytotoxicity of modern cancer treatments may induce hair loss and drug-induced malignancies, thus justifying the need for development of innovative cancer therapies.[15,16]

Cancer can affect any part of the body, and arises from the uncontrolled growth and spread of abnormal cells.[17–19] Cancer treatment has markedly evolved over time.[20] Historically, surgical removal of tumors was the primary treatment option.[21] More options were introduced with the development of radiation therapy and chemotherapy.[22] Although various chemotherapeutic options are available, in recent years, sulfonamide has emerged as a promising therapy[23] that may potentially target cancer cells more effectively, with fewer adverse effects, than traditional treatments.[24] Some studies have suggested that sulfonamides inhibit carbonic anhydrase, an enzyme overexpressed in numerous cancers, and that this inhibition selectively targets apoptotic pathways in cancerous cells but has lesser effects on healthy cells.[24,25] Therefore, sulfonamide must be explored as a possible therapy for treating cancers.[26]

Sulfonamides are a class of antibiotics used for more than 80 years to treat bacterial infections.[27] These drugs inhibit the growth and proliferation of cancer cells *in vitro* and *in vivo*.[28,29] Molecular docking investigations have been used to virtually screen sulfonamides, and predict and rank potential ligands according to their binding scores.[30] This process has facilitated the identification of promising lead compounds with high probabilities of efficacy against specific target proteins, such as carbonic anhydrases. By inhibiting carbonic anhydrases, sulfonamides block the formation of new blood vessels necessary for tumor growth, and thus may be a potentially effective anticancer therapy.[31] Additionally, some sulfonamides work synergistically with other cancer treatments, such as radiation therapy, thus increasing therapeutic efficacy. Sulfonamides, which are accessible scaffolds derived from sulfonyl chloride and amine derivatives, are a prominent class of pharmaceutical compounds with numerous biological applications, such as anti-diabetic, anticancer, antimalarial, antibacterial, antiviral, antiglaucoma, and anti-inflammatory properties.[32] Metalloprotease, carbonic anhydrase, heat shock protein, and jack bean urease are all inhibited by sulfonamides.[33] These compounds have attracted substantial interest in organic synthesis and drug discovery, because of their broad spectrum of medicinal uses. They can be synthesized in various ways, including reaction of 5-(dimethylamino) naphthalene-1-sulfonyl chloride (dansyl chloride) with aromatic amines. Dansyl chloride is a fluorescent dye that forms sulfonamides when combined with amines.[34] This process is frequently used in biological research to label and identify proteins and other macromolecules. Overall, sulfonamide synthesis is a versatile, frequently used approach for preparing prospective anticancer drugs. Positioned at the forefront of innovative future cancer treatments, they are excellent for producing innovative chemical systems for cancer and tumor reduction and eradication research.

This work describes how sulfonamide derivatives were designed, synthesized, and analyzed as potential anticancer therapeutics. In addition, the binding affinities of the synthesized ligands were determined via molecular docking against the potential target 1AZM, thus providing insights for subsequent validation of the results through *in vitro* and *in vivo* studies. This classical synthetic route is a convenient method for synthesizing sulfonamides in a single step by coupling sulfonyl chloride with a series of primary amines. Specifically, in this project, dansyl chloride coupled with a series of primary amines yielded a small library of sulfonamides as potential anticancer drugs. These 5-(dimethylamino) naphthalene-substituted sulfonamides are the main intermediates for several therapeutic agents.[35] On the basis of the literature, the development of anticancer therapeutics, some of which are sulfonamide-based molecules, has yielded positive results.[36,37]

Methods

All reactions were performed in flame-dried reaction flasks under an inert atmosphere and monitored with TLC

precoated plates (60, F254, Merck 5735, and 5554). CC was used to purify the products with silica gel 60 (Merck-109385) or neutral alumina (Merck-101077), and the eluent was a mixture of ethyl acetate and hexanes. A JEOL 400 MHz NMR spectrometer (JEOL, Ltd., Japan) and LC–MS spectrometer (Agilent Technologies, Santa Clara, CA, USA) were used to obtain NMR and MS spectra. All analytical grade reagents and solvents were acquired from commercial providers (Merck, Sigma-Aldrich, TCI), and used as received.

