Selective Carbonic Anhydrase IX and XII Inhibitors Based Around a Functionalized Coumarin Scaffold

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Abstract

Inhibition of specific carbonic anhydrase (CA) enzymes is a validated strategy for the development of agents to target cancer. The CA isoforms IX and XII are overexpressed in various human solid tumors wherein they play a critical role in regulating extracellular tumor acidification, proliferation, and progression. A series of novel sulfonamides based on the coumarin scaffold were designed, synthesized and characterized as potent and selective CA inhibitors. Selected compounds show significant activity and selectivity over CA I and CA II to target the tumor-associated CA IX and CA XII with high inhibition activity at the single digit nanomolar level. Twelve compounds were identified to be more potent compared with acetazolamide (AAZ) control to inhibit CA IX while one was also more potent than AAZ to inhibit CA XII. Compound **18f** (Ki's = 955 nM, 515 nM, 21 nM and 5 nM for CA's I, II, IX and XII respectively) is highlighted as a novel CA IX and XII inhibitor for further development.

Keywords:

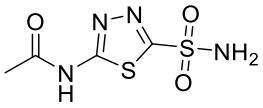
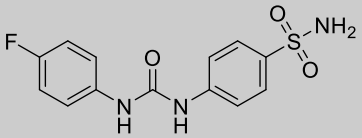
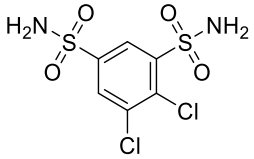
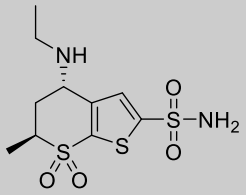
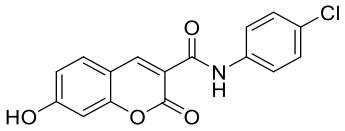
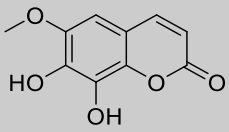
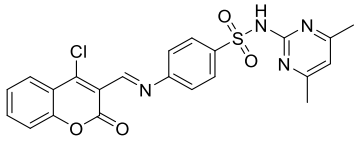
Carbonic anhydrase IX inhibitors, Carbonic anhydrases XII inhibitor, Coumarin, Structure-activity relationship

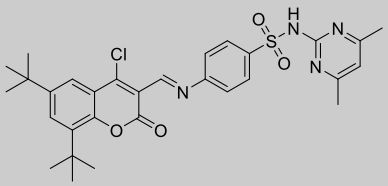
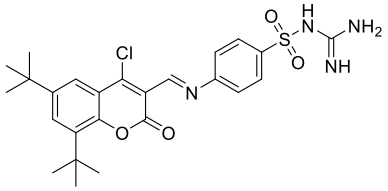
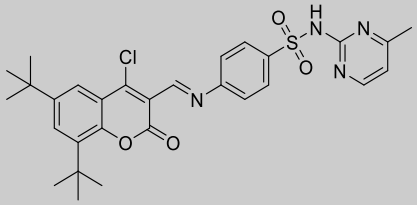
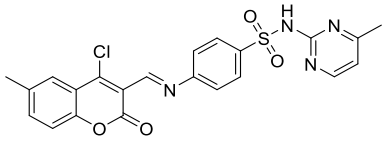
1. Introduction

The carbonic anhydrases (CA) are a family of ubiquitous zinc enzymes which play a catalytic role in the reversible hydration of carbon dioxide (CO_2) and water (H_2O) to bicarbonate (HCO_3) and a proton.(Supuran, 2008) In humans, the carbonic anhydrase enzymes have 15 isoforms that vary by localization and catalytic activity including; the cytosolic CAs; CA I, CA II, CA III, CA VII, CA XIII; the membrane-bound CAs; CA IV, CA IX, CA XII, CA XIV, CA XV (not present in primates, only in rodents and other animals/fish); CA VA and CA VB are mitochondrial, and CA VI is secreted in saliva and colostrum. In addition, three catalytically inactive forms of CA are known (CA VIII, CA X, and CA XI) referred to as CA related-proteins.(Lomelino, Supuran, & McKenna, 2016) *Carbonic anhydrase IX* and *CA XII* are highly overexpressed genes in response to hypoxia in a variety of human solid tumors such as breast, colorectal, glioblastoma and lung and they play a critical role in regulating tumor acidification, proliferation, and progression.(Lee & Griffiths, 2020; Neri & Supuran, 2011)

The overexpression of CA IX and CA XII induces cancer cell growth, activation of the metastatic cascade, and reduced response to chemotherapy.(Supuran, 2010) Targeting both CA IX and CA XII in cancers that overexpress these biomarkers, and suppressing their activity has been shown to be therapeutically beneficial in the treatment of tumors.(McDonald, Chafe, Supuran, & Dedhar, 2022) The classic CA inhibitors contain the sulfonamide pharmacophore and have been determined to exhibit potent CA IX and XII inhibition with high potency to attenuate cancer cell growth both *in vitro* and *in vivo*. Examples of this class include acetazolamide, dichlorphenamide and dorzolamide (**Table 1)**.(Supuran, 2008) Recently, coumarin-based small molecules (**1.1** and **1.2**) were reported as non-classical CA inhibitors with high efficacy and selectively for the physiologically dominant tumor-associated isoenzymes CA IX and CA XII.(Maresca et al., 2010; Supuran, 2008, 2020; Thacker, Alvala, Arifuddin, Angeli, & Supuran, 2019; Touisni et al., 2011; Williams & Gieling, 2019) Carbonic anhydrase inhibitors with combined sulfonamide and coumarin moieties have been reported to possess high efficacy for inhibiting the enzymatic activity of CA IX (**1.3**-**1.7**, **Table 1)**.(Wang et al., 2013) Moreover, sulfonamides containing coumarin moieties have potent anticancer activity in the MCF-7 breast cancer cell line.(Wang et al., 2013) Although several substituted coumarins have been described in the literature, little information is known about the importance of these coumarin structures as CA inhibitors.

Herein, the synthesis of a series of CA inhibitors is reported based around the coumarin chemotype with substituted sulfonamide moieties to further investigate the amalgamation of these two pharmacophores as CA inhibitors. Several of the synthesized derivatives possess high potency for inhibiting the tumor-associated CA IX and CA XII with nanomolar activity while possessing selectivity over CA I and II.

Compound	Structure	K_i (μM)		
		CA II	CA IX	CA XII
Acetazolamide (AAZ)		0.012	0.026	0.006
SLC-0111 (U-104)		9.6	0.045	0.004
Dichlorphenamide		0.038	0.05	0.05
Dorzolamide		0.009	0.052	0.004
1.1		>100	0.2	0.2
1.2		94.3	0.61	7.7
1.3		0.023	0.124	NA[a]

1.4		0.173	0.090	NA
1.5		0.103	0.074	NA
1.6		0.063	0.024	NA
1.7		0.061	0.048	NA

[a] Not available

Table 1. Structures and inhibition profile of selected known carbonic anhydrase inhibitors.

2 Experimental

2.1 Chemistry

All reactions were carried out in oven- or flame-dried glassware under positive nitrogen pressure unless otherwise noted. Reaction progress was monitored by thin-layer chromatography (TLC) carried out on silica gel plates (2.5 cm x 7.5 cm, 200 μm thick, 60 F254) and visualized by using UV (254 nm) or by potassium permanganate or phosphomolybdic acid stain as indicator. Flash column chromatography was performed with silica gel (40-63 μm, 60 Å) or on a Biotage® (Biotage® Selekt). Commercial grade solvents and reagents were purchased from Fisher Scientific (Houston, TX) or Sigma Aldrich (Milwaukee, WI) and were used without further purification except as indicated. Anhydrous solvents were purchased from Acros Organics and stored under an atmosphere of dry nitrogen over molecular sieves.

^{1}H and ^{13}C NMR spectra were recorded in the indicated deuterated solvent on a Bruker Advance III HD spectrometer at 400 or 500 for ^{1}H and 100 or 126 MHz for ^{13}C, respectively with solvent peak as an internal standard. Multiplicities are indicated by s (single), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br (broad). Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) in Hertz. High-resolution mass spectroscopy (HRMS) was performed on a 6230 LC/TOF (Agilent) using an ESI source conducted. The spectral data was extracted from total ion chromatogram (TIC).

Ethyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (2a): To a mixture of 2,4-dihydroxybenzaldehyde (1a, 2 g, 14.48 mmol) in ethanol (5 mL) at room temperature, the diethyl malonate (5.81 g, 2.43 mL, 15.23 mmol) was added along with piperidine (0.29 mL, 2.9 mmol). The mixture was stirred and heated to 60 °C for 2 h. Then, it was cooled to room temperature and filtered off washed with water and ethanol and air dried to yield a yellow powder (2.954 g, 87% yield):^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.30 (t, *J*=7.1 Hz, 3H), 4.24-4.29 (q, *J*=7.1 Hz, 2H), 6.73 (s, 1H), 6.86 (d, *J*=8.5 Hz, 1H), 7.77 (d, *J*=8.5 Hz, 1H), 8.67 (s, 1H), 11.06 (s, 1H).^{13}C NMR (100 MHz, DMSO-d_6): δ= 14.6, 61.2, 102.2, 110.8, 112.5, 114.4, 132.5, 149.8, 156.8, 157.5, 163.4, 164.5. HRMS (ESI): *m/z* calcd for $C_{12}H_{10}O_5$ [*M*+Na]$^{+}$: 257.1939, found: 257.1932.

2-Chloro-*N*-(4-sulfamoylphenyl)acetamide (4a). Potassium carbonate (K_2CO_3) (1.2 g, 8.72 mmol) was added to a solution of sulfanilamide (**3a**, 1 g, 5.81 mmol) in THF (20 mL). Chloroacetyl chloride (0.56 mL, 6.97 mmol) was added to the above solution dropwise and under N_2 atmosphere at 0 °C with stirring. After that, the reaction mixture was stirred for 2 h and then the water was added to quench the reaction. The reaction mixture was extracted with ethyl acetate, the organic layer washed with brine and dried over sodium sulfate, the solvent was removed *in vacuo* and purified by recrystallization in hexane:MeOH to yield a white powder (1.349 g, 93% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 4.30 (s, 2H), 7.27 (s, 2H), 7.74-7.81 (q, *J*=7.8 Hz, 4H), 10.61 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 44.0, 119.4, 127.2, 139.4, 141.7, 165.6. HRMS (ESI): *m/z* calcd for $C_8H_9ClN_2O_3S$ [*M*+Na]$^{+}$: 271.6736, found: 271.6731.

2-Chloro-*N*-(4-(*N*-(5,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)acetamide (4b). The powder compound was obtained from sulfadoxine (**3b**, 1 g, 3.22 mmol) by following the experimental conditions described for **4a** (0.983 g, 79% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.70 (s, 3H), 3.90 (s, 3H), 4.29 (s, 2H), 7.78 (d, *J*=8.7 Hz, 2H), 7.96 (d, *J*=8.7 Hz, 2H), 8.11 (s, 1H), 10.67 (s, 1H), 11.04 (br-s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ=

43.9, 54.5, 60.6, 119.2, 127.7, 129.3, 135.5, 142.8, 150.7, 151.0, 162.1, 165.7. HRMS (ESI): *m/z* calcd for $C_{14}H_{15}ClN_4O_5S$ [*M*+Na]$^+$: 409.7968, found: 409.7947.

***N*-(4-(*N*-Acetylsulfamoyl)phenyl)-2-chloroacetamide (4c).** The white powder was obtained from sulfacetamide (**3c**, 1 g, 4.67 mmol) by following the experimental conditions described for **4a** (1.076 g, 80% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.32 (s, 3H), 4.31 (s, 2H), 7.78 (d, *J*=8.7 Hz, 2H), 7.89 (d, *J*=8.7 Hz, 2H),10.72 (s, 1H), 11.99 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 23.6, 44.0, 119.3, 129.4, 134.0, 143.4, 165.8, 169.1. HRMS (ESI): *m/z* calcd for $C_{10}H_{11}ClN_2O_4S$ [*M*+Na]$^+$: 313.7102, found: 313.7101.

Ethyl 2-oxo-7-(2-oxo-2-((4-sulfamoylphenyl)amino)ethoxy)-2*H*-chromene-3-carboxylate (5a). To a solution of ethyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**2a** 0.2 g, 0.85 mmol) in dry *N,N*-dimethylformamide (15 mL), anhydrous K_2CO_3 (0.18 g, 1.28 mmol) was added. The solution was stirred for 15 mins at 70-80 ^{o}C and 2-chloro-*N*-(4-sulfamoylphenyl) acetamide (**4a**, 0.23 g, 0.94 mmol) was added, followed by a pinch of potassium iodide (KI), and heated overnight. After that, the water (10 mL) was added to the reaction mixture, followed by 1 mL 6N HCl. The resulting solid was filtered, washed with water, and air dried and purification by flash column chromatography (hexane/EtOAc 20:1) afforded the title compound as a brown powder (0.21 g, 55% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.30 (t, *J*=7.1 Hz, 3H), 3.70 (s, 3H), 4.90 (s, 3H), 4.27-4.29 (q, *J*=7.3 Hz, 2H), 4.95 (s, 2H), 7.09 (dd, *J*=7.1, 2.2 Hz, 2H), 7.27 (s, 2H), 7.79 (s, 4H), 7.87 (d, *J*=8.5 Hz, 1H), 8.73 (s, 1H), 10.52 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 14.5, 61.4, 67.7, 101.6, 112.4, 113.9, 114.2, 119.7, 127.1, 132.1, 139.3, 141.6, 149.5, 156.6, 157.1, 163.2, 163.6, 166.6. HRMS (ESI): *m/z* calcd for $C_{20}H_{18}N_2O_8S$ [*M*+Na]$^+$: 469.4179, found: 469.4138.

Ethyl 7-(2-((4-(*N*-(5,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)amino)-2-oxoethoxy)-2-oxo-2*H*-chromene-3-carboxylate (5b). The yellow powder was obtained from 2-chloro-*N*-(4-(*N*-(5,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)acetamide (**4b**) by following the experimental conditions described for **5a** (0.197 g, 61% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.31 (t, *J*=7.0 Hz, 3H), 4.25-4.30 (q, *J*=7.0 Hz, 2H), 4.95 (s, 2H), 7.07 (dd, *J*=7.3, 2.1 Hz, 2H), 7.81 (d, *J*=8.8 Hz, 2H), 7.87 (d, *J*=8.5 Hz, 1H), 7.95 (d, *J*=8.8 Hz, 2H), 8.11 (s, 1H), 8.73 (s, 1H), 10.56 (s, 1H), 11.04 (br-s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 14.5, 54.5, 60.7, 61.4, 67.7, 101.6, 112.4, 113.9, 114.2, 119.4, 127.6, 129.3, 132.1, 135.4, 149.5, 150.8, 151.1, 157.1, 162.1, 163.2, 163.6, 166.7. HRMS (ESI): *m/z* calcd for $C_{26}H_{24}N_4O_{10}S$ [*M*+Na]$^+$: 607.5418, found: 607.5406.

Ethyl 7-(2-((4-(*N*-acetylsulfamoyl)phenyl)amino)-2-oxoethoxy)-2-oxo-2*H*-chromene-3-carboxylate (5c). The yellow powder was obtained from *N*-(4-(*N*-acetylsulfamoyl) phenyl)-2-chloroacetamide (**4c**) by following the experimental conditions described for **5a** (0.22 g, 58% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.28-1.32 (m, 3H), 1.91 (s, 1H), 4.25-4.30 (q, *J*=7.3 Hz, 2H), 4.96 (s, 2H), 7.08 (d, *J*=9.9 Hz, 2H), 7.83-7.89 (m, 5H), 8.73 (d, *J*=2.3 Hz, 1H), 10.63 (s, 1H), 11.98 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 14.5, 23.6, 61.4, 67.6, 101.6, 112.4, 113.9, 114.2, 119.5, 119.6, 129.3, 132.1, 133.9, 149.5, 156.6, 157.1, 163.2, 163.6, 166.8, 169.1. HRMS (ESI): *m/z* calcd for $C_{22}H_{20}N_2O_9S$ [*M*+Na]$^+$: 511.4545, found: 511.4518.