Structural design of a sulfonamide library

A library of fluorescent sulfonamide adducts as potential anticancer agents was generated via a structure-guided drug design approach, as presented below. This approach can decrease the time and costs of discovering new effects and potential therapeutic lead compounds (see Scheme 1).[38]

The 5-(dimethylamino) *N*-substituted naphthalene-1-sulfonamides were synthesized according to a method in the literature, with slight modifications[39,40]

General experimental procedure

To an amine **1** (1.0 mmol) dissolved in 1 mL of anhydrous pyridine, 1.1 mmol of 5-(dimethylamino)naphthalene-1-sulfonyl chloride **2** was added dropwise, with stirring, at 0 °C. The reaction mixture was agitated at room temperature under N_2 until TLC confirmed the complete consumption of the limiting reagent. After completion of the reaction, the reaction mixture was carefully poured into a bath of ice-cold water. Consequently, precipitation led to the formation of solid particles. These sticky solids were subsequently subjected to thorough filtration and subsequent drying. The solid was carefully washed with hexane, to ensure its purity. The resulting products (**3a–i**) were further purified via column chromatography with ethyl acetate:hexane as an eluent in most cases, thus affording (**3a–i**) with good to excellent yields (see Figure 1).

5-(Dimethylamino)-N-(2-fluoro-3-(trifluoromethyl)phenyl) naphthalene-1-sulfonamide (3a)

Yield 85% (brown, semi-solid), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.55 (d, *J* = 8.6, 1.1 Hz, 1H), 8.28 (d, *J* = 8.7, 1.0 Hz, 1H), 8.23 (dd, *J* = 7.4, 1.2 Hz, 1H), 7.68 (t, *J* = 7.8 Hz, 1H), 7.58 (t, *J* = 8.5, 7.5, 1.0 Hz, 1H), 7.46 (t, *J* = 8.3, 7.3, 1.0 Hz, 1H), 7.28–7.15 (m, 2H), 7.15–7.05 (m, 2H), 2.86 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 152.31, 133.59, 131.75, 130.45, 130.04, 129.61, 129.10, 126.51, 126.40, 125.58, 124.51, 123.45, 123.04, 122.40, 118.18, 115.66, 45.50. LCMS (ESI+): calc. for $C_{19}H_{16}F_4N_2O_2S$ $[M + H]^+$: 412.09, found: 413.00.

N-(4-chloro-3-(trifluoromethyl)phenyl)-5-(dimethylamino) naphthalene-1-sulfonamide (3b)

Yield 81% (yellow, semi-solid), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.53 (d, J = 8.6, 1.0 Hz, 1H), 8.30 (d, J = 8.6, 0.9 Hz, 1H), 8.20 (d, J = 7.3, 1.1 Hz, 1H), 7.57 (t, J = 8.5, 7.6, 0.9 Hz, 1H), 7.46 (t, J = 8.3, 7.3, 0.8 Hz, 1H), 7.33 (s, 1H), 7.23 (d, J = 8.6 Hz, 1H), 7.19–7.16 (m, 2H), 7.11–7.06 (m, 1H), 2.86 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 152.19, 135.24, 132.99, 132.06, 131.40, 130.49, 129.67, 129.24, 128.90, 128.18, 124.98, 122.91, 120.03, 119.97, 117.67, 115.28, 45.19. LCMS (ESI+): calc. for $C_{19}H_{16}ClF_3N_2O_2S$ $[M + H]^+$: 428.06, found: 429.00.

5-(Dimethylamino)-N-phenylnaphthalene-1-sulfonamide (3c)

Yield 78% (pale green, sticky solid), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.48 (d, *J* = 8.6, 1.2 Hz, 1H), 8.33 (d, *J* = 8.6, 1.0 Hz, 1H), 8.16 (dd, *J* = 7.3, 1.2 Hz, 1H), 7.59–7.54 (m, 1H), 7.46–7.37 (m, 1H), 7.17 (d, *J* = 7.7, 1.1 Hz, 1H), 7.15–7.06 (m, 2H), 7.06–6.97 (m, 1H), 6.91 (dd, 2H), 6.85 (s, 1H), 2.89–2.83 (m, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 152.18, 136.58, 134.23, 130.97, 130.46, 129.91, 129.74, 129.26, 128.77, 125.28, 123.22, 121.54, 118.60, 115.35, 45.52. LCMS (ESI+): calc. for $C_{18}H_{18}N_2O_2S$ $[M + H]^+$: 326.11, found: 327.00.

5-(Dimethylamino)-N-(3-methoxyphenyl)naphthalene-1-sulfonamide (3d)

Yield 91% (yellow, sticky solid), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.50 (d, J = 8.4 Hz, 1H), 8.34 (d, J = 8.3, 3.9 Hz, 1H), 8.20 (d, J = 7.4, 1.3 Hz, 1H), 7.62–7.56 (m, 1H), 7.49–7.40 (m, 1H), 7.18 (d, J = 7.5 Hz, 1H), 7.00 (t, J = 8.9, 7.1 Hz, 1H), 6.85 (d, J = 41.4 Hz, 1H), 6.57–6.53 (m, 2H), 6.50–6.45 (m, 1H), 3.64 (s, 3H), 2.87 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 159.85, 151.78, 137.53, 133.92, 130.66, 130.13, 129.62, 129.56, 129.42, 128.46, 122.87, 118.32, 115.04, 112.84, 110.46, 106.28, 54.95, 45.16. LCMS (ESI+): calc. for $C_{19}H_{20}N_2O_3S$ $[M + H]^+$: 356.12, found: 357.00.

N-(2,3-dihydro-1H-inden-5-yl)-5-(dimethylamino)naphthalene-1-sulfonamide (3e)

Yield 77% (pale green, sticky solid), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.48 (d, J = 8.4, 1.1 Hz, 1H), 8.34 (d, J = 8.8, 1.0 Hz, 1H), 8.12 (dd, J = 7.4, 1.3 Hz, 1H), 7.57 (t, J = 8.6, 7.6 Hz, 1H), 7.41 (t, J = 8.5, 7.3 Hz, 1H), 7.17 (d, J = 7.5, 1.0 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 6.80 (s, 1H), 6.66 (s, 1H), 6.59 (dd, J = 8.0, 2.1 Hz, 1H), 2.87 (s, 6H), 2.73 (q, J = 7.9 Hz, 4H), 1.96 (p, J = 7.4 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 152.10, 145.49, 141.69, 134.49, 134.45, 130.73, 130.34, 129.88, 129.80, 128.65, 124.69, 123.24, 120.28, 118.78, 118.69, 115.28, 45.52, 32.87, 32.31, 25.55. LCMS (ESI+): calc. for $C_{21}H_{22}N_2O_2S$ $[M + H]^+$: 366.14, found: 367.00.

5-(Dimethylamino)-N-(3-phenoxyphenyl)naphthalene-1-sulfonamide (3f)

Yield 99% (light green, sticky solid), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.50 (d, J = 8.6, 1.1 Hz, 1H), 8.31 (d, J = 8.6, 0.8 Hz, 1H), 8.15 (d, J = 7.4, 1.2 Hz, 1H), 7.53 (t, 1H), 7.41 (t, 1H), 7.29–7.24 (m, 2H), 7.16 (d, J = 7.7, 0.9 Hz, 1H), 7.12–7.07 (m, 2H), 7.06–7.02 (m, 1H), 6.79 (s, 1H), 6.77 (s, 1H), 6.71–6.67 (m, 1H), 6.64–6.60 (m, 1H), 6.57 (t, J = 2.2 Hz, 1H), 2.86 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 157.67, 156.19, 151.84, 137.73, 133.69, 130.74, 130.31, 129.99, 129.58, 129.53, 129.38, 128.48, 123.38, 122.90, 118.83, 118.20, 115.25, 115.06, 114.78, 110.72, 45.19. LCMS (ESI+): calc. for $C_{24}H_{22}N_2O_3S$ $[M + H]^+$: 418.14, found: 419.00.