7-Hydroxy-2-oxo-2*H*-chromene-3-carboxylic acid (7a). To a solution of ethyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**2a**, 2 g, 8.54 mmol) in MeOH (15 mL) and water (12 mL) was added 2N NaOH solution (40 mL). The solution was heated to reflux for 12 h, then cooled and concentrated in vacuo. The crude product was diluted with water (10 mL) and acidified with an aqueous solution of 6N HCl. The resulting solid was filtered, washed with water, and air-dried to provide to the product as a yellow solid. (1.71 g, 97% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 6.73 (s, 1H), 6.85 (dd, *J*=8.6,2.1 Hz, 1H), 7.72 (d, *J*=8.6 Hz, 1H), 8.66 (s, 1H), 11.10 (br-s, 1H), 12.93 (br-s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 103.1, 111.0, 113.0, 114.5, 132.4, 149.7, 157.4, 158.3, 164.1, 164.5ppm. HRMS (ESI): *m/z* calcd for $C_{10}H_6O_5$ [*M*+Na]$^+$: 229.1406, found: 229.1401.

6-Chloro-2-oxo-2*H*-chromene-3-carboxylic acid (7b). The white powder was obtained from ethyl 6-chloro-2-oxo-2*H*-chromene-3-carboxylate (**2b**, 2 g, 7.92 mmol) by following the experimental conditions described for **7a** (1.725 g, 97% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.49 (d, *J*=8.8 Hz, 1H), 7.75 (dd, *J*=8.8, 2.5 Hz, 1H), 8.04 (d, *J*=2.5 Hz, 1H), 8.69 (s, 1H), 13.38 (br-s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 118.6, 119.8, 120.0, 128.8, 129.4, 134.0, 147.4, 153.5, 156.5, 164.1. HRMS (ESI): *m/z* calcd for $C_{10}H_5ClO_4$ [*M*+Na]$^+$: 247.5861, found: 247.5859.

6-Bromo-2-oxo-2*H*-chromene-3-carboxylic acid (7c). The yellow powder was obtained from ethyl 6-bromo-2-oxo-2*H*-chromene-3-carboxylate (**2c**, 2 g, 6.73 mmol) by following the experimental conditions described for **7a** (1.8 g, 99% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.42 (d, *J*=8.8 Hz, 1H), 7.86 (dd, *J*=8.8, 2.5 Hz, 1H), 8.17 (d, *J*=2.5 Hz, 1H), 8.69 (s, 1H), 13.37 (br-s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 118.6, 119.8, 120.1, 128.9, 129.3, 134.1, 148.0, 153.1, 156.3, 164.6. RMS (ESI): *m/z* calcd for $C_{10}H_5BrO_4$ [*M*+Na]$^+$: 292.0370, found: 292.0361.

6,8-Dichloro-2-oxo-2*H*-chromene-3-carboxylic acid (7d). The yellow powder was obtained from ethyl 6,8-dichloro-2-oxo-2*H*-chromene-3-carboxylate (**2d**, 2 g, 6.97 mmol) by following the experimental conditions described for **7a** (1.65 g, 91% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 8.04 (d, *J*=2.5 Hz, 1H), 8.06 (d, *J*= 2.5 Hz, 1H), 8.71 (s,1H), 13.51 (br-s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 116.7, 118.44, 120.02\, 129.0, 129.6, 133.9, 148.5, 153.4, 156.8, 164.0. HRMS (ESI): *m/z* calcd for $C_{10}H_4Cl_2O_4$ [*M*+Na]$^+$: 282.0308 , found: 282.0301.

2-Oxo-2*H*-chromene-3-carboxylic acid (7e). The white powder was obtained from ethyl 2-oxo-2*H*-chromene-3-carboxylate (**2e**, 2 g, 9.17 mmol) by following the experimental conditions described for **7a** (1.7 g, 98% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.37-7.44 (m, 2H), 7.73 (t, *J*=7.6 Hz, 1H), 7.91 (d, *J*=7.7 Hz, 1H), 8.74 (s,1H), 13.24 (br-s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 116.5, 118.4, 118.7, 125.3, 130.6, 134.6, 148.7, 154.8, 157.1, 164.3. HRMS (ESI): *m/z* calcd for $C_{10}H_6O_4$ [*M*+Na]$^+$: 213.1414 , found: 213.1410.

6-Methoxy-2-oxo-2*H*-chromene-3-carboxylic acid (7f). The yellow powder was obtained from ethyl 6-methoxy-2-oxo-2*H*-chromene-3-carboxylate (**2f**, 2 g, 8.06 mmol) by following the experimental conditions described for **7a** (1.71 g, 96% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.80 (s, 3H), 4.78 (br-s, 1H), 7.31 (dd, *J*=9, 3 Hz, 1H), 7.35 (d, *J*=9 Hz, 1H), 7.44 (d, *J*=3 Hz, 1H), 8.66 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 56.2, 112.3, 117.7, 118.8,118.9, 122.5, 148.5, 149.3, 156.1, 157.4, 164.4. HRMS (ESI): *m/z* calcd for $C_{11}H_8O_5$ [*M*+Na]$^+$: 243.1673, found: 243.1652.

7-(Diethylamino)-2-oxo-2*H*-chromene-3-carboxylic acid (7g). The brown powder was obtained from ethyl 7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylate (**2g**, 2 g, 6.91 mmol) by following the experimental conditions described for **7a** (1.53 g, 84% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.14 (t, *J*=7.0 Hz, 6H), 3.47 (q, *J*=7.0 Hz, 4H), 6.56 (d, *J*=2.0 Hz, 1H), 6.81 (dd, *J*=9.0, 2.0 Hz, 1H), 7.63 (d, *J*=9.0 Hz, 1H), 8.58 (s, 1H), 12.49 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 12.7, 44.8, 96.3, 107.6, 107.8, 110.5, 132.3, 149.9, 153.3, 158.3, 159.9, 164.9. HRMS (ESI): *m/z* calcd for $C_{14}H_{15}NO_4$ [*M*+Na]$^+$: 284.2621, found: 284.2619.

7-Methoxy-2-oxo-2*H*-chromene-3-carboxylic acid (7h). The yellow powder was obtained from ethyl 7-methoxy-2-oxo-2*H*-chromene-3-carboxylate (**2h**, 2 g, 8.06 mmol) by following the experimental conditions described for **7a** (1.54 g, 91% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.81 (s, 3H), 7.33 (dd, *J*=8.8, 2.5 Hz, 1H), 7.40 (d, *J*=8.9 Hz, 1H), 7.48 (d, *J*=2.5 Hz, 1H), 8.69 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 56.3, 112.3,

117.7, 118.8,119.0, 122.4, 148.5, 149.4, 156.2, 157.4, 164.4. HRMS (ESI): *m/z* calcd for $C_{11}H_8O_5$ [*M*+Na]$^+$: 243.1673, found: 243.1631.

6-Fluoro-2-oxo-2*H*-chromene-3-carboxylic acid (7i). The white powder was obtained from ethyl 6-fluoro-2-oxo-2*H*-chromene-3-carboxylate (**2i**, 2 g, 7.92 mmol) by following the experimental conditions described for **7a** (1.62 g, 95% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.40 (d, *J*=8.8 Hz, 1H), 7.86 (dd, *J*=8.8, 2.5 Hz, 1H), 8.17 (d, *J*=2.5 Hz, 1H), 8.69 (s, 1H), 13.37 (br-s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ= 115.4, 115.6, 118.6, 118.7, 119.3, 119.3, 119.9, 121.8, 122.0, 147.7, 147.8, 151.4, 151.4, 156.8, 157.5, 159.4, 164.3. HRMS (ESI): *m/z* calcd for $C_{10}H_5FO_4$ [*M*+Na]$^+$: 231.1328, found: 231.1318.