N-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-(dimethylamino) naphthalene-1-sulfonamide (3g)

Yield 71% (yellow, semi-solid), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.49 (d, J = 8.5, 1.0 Hz, 1H), 8.30 (d, J = 8.6, 0.9 Hz, 1H), 8.11 (dd, J = 7.3, 1.1 Hz, 1H), 7.56 (t, J = 8.6, 7.6, 0.9 Hz, 1H), 7.41 (t, J = 8.5, 7.3, 0.9 Hz, 1H), 7.17 (dd, J = 7.6, 0.8 Hz, 1H), 6.59−6.53 (m, 2H), 6.47 (d, J = 2.5, 0.9 Hz, 1H), 6.31 (m, J = 8.6, 2.6, 0.7 Hz, 1H), 4.14−4.10 (m, 4H), 2.87 (s, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ: δ 151.43, 143.18, 140.08, 134.91, 130.81, 130.01, 129.65, 129.06, 128.95, 128.12, 123.58, 118.73, 117.23, 115.26, 113.15, 108.96, 64.07, 63.77, 45.07. LCMS (ESI+): calc. for $C_{20}H_{20}N_2O_4S$ $[M + H]^+$: 384.11, found: 385.00.

5-(Dimethylamino)-N-(3-isopropoxyphenyl)naphthalene-1-sulfonamide (3h)

Yield 98% (brown, sticky solid), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.49 (d, 1H), 8.34 (d, J = 8.9 Hz, 1H), 8.20 (dd, J = 7.4, 1.7 Hz, 1H), 7.57 (t, J = 8.7, 7.7 Hz, 1H), 7.43 (t, J = 8.5, 7.5 Hz, 1H), 7.17 (d, J = 7.8 Hz, 1H), 6.98 (t, J = 8.0 Hz, 1H), 6.93 (s, 1H), 6.53 (dd, J = 8.1, 2.4 Hz, 1H), 6.50 (t, J = 2.2 Hz, 1H), 6.47 (dd, J = 8.0, 2.0 Hz, 1H), 4.37−4.27 (m, 1H), 2.86 (s, 6H), 1.19 (s, 3H), 1.17 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 158.54, 152.22, 137.65, 134.22, 130.96, 130.51, 129.98, 129.94, 129.77, 128.76, 123.23, 118.58, 115.36, 113.54, 113.20, 108.87, 70.08, 45.53, 21.96. LCMS (ESI+): calc. for $C_{21}H_{24}N_2O_3S$ $[M - H]^+$: 384.15, found: 383.00.

5-(Dimethylamino)-N-(quinolin-3-yl)naphthalene-1-sulfonamide (3i)

Yield 78% (yellow, sticky solid), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.49 (d, J = 8.5, 1.1 Hz, 1H), 8.40−8.34 (m, 2H), 8.19 (dd, J = 7.4, 1.3 Hz, 1H), 7.95 (d, J = 8.5, 1.4, 0.8 Hz, 1H), 7.91 (dd, J = 2.6, 0.8 Hz, 1H), 7.70−7.66 (m, 1H), 7.63−7.57 (m, 2H), 7.52−7.47 (m, 1H), 7.42−7.37 (m, 1H), 7.32 (s, 1H), 7.18 (d, J = 7.7, 0.9 Hz, 1H), 2.86 (s, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 150.95, 143.58, 143.47, 133.46, 130.84, 129.90, 129.56, 128.37, 128.25, 127.89, 127.85, 127.62, 126.91, 126.85, 126.74, 122.95, 121.17, 117.78, 114.78, 44.40. LCMS (ESI+): calc. for $C_{21}H_{19}N_3O_2S$ $[M + H]^+$: 377.12, found: 378.00.

Computational study

Computational studies encompass various computational techniques used to investigate and analyze scientific problems. In computational studies, computer simulations, algorithms, or mathematical models are used to gain insights into complex phenomena and make predictions. Molecular docking studies are a specific type of computational study focused on predicting the binding affinity and orientation of small molecules (ligands) within a receptor or target protein. Molecular docking involves simulating the docking process between the ligand and protein, and evaluating their interactions and compatibility. These studies are particularly useful in drug discovery and design, because they can help identify potential drug candidates, on the basis of the ability to bind a specific target protein.[41]

Molecular docking

Molecular docking analysis is commonly used for investigating interactions among complexes, DNA, and proteins. This method can indicate the optimal mechanism for drug and biomolecule interaction, on the basis of prediction of the binding affinity between these molecules and proteins.[42] Here, we used molecular docking tools to gain insight into the molecular interactions between small molecules (e.g., sulfonamide compounds) and particular protein targets (e.g., carbonic anhydrase), and to determine or validate their potential as anticancer agents.