8-(*Tert*-butyl)-2-oxo-*2H*-chromene-3-carboxylic acid (7j). The white powder was obtained from ethyl 8-(tert-butyl)-2-oxo-2H-chromene-3-carboxylate (**2j**, 2 g, 7.92 mmol) by following the experimental conditions described for **7a** (1.42 g, 83% yield): ^{1}H NMR (500 MHz, DMSO-d_6): δ= 1.46 (s, 9H), 7.35 (t, *J*=8.0 Hz, 1H), 7.68 (d, *J*=7.5 Hz, 1H), 7.78 (d, *J*=8.0 Hz, 1H), 8.74 (s, 1H), 13.24 (br-s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ= 29.9, 35.0, 117.9, 119.0, 124.9, 129.2, 132.0, 137.0, 149.7, 153.6, 156.8, 164.4. HRMS (ESI): *m/z* calcd for $C_{14}H_{14}O_4$ [*M*+Na]$^+$: 269.2488, found: 269.2453.

7-Hydroxy-2-oxo-*N*-(4-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (9a). A solution of 7-hydroxy-2-oxo-2*H*-chromene-3-carbonyl chloride (**8a**, 0.2 g. 0.89 mmol) and sulfanilamide (**3a**, 0.17 g, 0.98 mmol) in the presence pyridine or triethylamine (1 mL) in DMF (5 mL) was stirred under reflux for 12 h. The solution was cooled, and 5 mL of 6N HCl was added, and the resulting solid was filtered off and washed with water (10 mL) and air dried to yield a white powder (0.211 g, 66% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ= 6.84 (d, *J*=2.4 Hz, 1H), 6.90 (dd, *J*=8.6, 2.4 Hz, 1H), 7.30 (t, *J*=9.1, 2H), 7.81-7.92 (m, 5H), 8.87 (d, *J*=8.6 Hz, 1H), 10.89 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 102.4, 106.4, 111.5, 113.9, 115.2, 120.0, 127.3, 132.7, 139.5, 141.3, 149.1, 157.0, 161.2, 161.7, 165.0. HRMS (ESI): *m/z* calcd for $C_{16}H_{12}N_2O_6S$ [*M*+Na]$^+$: 383.3285, found: 383.3253.

6-Chloro-2-oxo-*N*-(4-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (9b). The white powder was obtained from 6-chloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8b**, 0.2 g, 0.82 mmol) by following the experimental conditions described for **9a** (0.287 g, 92% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.32 (s, 2H), 7.61 (d, *J*=8.9 Hz, 1H), 7.83 (d, *J*=8.7 Hz, 2H), 7.85- 7.95 (m, 3H), 7.89 (d, *J*=8.9 Hz, 2H), 8.16 (d, *J*=2.6 Hz, 1H), 8.78 (s, 1H), 10.86 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 118.8, 120.1, 120.2, 121.6, 127.3, 129.4, 129.6, 134.2, 139.9,

141.1, 146.7, 153.0, 160.2, 160.6. HRMS (ESI): *m/z* calcd for $C_{16}H_{11}ClN_2O_5S$ [*M*+Na]$^+$: 401.7737, found: 401.7717.

6-Bromo-2-oxo-*N*-(4-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (9c). The white powder was obtained from 6-bromo-2-oxo-2*H*-chromene-3-carbonyl chloride (**8c**, 0.2 g, 0.70 mmol) by following the experimental conditions described for **9a** (0.243 g, 83% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.32 (s, 2H), 7.61 (d, *J*=8.8 Hz, 1H), 7.83 (dd, *J*=8.8, 2.1 Hz, 2H), 7.85 (s, 1H), 7.89 (d, *J*=8.5 Hz, 2H), 8.15 (d, *J*=2.5 Hz, 1H), 8.87 (s, 1H), 10.86 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 117.2, 119.0, 120.1, 120.7, 121.6, 127.3, 132.6, 137.0, 139.9, 141.1, 146.6, 153.4, 160.1, 160.6. HRMS (ESI): *m/z* calcd for $C_{16}H_{11}BrN_2O_5S$ [*M*+Na]$^+$: 446.2247, found: 446.2236.

6,8-Dichloro-2-oxo-*N*-(4-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (9d). The white powder was obtained from 6,8-dichloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8d**, 0.2 g, 0.72 mmol) by following the experimental conditions described for **9a** (0.214 g, 72% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.33 (s, 2H), 7.86 (d, *J*=8.8 Hz, 2H), 7.89 (d, *J*=8.8 Hz, 2H), 8.14 (s, 2H), 8.84 (s, 1H), 10.78 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 120.2, 121.2, 121.4, 122.7, 127.3, 128.7, 129.3, 133.5, 140.0, 141.0, 148.7, 159.1, 160.4, 162.7. HRMS (ESI): *m/z* calcd for $C_{16}H_{10}Cl_2N_2O_5S$ [*M*+Na]$^+$: 436.2178, found: 436.2172.

6-Methoxy-2-oxo-*N*-(4-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (9f). The yellow powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8f**, 0.2 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.298 g, 95% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.84 (s, 1H), 7.32 (s, 2H), 7.39 (d, *J*=8.2 Hz, 1H), 7.49 (d, *J*=8.9 Hz, 1H), 7.59 (d, *J*=2.2 Hz, 1H), 7.85 (d, *J*=8.6 Hz, 2H), 7.90 (d, *J*=8.6 Hz, 2H), 8.89 (s, 1H), 10.95 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 56.3, 112.3, 117.9, 119.3, 120.1, 120.2, 122.6, 127.3, 139.8, 141.1, 148.1, 148.9, 156.5, 160.8, 160.9. HRMS (ESI): *m/z* calcd for $C_{17}H_{14}N_2O_6S$ [*M*+Na]$^+$: 397.3553, found: 397.3518.

7-(Diethylamino)-2-oxo-*N*-(4-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (9g). To a solution of 7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylic acid (**7g**, 0.2 g, 0.77 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (0.14 g, 0.92 mmol), 1-hydroxybenzotriazole hydrate (HOBt•H2O) (0.124 g, 0.92 mmol) and triethylamine (TEA) (0.5 mL) in DMF (7 mL) was added sulfanilamide (**3a**, 0.132 g, 0.77 mmol). The solution was stirred at room temperature for 12 h. The water (5 mL) was added to the

solution with few drops of 6N HCl and the resulting solid was filtered off and washed with water and air-dried to yield a yellow powder (0.233 g, 73% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.15 (t, *J*=7.1 Hz, 6H), 3.52 (q, *J*=9.1 Hz, 4H), 6.68 (d, *J*=2.1 Hz, 1H), 6.86 (dd, *J*=8.9, 2.1 Hz, 1H), 7.29 (s, 2H), 7.73 (d, *J*=9 Hz, 1H), 7.82 (d, *J*=8.6 Hz, 2H), 7.88 (, d, *J*=8.6 Hz, 2H), 8.77 (s, 1H), 10.98 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 12.8, 44.9, 96.4, 108.4, 109.1, 111.0, 119.8, 127.3, 132.4, 139.3, 141.5, 148.9, 153.4, 157.9, 161.6, 162.6. HRMS (ESI): *m/z* calcd for $C_{20}H_{21}N_3O_5S$ [*M*+Na]$^+$: 438.4500, found: 438.4498.

7-Methoxy-2-oxo-*N*-(4-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (9h). The yellow powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8h**, 0.2 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.283 g, 91% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.93 (s, 1H), 7.09 (d, *J*=2.2 Hz, 1H), 7.20 (d, *J*=8.2 Hz, 1H), 7.31 (s, 2H), 7.84 (d, *J*=8.2 Hz, 2H), 7.92 (d, *J*=2.6 Hz, 2H), 7.98 (d, *J*=8.6 Hz, 1H), 8.93 (s, 1H), 10.89 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 56.8, 100.9, 112.6, 114.4, 115.7, 120.1, 127.3, 132.2, 139.7, 141.3, 148.9, 156.8, 161.1, 161.4, 165.3. HRMS (ESI): *m/z* calcd for $C_{17}H_{14}N_2O_6S$ [*M*+Na]$^+$: 397.3553, found: 397.3537.