Molecular docking methods: identification of active sites in the protein

The protein crystal structure of a sulfonamide drug complexed with CA I was obtained from the Protein Data Bank (PDB ID: 1AZM), downloaded from https://www.rcsb.org. In Discovery Studio software, the receptor was visualized, thus revealing a receptor ready to be docked with a ligand molecule (Figure 2). The scientific motivation for choosing 1AZM was based on its relevance to the interactions between sulfonamide ligands and carbonic anhydrase. This choice enabled exploration the structural and functional aspects of these binding affinities, and provided insights for further validation of the synthesized sulfonamides via *in vitro* and *in vivo* studies.

This ligand−protein complex interaction allowed us to identify active sites within the protein. The active sites were determined to be His200, Leu131, Leu198, His94, Phe91, Ala121, and Gln92. These sites participate in various types of bonds, including conventional hydrogen bonds, donor−donor bonds, acceptor−acceptor bonds, pi−sigma interactions, pi−pi stacking, and pi−alkyl interactions.

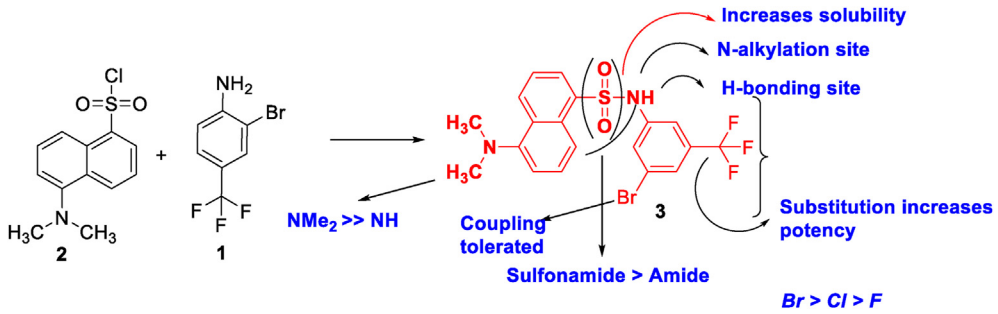
Molecular docking analysis: protein-ligand preparation

The receptor obtained from RCSB was in PDB file format and was prepared in Discovery Studio software. During this process, residues such as ligands, water molecules, and other associated traces were eliminated from the receptor protein. The ligand, represented by the optimized compound (Figure 2b), was also in PDB file format. The prepared receptor (Figure 2a) and the ligand were imported into PyRx docking software to perform the docking simulation.[43]

Results and discussion

Chemistry

The compounds **3a**−**3i** were synthesized as summarized in Scheme 2. The structures of the synthesized products were elucidated by ^{1}H NMR, ^{13}C NMR, and mass spectroscopic techniques. In the ^{1}H NMR spectra of the sulfonamides, the characteristic signals supporting the synthesized products were primarily the aromatic CH signals resonating at approximately 6.43−8.55 ppm. Furthermore, dimethyl CH_3



Scheme 1: Structure based design of the target sulfonamide series.

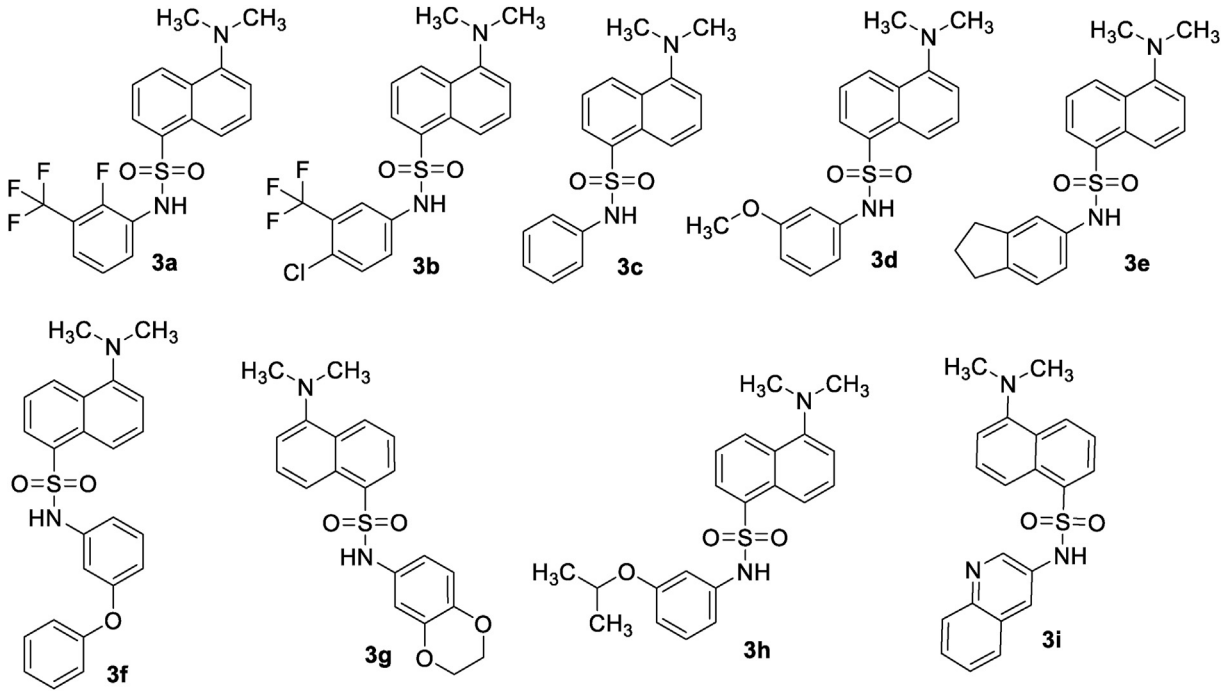
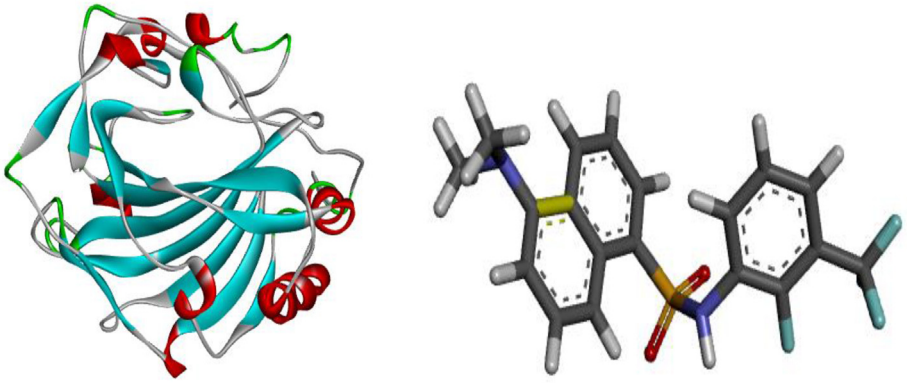


Figure 1: Structures of sulfonamide derivatives (**3a**–**3i**).



(a) Prepared receptor (b) Prepared ligand

Figure 2: (a) Prepared receptor, (b) Prepared ligand.

Table 1: Molecular docking results of compounds 3a–i against carbonic anhydrase I (PDB ID 1AZM).

S/no.	Ligand	Binding affinity (k/mol)	Hydrogen bond (amino acid)	Residual interactions (amino acid)
1	**3a**	−6.90	Gln92	His64, His67, Leu198, Phe91, Ala121, His94, His200, Val62
2	**3b**	−7.10	Gln92	Ala121, His64, His67, His94, Leu198, Phe91, His200, Val62
3	**3c**	−7.10	Gln92	Ala135, His200, His94, Ala121, Leu198, Phe91, Val62, Leu131
4	**3d**	−6.90	Gln92	Ala121, Leu141, Leu131, Ala135, Leu198, His200, His94, Phe91
5	**3e**	−7.50	–	Leu131, Phe91
6	**3f**	−8.20	–	Leu131, Phe91, Ala121, Leu198, His200, His94
7	**3g**	−7.30	–	Leu131, Leu198, Phe91
8	**3h**	−7.0	–	Leu198, His200, His94, Leu131, Phe91, Ala121
9	**3i**	−7.7	–	Leu198, Gln92, Phe91