6-Fluoro-2-oxo-*N*-(4-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (9i). The white powder was obtained from 6-fluoro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8i**, 0.2 g, 0.82 mmol) by following the experimental conditions described for **9a** (0.294 g, 94% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.32 (s, 2H), 7.64- 7.69 (m, 2H), 7.85 (d, *J*=8.7 Hz, 2H), 7.90- 7.93 (m, 3H), 8.89 (s, 1H), 10.89 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 115.5, 115.8, 118.9, 119.8, 120.1, 121.5, 122.1, 127.3, 139.9, 141.1, 147.1, 150.8, 159.9, 160.4, 160.7. HRMS (ESI): *m/z* calcd for $C_{16}H_{11}ClN_2O_5S$ [*M*+Na]$^+$: 385.3238, found: 385.3213.

8-(tert-butyl)-2-oxo-*N*-(4-sulfamoylphenyl)-*2H*-chromene-3-carboxamide (9j). The white powder was obtained from 8-(tert-butyl)-2-oxo-*2H*-chromene-3-carbonyl chloride (**8j**, 0.2 g, 0.82 mmol) by following the experimental conditions described for **9a** (0.196 g, 71% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.49 (s, 9H), 7.32 (s, 2H), 7.42 (t, *J*=8.7 Hz, 1H), 7.73 (dd, *J*=7.6, 2.1 Hz, 1H), 7.83- 7.92 (m, 5H), 8.89 (s, 1H), 10.91 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 29.9, 35.0, 119.39, 119.47, 120.1, 125.4, 127.3, 129.3, 132.2, 137.2, 139.8, 141.3, 148.9, 153.0, 160.2, 160.9. HRMS (ESI): *m/z* calcd for $C_{20}H_{20}N_2O_5S$ [*M*+Na]$^+$: 423.4398, found: 423.4351.

***N*-(4-(*N*-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-7-hydroxy-2-oxo-2*H*-chromene-3-carboxamide (10a).** The white powder was obtained from 7-hydroxy-2-oxo-2*H*-chromene-3-carbonyl chloride (**8a**, 0.2 g, 0.84

mmol) and sulfamethazine (**3e**, 0.233 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.21 g, 54% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 2.26 (s, 6H), 6.76 (s, 1H), 6.85 (d, *J*=2.1 Hz, 1H), 6.92 (dd, *J*=8.6, 2.1 Hz, 1H), 7.85-7.89 (m, 3H), 7.98 (d, *J*=8.7 Hz, 2H), 8.86 (s, 1H), 10.89 (s, 1H), 11.18 (s, 1H), 11.66 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 23.3, 102.4, 111.6, 114.3, 115.0, 119.4, 129.8, 132.7, 135.9, 141.9, 149.1, 156.6, 156.9, 161.1, 161.6, 164.6. HRMS (ESI): *m/z* calcd for $C_{22}H_{18}N_4O_6S$ [*M*+Na]$^+$: 489.4538, found: 489.4517.

6-Chloro-*N*-(4-(*N*-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (10b). The white powder was obtained from 6-chloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8b**, 0.2 g, 0.82 mmol) and sulfamethazine (**3e**, 0.228 g, 0.82 mmol) by following the experimental conditions described for **9a** (0.254 g, 63% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 2.26 (s, 6H), 6.75 (s, 1H), 7.60 (d, *J*=8.8 Hz, 1H), 7.79 (dd, *J*=8.6, 2.5 Hz, 1H), 7.89 (d, *J*=8.8 Hz, 2H), 8.00 (d, *J*=8.8 Hz, 2H), 8.14 (d, *J*=2.5 Hz, 1H), 8.84 (s, 1H), 10.85 (s, 1H),11.81 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 23.3, 112.5, 118.7, 119.4, 119.8, 120.1, 121.6, 129.4, 129.6, 129,8, 130.3, 134.2, 136.3, 141.7, 146.7, 150.2, 152.9, 156.6, 160.1, 160.6. HRMS (ESI): *m/z* calcd for $C_{22}H_{17}ClN_4O_5S$ [*M*+Na]$^+$: 507.8985, found: 507.8975.

6-Bromo-*N*-(4-(*N*-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (10c). The white powder was obtained from 6-bromo-2-oxo-2*H*-chromene-3-carbonyl chloride (**8c**, 0.2 g, 0.70 mmol) and sulfamethazine (**3e**, 0.194 g, 0.70 mmol) by following the experimental conditions described for **9a** (0.269 g, 72% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 2.26 (s, 6H), 6.76 (s, 1H), 7.54 (d, *J*=8.8 Hz, 1H), 7.89 (d, *J*=8.8 Hz, 2H), 7.94 (dd, *J*=8.8, 2.3 Hz, 1H), 8.00 (d, *J*=8.8 Hz, 2H), 8.27 (d, *J*=2.3 Hz, 1H), 8.84 (s, 1H), 10.85 (s, 1H),11.68 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 23.3, 112.5, 117.2, 119.0, 119.5, 120.6, 121.6, 129.8, 129.9, 132.6, 137.0, 141.7, 146.6, 153.4, 156.6, 160.1, 160.6 ppm. HRMS (ESI): *m/z* calcd for $C_{22}H_{17}BrN_4O_5S$ [*M*+Na]$^+$: 552.3499, found: 552.3443.

***N*-(4-(*N*-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-6-methoxy-2-oxo-2*H*-chromene-3-carboxamide (10f).** The white powder was obtained from 6-methoxy-2-oxo-2*H*-chromene-3-carbonyl chloride (**8f**, 0.2 g, 0.84 mmol) and sulfamethazine (**3e**, 0.234 g, 0.84 mmol) by following the experimental conditions described for **5.9a** (0.367 g, 91% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 2.27 (s, 6H), 3.84 (s, 3H), 6.77 (s, 1H), 7.39 (dd, *J*=9.0, 2.9 Hz, 1H), 7.50 (d, *J*=9.0 Hz, 1H), 7.59 (d, *J*=2.9 Hz, 1H), 7.90 (d, *J*=8.8 Hz, 2H), 8.02 (d, *J*=8.8 Hz, 2H), 8.87

(s, 1H), 10.96 (s, 1H), 11.59 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 23.3, 56.3, 112.3, 113.8, 117.8, 119.3, 119.4, 120.1, 122.8, 123.4, 136.2, 141.8, 148.1, 148.9, 156.5, 160.8, 160.9. HRMS (ESI): *m/z* calcd for $C_{23}H_{20}N_4O_6S$ [*M*+Na]$^+$: 503.4807, found: 503.4802.

7-Hydroxy-*N*-(4-(*N*-(6-methoxypyridazin-3-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (11a). The white powder was obtained from 7-hydroxy-2-oxo-2*H*-chromene-3-carbonyl chloride (**8a**, 0.2 g, 0.84 mmol) and sulfamethoxypyridazine (**3f**, 0.235 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.237 g, 60% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.84 (s, 3H), 6.85 (d, *J*=2.0 Hz, 1H), 6.91 (dd, *J*=8.6, 2.0 Hz, 1H), 7.38 (d, *J*=8.5 Hz, 1H), 7.76 (br-s, 1H), 7.82-7.88 (m, 6H), 8.87 (s, 1H), 10.87 (s, 1H), 11.22 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 54.9, 102.3, 111.1, 111.6, 114.2, 115.1, 120.2, 127.9, 132.7, 141.5, 149.1, 153.6, 156.9, 161.1, 161.6, 164.7. HRMS (ESI): *m/z* calcd for $C_{21}H_{16}N_4O_7S$ [*M*+Na]$^+$: 491.4265, found: 491.4241.

6-Chloro-*N*-(4-(*N*-(6-methoxypyridazin-3-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (11b). The white powder was obtained from 6-chloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8b**, 0.2 g, 0.82 mmol) and sulfamethoxypyridazine (**3f**, 0.228 g, 0.82 mmol) by following the experimental conditions described for **9a** (0.326 g, 82% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.84 (s, 3H), 7.39 (br-s, 1H), 7.62 (d, *J*=8.7 Hz, 1H), 7.82-7.91 (m, 5H), 8.15 (d, *J*=2.2 Hz, 1H), 8.85 (s, 1H), 10.84 (s, 1H), 13.84 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 54.9,118.8, 120.2, 120.7, 121.6, 127.8, 129.4, 129.7, 134.2, 141.4, 146.1, 146.7, 153.0, 160.1, 160.6. HRMS (ESI): *m/z* calcd for $C_{21}H_{15}ClN_4O_6S$ [*M*+Na]$^+$: 509.8711, found: 509.8709.