signals from the 5-(dimethylamino)naphthalene portion of products **3a–i** resonated at approximately 2.85–290 ppm. For instance, the proton NMR spectrum of sulfonamide **3a** displayed a doublet at 8.55 and 8.28 ppm; doublets of a doublet at 8.23 ppm; triplets at 7.68, 7.58, and 7.46 ppm; and multiplets ranging from 7.28 to 7.05 ppm, representing the aromatic protons from the naphthalene core as well as the precursor aromatic amines. The proton NMR spectrum of sulfonamide **3a** showed a doublet at 8.55 and 8.28 ppm; doublets of a doublet at 8.23 ppm; triplets at 7.68, 7.58, and 7.46 ppm; and multiplets at 7.28–7.05 ppm representing the aromatic protons from the naphthalene core as well as the precursor aromatic amines. Notably, the NH group of the sulfonamide linkage exhibited a greater chemical shift than the precursor amine. In the ^{13}C NMR spectra of the sulfonamides, the characteristic signals supporting the synthesized products were primarily the aromatic CH signals resonating at approximately 106–109 ppm. Furthermore, dimethyl CH_3 signals from the 5-(dimethylamino)naphthalene portion of products **3a–j** resonated at approximately 45.0 ppm in most cases. However, several derivatives showed characteristic aliphatic peaks resonating primarily at lower frequencies. For instance, the carbon NMR spectrum of compound **3a** showed the following characteristic aromatic peaks at δ: 152.31, 133.59, 131.75, 130.45, 130.04, 129.61, 129.10, 126.51, 125.58, 124.51, 123.45, 123.04, 122.40, 118.18, and 115.66 ppm. The dimethyl CH_3 carbon peaks from the 5-(dimethylamino)naphthalene portion of product **3a** resonated at approximately 45.50 ppm. In LCMS, all calculated masses matched the experimental values well.

Docking results

Docking analysis was performed to investigate the binding affinities and interactions between novel sulfonamides, downloaded from Protein Data Bank accession code 1AZM, and carbonic anhydrase I as potential anticancer agents. Interaction of the sulfonamide derivatives with carbonic anhydrase (receptor–ligand interaction) and the amino acid residues involved are shown in the supplementary figure (Figure S1).

The docking results clearly demonstrated the binding interactions and affinities of these ligands to their receptor, including the strength and readiness of these interactions. The binding affinities of the compounds, ranging from −6.8 to −8.2 kcal/mol, are summarized in Table 1. The interactions between compound **3a** and the target protein 1AZM showed a binding affinity of **−6.90** kcal/mol

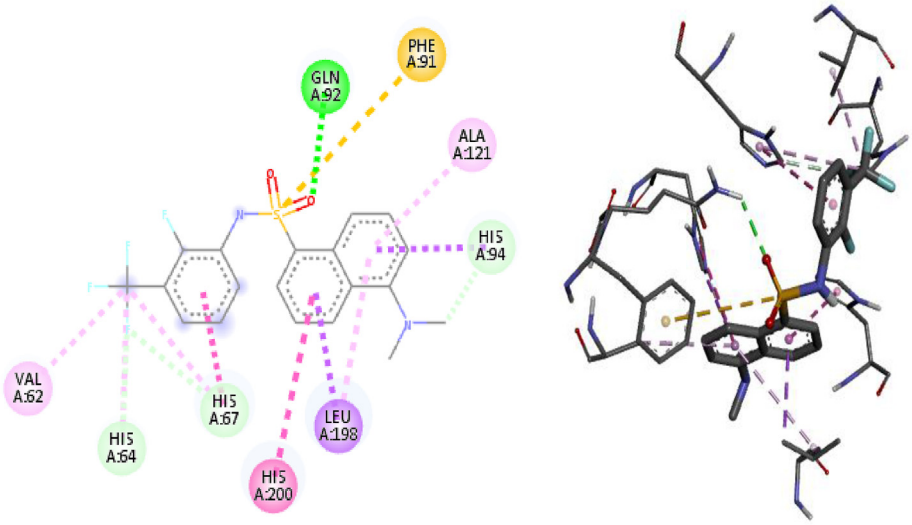
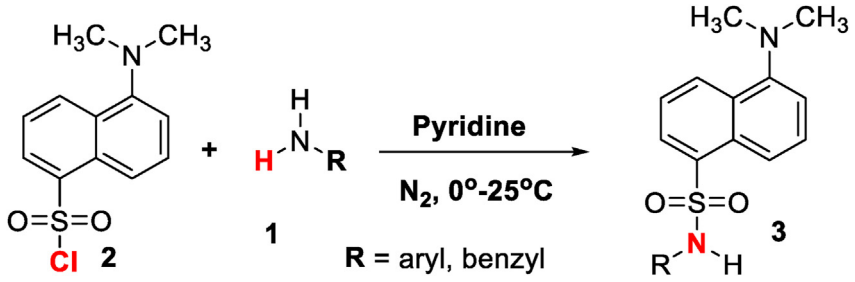