6-Bromo-*N*-(4-(*N*-(6-methoxypyridazin-3-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (11c). The white powder was obtained from 6-bromo-2-oxo-2*H*-chromene-3-carbonyl chloride (**8c**, 0.2 g, 0.70 mmol) and sulfamethoxypyridazine (**3f**, 0.196 g, 0.70 mmol) by following the experimental conditions described for **9a** (0.309 g, 83% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.84 (s, 3H), 7.40 (br-s, 1H), 7.55 (d, *J*=8.8 Hz, 1H), 7.84- 7.94 (m, 4H), 7.92 (dd, *J*=8.8, 2.4 Hz, 2H), 8.28 (d, *J*=2.4 Hz, 1H), 8.84 (s, 1H), 10.84 (s, 1H), 13.81 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 54.9,117.2, 119.0, 120.2, 120.7, 121.6, 127.8, 132.6, 137.0, 141.3, 146.6, 153.4, 160.1, 160.6 ppm. HRMS (ESI): *m/z* calcd for $C_{21}H_{15}BrN_4O_6S$ [*M*+Na]$^+$: 554.3226, found: 554.3213.

6,8-Dichloro-*N*-(4-(*N*-(6-methoxypyridazin-3-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (11d). The white powder was obtained from 6,8-dichloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8d**, 0.2 g, 0.72

mmol) and sulfamethoxypyridazine (**3f**, 0.202 g, 0.72 mmol) by following the experimental conditions described for **9a** (0.263 g, 70% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.85 (s, 3H), 7.40 (br-s, 1H), 7.87- (m, 5H), 8.13 (s, 2H), 7.82 (s, 1H), 10.77 (s, 1H),13.83 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.9, 120.2, 121.2, 121.4, 122.7, 127.9, 128.2, 128.6, 129.3, 133.4, 141.3, 146.1, 148.7, 151.4, 153.9, 159.1, 160.4. HRMS (ESI): *m/z* calcd for $C_{21}H_{14}Cl_2N_4O_6S$ [*M*+Na]$^+$: 544.3155, found: 544.3108.

6-Methoxy-*N*-(4-(*N*-(6-methoxypyridazin-3-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (11f). The white powder was obtained from 6-methoxy-2-oxo-2*H*-chromene-3-carbonyl chloride (**8f**, 0.2 g, 0.84 mmol) and sulfamethoxypyridazine (**3f**, 0.235 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.328 g, 81% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.83 (d, *J*=4.0 Hz 6H), 7.37 (dd, *J*=6.4, 2.1 Hz, 2H), 7.47 (d, *J*=8.8 Hz, 1H), 7.56 (d, *J*=4.0 Hz, 1H), 7.84-7.89 (m, 4H), 7.89 (s, 1H), 8.88 (s,1H), 10.93 (s, 1H), 13.93 (be-s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 54.9, 56.3, 107.2, 112.3, 117.8, 119.3, 120.1, 122.8, 127.9, 129.8, 130.1, 135.6, 141.4, 148.0, 148.9, 156.5, 160.8. HRMS (ESI): *m/z* calcd for $C_{22}H_{18}N_4O_7S$ [*M*+Na]$^+$: 505.4531, found: 505.4522.

6-Chloro-2-oxo-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)-2*H*-chromene-3-carboxamide (12b). The white powder was obtained from 6-chloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8b**, 0.2 g, 0.82 mmol) and sulfapyridine (**3d**, 0.204 g, 0.82 mmol) by following the experimental conditions described for **9a** (0.298 g, 80% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 6.88 (t, *J*=7.5 Hz, 1H), 7.17 (d, *J*=8.6 Hz, 1H), 7.61 (d, *J*=8.6 Hz, 1H), 7.72 (t, *J*=8.5 Hz, 1H), 7.83-7.89 (m, 6H), 8.02 (br-s, 1H), 8.15 (s, 1H), 8.84 (s, 1H),10.84 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 101.9, 113.1, 119.0 119.7, 120.4, 120.8, 121.6, 128.2, 132.6, 137.7, 141.2, 149.6, 153.4, 160.1, 160.9. HRMS (ESI): *m/z* calcd for $C_{21}H_{14}ClN_3O_5S$ [*M*+Na]$^+$: 478.8573, found: 478.8539.

6-Bromo-2-oxo-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)-2*H*-chromene-3-carboxamide (12c). The white powder was obtained from 6-bromo-2-oxo-2*H*-chromene-3-carbonyl chloride (**8c**, 0.2 g, 0.70 mmol) and sulfapyridine (**3d**, 0.18 g, 0.70 mmol) by following the experimental conditions described for **9a** (0.288 g, 82% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 6.88 (t, *J*=7.5 Hz, 1H), 7.17 (d, *J*=8.6 Hz, 1H), 7.54 (d, *J*=8.6 Hz, 1H), 7.72 (t, *J*=7.5 Hz, 1H), 7.85-7.94 (m, 6H), 8.02 (br-s, 1H), 8.28 (d, *J*=2.4 Hz, 1H), 8.84 (s, 1H),10.3 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 101.6, 112.8, 117.2, 119.0, 120.1, 120.7, 121.6, 128.3, 132.6, 137.0, 141.5, 146.5, 153.4, 160.0, 160.6. HRMS (ESI): *m/z* calcd for $C_{21}H_{14}BrN_3O_5S$ [*M*+Na]$^+$: 523.3089, found: 523.3059.

6,8-Dichloro-2-oxo-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)-2*H*-chromene-3-carboxamide (12d). The white powder was obtained from 6,8-dichloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8d**, 0.2 g, 0.72 mmol) and sulfapyridine (**3d**, 0.18 g, 0.72 mmol) by following the experimental conditions described for **9a** (0.210 g, 59% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 6.88 (t, *J*=6.6 Hz, 1H), 7.17 (d, *J*=8.4 Hz, 1H), 7.73 (dt, *J*=7.1, 1.9 Hz, 1H), 7.84- 7.91 (m, 4H), 8.02 (d, *J*=2.4 Hz, 1H), 8.12 (s, 2H), 8.81 (s, 1H),10.76 (s,1H), 12.02 (br-s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 114.1, 120.1, 121.2,121.4, 122.7, 128.3, 128.6, 129.3, 133.4, 137.6, 140.9, 141.4, 146.1, 148.7, 153.5, 159.0, 160.4. HRMS (ESI): *m/z* calcd for $C_{21}H_{13}Cl_2N_3O_5S$ [*M*+Na]$^+$: 513.3020, found: 513.3007.

2-Oxo-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)-2*H*-chromene-3-carboxamide (12e). The white powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8e**, 0.2 g, 0.96 mmol) and sulfapyridine (**3d**, 0.24 g, 0.96 mmol) by following the experimental conditions described for **9a** (0.389 g, 96% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 6.87 (t, *J*=6.2 Hz, 1H), 7.17 (d, *J*=8.6 Hz, 1H), 7.47 (t, *J*=7.4 Hz, 1H), 7.56 (d, *J*=8.4 Hz, 1H), 7.78 (dt, *J*=6.3 Hz 1H), 7.84- 7.90 (q, *J*=6.2, 4H), 8.02 (d, *J*=7.0 Hz, 2H), 8.90 (s, 1H), 10.88 (s, 1H),11.94 (br,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 114.1, 116.0, 116.7, 118.8, 120.0, 120.3, 120.4, 125.7, 128.3, 130.8, 134.9, 137.3, 140.7, 141.6, 148.1, 154.4, 160.6, 160.9. HRMS (ESI): *m/z* calcd for $C_{21}H_{15}N_3O_5S$ [*M*+Na]$^+$: 444.4133, found: 444.4103.