Figure 3: 2D and 3D interactions between **3a** and the receptor **1AZM**.



Scheme 2: Experimental procedure for the synthesis of sulfonamide analogues.

(Figure 3). The interactions included conventional hydrogen bond, van der Waals, pi-sigma, pi-sulfur, pi-alkyl, pi−pi stacked, pi−pi T-shaped, and pi−alkyl interactions. Notably, compounds **3i** and **3f** exhibited high binding affinities of **−7.70** and **−8.20** kcal/mol, respectively, thus suggesting their potential as promising lead agents for derivatization (Figure S1). The ligand−protein complex interactions facilitated the identification of active sites within the protein. Various types of bonding interactions were observed, including hydrophobic interactions, conventional hydrogen bonds, donor−donor bonds, acceptor−acceptor bonds, pi−sigma interactions, pi−pi stacking interactions, and pi−alkyl interactions within the ligand−protein complex. The active sites within the protein involved different amino acid residues, such as Leu131, His200, His94, Leu198, Gln92, Ala121, and Phe91.

Although all synthesized and docked compounds demonstrated higher affinity for binding the receptor than the reference drug, acetazolamide (−5.25 kcal/mol), compound **3f** had the highest affinity for binding the target enzyme, with a binding affinity of −8.20 kcal/mol. However, examination of the binding diagrams revealed that compound **3f** did not form hydrogen bonds with Gln92, in contrast to those formed by acetazolamide. Moreover, the molecules 3e, 3g, 3h, and 3i (Figure S1), despite having the best binding affinity towards the target, also did not form hydrogen bonds with Gln92. Nevertheless, all the synthesized compounds interact with the enzyme structure via van der Waals, electrostatic, or hydrophobic interactions.

Conclusions

This report demonstrated that N-substituted sulfonamides can be synthesized under mild conditions from dansyl chloride and a series of aromatic amines. The newly synthesized anticancer motifs were characterized through ^{1}HNMR, ^{13}CNMR, and MS analyses. Molecular docking analysis revealed that the tested derivatives had binding affinities between −6.8 and −8.2 kcal/mol. Remarkably, all derivatives exhibited higher binding affinities than the prescribed drug acetazolamide, which has a binding affinity of −5.25 kcal/mol. With additional experimental validation and the overexpression of carbonic anhydrase in hypoxic tumors, sulfonamides may become a promising new cancer treatment option. Although molecular docking revealed the extent to which the sulfonamides bind the target protein in this work, it does not account for the broader biological context, such as protein−protein interactions, and the cellular environment and dynamics. Although these factors can influence the actual behavior of the compounds in a living system, molecular docking predictions should ideally be experimentally tested with techniques such as binding assays. These findings have substantial implications for the development of novel, highly promising anticancer analogues. Therefore, our future studies will aim at confirming the predicted binding affinities and evaluating the genuine anti-cancer capabilities of sulfonamides by exploring the *in vitro* and *in vivo* bioactivities of this newly discovered scaffold, including structure−activity relationships, and additional computational assessment of the most active analogs.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The section is not applicable to this research work.

Authors contributions

ITB and GS made equal contributions to the design, execution, and preparation of this submission. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtumed.2023.10.006.

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