2-Oxo-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)-2*H*-chromene-3-carboxamide (12f). The white powder was obtained from 6-methoxy-2-oxo-2*H*-chromene-3-carbonyl chloride (**8f**, 0.2 g, 0.84 mmol) and sulfapyridine (**3d**, 0.21 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.249 g, 66% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.84 (s, 3H), 6.88 (t, *J*=6.1 Hz, 1H), 7.18 (d, *J*=8.6 Hz, 1H), 7.38 (dd, *J*=7.4, 2.0 Hz, 1H), 7.50 (d, *J*=7.6 Hz, 1H), 7.59 (d, *J*=2.1 Hz 1H), 7.73 (dt, *J*=6.6,1.9 Hz, 1H), 7.86- 7.91 (q, *J*=7.3 Hz 4H), 8.02 (d, *J*=7.0 Hz, 2H), 8.87 (s, 1H), 10.93 (s, 1H), 11.93 (br-s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 56.3, 112.3, 114.1, 117.8, 119.3, 120.1, 120.2, 122.8, 128.4, 140.8, 141.5, 148.1, 148.9, 156.5, 160.8. HRMS (ESI): *m/z* calcd for $C_{22}H_{17}N_3O_6S$ [*M*+Na]$^+$: 474.4393, found: 474.4329.

6-Chloro-*N*-(4-(*N*-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (13b). The white powder ound was obtained from 6-chloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8b**, 0.2 g, 0.82 mmol) and sulfamethizole (**3g**, 0.222 g, 0.82 mmol) by following the experimental conditions described for

9a (0.288 g, 74% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 2.47 (s, 3H), 7.62 (d, *J*=8.8 Hz, 1H), 7.80 (d, *J*=8.8 Hz, 2H), 7.88 (d, *J*=8.8 Hz, 3H), 8.15 (d, *J*=2.4 Hz 1H), 8.84 (s, 1H), 10.86 (s, 1H), 13.94 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6):δ= 16.5, 118.8, 120.0, 120.3, 121.6, 127.5, 129.4, 129.6, 134.2, 137.5, 141.6, 146.7, 153.0, 155.1, 160.1, 160.6, 168.3. HRMS (ESI): *m/z* calcd for $C_{19}H_{13}ClN_4O_5S_2$ [*M*+Na]$^{+}$: 499.8982, found: 499.8936.

6-Bromo-*N*-(4-(*N*-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (13c). The white powder was obtained from 6-bromo-2-oxo-2*H*-chromene-3-carbonyl chloride (**8c**, 0.2 g, 0.70 mmol) and sulfamethizole (**3g**, 0.189 g, 0.70 mmol) by following the experimental conditions described for **9a** (0.277 g, 76% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 2.47 (s, 3H), 7.55 (d, *J*=8.8 Hz, 1H), 7.82 (d, *J*=8.8 Hz, 2H), 7.90 (d, *J*=7.8 Hz, 2H), 7.94 (dd, *J*=7.4, 2.1 Hz, 1H), 8.28 (d, *J*=2.4 Hz, 1H), 8.84 (s, 1H), 10.86 (s, 1H), 13.94 ppm (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 16.5, 117.2, 119.0, 120.3, 120.7, 121.6, 127.5, 132.6, 137.0, 137.5, 141.7, 146.6, 153.4, 155.0, 160.1, 160.6, 168.3. HRMS (ESI): *m/z* calcd for $C_{19}H_{13}BrN_4O_5S_2$ [*M*+Na]$^{+}$: 544.3507, found: 544.3501.

6-Chloro-*N*-(4-(*N*-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (14b). The white powder was obtained from 6-chloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8b**, 0.2 g, 0.82 mmol) and sulfadimethoxine (**3h**, 0.254 g, 0.82 mmol) by following the experimental conditions described for **9a** (0.312 g, 73% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.77 (s, 3H), 3.80 (s,3H), 5.96 (s, 1H), 7.61 (d, *J*=8.8 Hz, 1H), 7.81 (dd, *J*=8.8, 2.4 Hz, 1H), 7.92- 7.97 (q, *J*=7.6, 4H), 8.14 (d, *J*= 2.4 Hz, 1H), 8.85 (s, 1H), 10.90 (s, 1H), 11.59 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 54.2, 55.0, 85.0, 118.8, 120.1, 120.2, 121.6, 129.1, 129.2, 129.6, 134.2, 135.2, 142.4, 146.7, 153.0, 160.0, 160.3, 160.8, 164.6, 172.1. HRMS (ESI): *m/z* calcd for $C_{22}H_{17}ClN_4O_7S$ [*M*+Na]$^{+}$: 539.8971, found: 539.8957.

6-Bromo-*N*- (4-(*N*-(2,6-dimethoxypyrimidin-4-yl) sulfamoyl)phenyl) -2-oxo-2*H*-chromene -3-carboxamide (14c). The white compound was obtained from 6-bromo-2-oxo-2*H*-chromene-3-carbonyl chloride (**8c**, 0.2 g, 0.70 mmol) and sulfadimethoxine (**3h**, 0.217 g, 0.70 mmol) by following the experimental conditions described for **9a** (0.322 g, 82% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.77 (s, 3H), 3.80 (s,3H), 5.96 (s, 1H), 7.54 (d, *J*=8.8 Hz, 1H), 7.91- 7.98 (m, 5H), 8.15 (d, *J*=2.4 Hz, 1H), 8.84 (s, 1H), 10.90 (s, 1H), 11.56 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 54.2, 55.0, 85.0, 117.2, 119.0, 120.2, 120.6, 121.6, 129.1, 132.6, 137,0, 142.4, 146.6, 153.4,

160.0, 160.3, 160.8, 162.7, 172.1. HRMS (ESI): *m/z* calcd for $C_{22}H_{17}BrN_4O_7S$ [*M*+Na]$^+$: 584.3486, found: 584.3497.

***N*-(4-(*N*-(2,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (14e).** The white powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8e**, 0.2 g, 0.96 mmol) and sulfadimethoxine (**3h**, 0.298 g, 0.96 mmol) by following the experimental conditions described for **9a** (0.377 g, 81% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.77 (s, 3H), 3.80 (s,3H), 5.96 (s, 1H), 7.47 (t, *J*=7.6 Hz, 1H), 7.56 (d, *J*=8.4 Hz, 1H), 7.56 (t, *J*=8.6 Hz, 1H), 7.92- 8.01 (m, 5H), 8.90 (s, 1H), 10.94 (s, 1H), 11.57 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 54.2, 55.0, 85.0, 116.7, 118.8, 120.2, 120.2, 125.7, 129.1, 130.8, 134,9, 135.1, 142.5, 148.1, 154.4, 160.3, 160.5, 151.1, 164.7, 172.1. HRMS (ESI): *m/z* calcd for $C_{22}H_{18}N_4O_7S$ [*M*+Na]$^+$: 505.4531, found: 505.4509.

***N*-(4-(*N*-acetylsulfamoyl)phenyl)-6-chloro-2-oxo-2*H*-chromene-3-carboxamide (15b).** The white powder was obtained from 6-chloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8b**, 0.2 g, 0.82 mmol) and sulfacetamide (**3c**, 0.176 g, 0.82 mmol) by following the experimental conditions described for **9a** (0.302 g, 88% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.93 (s, 3H), 7.61 (d, *J*=8.8 Hz, 1H), 7.81 (dd, *J*=8.8, 2.5 Hz, 1H), 7.91- 7.96 (m, 4H), 8.14 (d, *J*=2.5 Hz, 1H), 8.86 (s, 1H), 10.93 (s, 1H), 12.04 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 23.6, 116.6, 118.8, 120.1, 120.6, 121.7, 129.5, 134.3, 134.6, 139.0, 142.7, 146.7, 153.0, 160.1, 160.6, 169.2. HRMS (ESI): *m/z* calcd for $C_{18}H_{13}ClN_2O_6S$ [*M*+Na]$^+$: 443.8101, found: 443.8095.

***N*-(4-(*N*-acetylsulfamoyl)phenyl)-6-bromo-2-oxo-2*H*-chromene-3-carboxamide (15c).** The white powder was obtained from 6-bromo-2-oxo-2*H*-chromene-3-carbonyl chloride (**8c**, 0.2 g, 0.70 mmol) and sulfacetamide (**3c**, 0.15 g, 0.70 mmol) by following the experimental conditions described for **9a** (0.285 g, 87% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.93 (s, 3H), 7.54 (d, *J*=8.8 Hz, 1H), 7.90-7.95 (m, 5H), 8.28 (d, *J*=2.4 Hz, 1H), 8.85 (s, 1H), 10.93 (s, 1H), 12.05 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 23.6, 117.2, 119.1, 120.1, 120.6, 121.6, 129.4, 132.6, 134.6, 137.1, 142.7, 146.7, 153.4, 160.0, 160.8, 169.1. HRMS (ESI): *m/z* calcd for $C_{18}H_{13}BrN_2O_6S$ [*M*+Na]$^+$: 488.2614, found: 488.2601.

***N*-(4-(*N*-acetylsulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (15e).** The white powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8e**, 0.2 g, 0.96 mmol) and sulfacetamide (**5.3c**, 0.21 g, 0.96 mmol) by following the experimental conditions described for **9a** (0.329 g, 89% yield): ^{1}H NMR (400 MHz, DMSO-d_6):

δ= 1.93 (s, 3H), 7.49 (t, *J*=7.9 Hz, 1H), 7.58 (d, *J*=8.8 Hz, 1H), 7.80 (t, *J*=8.8 Hz, 1H), 7.91- 7.97 (q, *J*=7.6, 4H), 8.02 (dd, *J*=6.9, 2.1 Hz, 1H), 8.93 (s, 1H), 10.97 (s, 1H), 12.05 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 23.7, 116.7, 118.8, 120.1, 120.3, 125.8, 129.4, 130.8, 134.6, 134.9, 142.8, 148.2, 154.4, 160.5, 161.1, 169.2. HRMS (ESI): *m/z* calcd for $C_{18}H_{14}N_2O_6S$ [*M*+Na]$^+$: 409.3660, found: 409.3641.

***N*-(4-(*N*-acetylsulfamoyl)phenyl)-6-methoxy-2-oxo-2*H*-chromene-3-carboxamide (15f).** The white powder was obtained from 6-methoxy-2-oxo-2*H*-chromene-3-carbonyl chloride (**8f**, 0.2 g, 0.84 mmol) and sulfacetamide (**3c**, 0.18 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.297 g, 85% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.93 (s, 3H), 3.85 (s, 3H), 7.40 (dd, *J*=9.0, 2.8 Hz, 1H), 7.50 (d, *J*=9.0 Hz, 1H), 7.59 (dd, *J*=2.8 Hz, 1H), 7.91- 7.96 (m, 4H), 8.89 (s, 1H), 11.01 (s, 1H), 12.05 (br-s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 23.7, 56.3, 112.4, 117.9, 119.3, 120.1, 120.3, 122.8, 129.4, 134.5, 142.8, 148.1, 148.9, 156.5, 160.8, 161.1, 169.2. HRMS (ESI): *m/z* calcd for $C_{19}H_{16}N_2O_7S$ [*M*+Na]$^+$: 439.3920, found: 439.3901.

6-Chloro-2-oxo-*N*-(3-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (16b). The white powder was obtained from 6-chloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8b**, 0.2 g, 0.82 mmol) and 3-aminobenzenesulfonamide (**3i**, 0.141 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.273 g, 85% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.43 (s, 2H), 7.59- 7.62 (m, 3H), 7.82 (dd, *J*=8.8, 2.5 Hz, 1H), 7.87 (t, *J*=7.8, 1H), 8.16 (, d, *J*=2.5 Hz, 1H), 8.28 (s, 1H), 8.85 (s, 1H), 10.83 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 117.4, 118.8, 120.2, 121.8, 123.3, 129.4, 129.5, 130.2, 134.2, 138.7, 145.3, 146.4, 153.0, 160.1, 160.6, 162.7. HRMS (ESI): *m/z* calcd for $C_{16}H_{11}ClN_2O_5S$ [*M*+Na]$^+$: 401.7737, found: 401.7703.

6-Bromo-2-oxo-*N*-(3-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (16c). The white powder was obtained from 6-bromo-2-oxo-2*H*-chromene-3-carbonyl chloride (**8c**, 0.2 g, 0.70 mmol) and 3-aminobenzenesulfonamide (**3i**, 0.121 g, 0.70 mmol) by following the experimental conditions described for **9a** (0.267 g, 90% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.43 (s, 2H), 7.52- 7.60 (m, 2H), 7.92 (dd, *J*=8.8, 2.5 Hz, 3H), 8.29 (s, 2H), 8.84 (s, 1H), 10.82 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 117.2, 117.4, 119.0, 120.7, 121.8, 123.3, 130.2, 132.6, 136.6, 138.7, 145.3, 146.4, 153.0, 160.1, 160.5, 162.7. HRMS (ESI): *m/z* calcd for $C_{16}H_{11}BrN_2O_5S$ [*M*+Na]$^+$: 446.2247, found: 446.2211.

2-Oxo-*N*-(3-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (16e). The white powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8e**, 0.2 g, 0.96 mmol) and 3-aminobenzenesulfonamide (**3i**, 0.17 g, 0.96

mmol) by following the experimental conditions described for **9a** (0.248 g, 75% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.43 (s, 2H), (t, *J*= 8.3 Hz, 1H), 7.56- 7.61 (m, 3H), 7.80 (t, *J*= 8.3 Hz, 1H), 7.90 (d, *J*=6.7 Hz, 1H), 8.03 (d, *J*=7.7 Hz, 1H), 8.29 (s, 1H), 8.91 (s, 1H), 10.87 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 116.7, 117.3, 118.8, 120.5, 121.7, 123.3, 125.7, 130.2, 130.8, 134.8, 138.7, 145.3, 147.9, 154.3, 160.6, 160.8. HRMS (ESI): *m/z* calcd for $C_{16}H_{12}N_2O_5S$ [*M*+Na]$^+$: 367.3292, found: 367.3224.

6-Methoxy-2-oxo-*N*-(3-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (16f). The white powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8f**, 0.2 g, 0.84 mmol) and 3-aminobenzenesulfonamide (**3i**, 0.145 g, 0.96 mmol) by following the experimental conditions described for **9a** (0.211 g, 69% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.85 (s, 3H), 7.40 (dd, *J*=6.8, 2.1 Hz, 1H), 7.43 (s, 2H), 7.50 (dd, *J*=8.2, 2.1 Hz, 1H), 7.58- 7.61 (m, 3H), 7.89 (dd, *J*=8.7, 2.0 Hz, 1H), 8.30 (s, 1H), 8.88 (s, 1H), 10.91 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 56.3, 112.35, 117.3, 117.9, 119.3, 120.4, 121.7, 122.8, 123.3, 130.2, 138.7, 145.3, 147.8, 148.9, 156.5, 160.8. HRMS (ESI): *m/z* calcd for $C_{17}H_{14}N_2O_6S$ [*M*+Na]$^+$: 397.3553, found: 397.3516.

7-Methoxy-2-oxo-*N*-(3-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (16h). The white powder was obtained from 3-oxo-2*H*-chromene-3-carbonyl chloride (**8h**, 0.2 g, 0.84 mmol) and 3-aminobenzenesulfonamide (**3i**, 0.145 g, 0.96 mmol) by following the experimental conditions described for **9a** (0.246 g, 84% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.93 (s, 3H), 7.10 (dd, *J*=6.6, 2.0 Hz, 1H), 7.19 (d, *J*=7.6 Hz, 1H), 7.42 (s, 2H), 7.58 (d, *J*=7.4 Hz, 2H), 7.89 (d, *J*=7.6 Hz, 1H), 7.97 (d, *J*=7.6 Hz, 1H), 8.29 (s, 1H), 8.91 (s, 1H), 10.85 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 56.8, 100.92, 112.6, 114.3, 115.8, 117.3, 121.6, 123.3, 130.2, 132.2, 138.8, 145.3, 148.6, 156.8, 161.0, 161.3 165.2. HRMS (ESI): *m/z* calcd for $C_{17}H_{14}N_2O_6S$ [*M*+Na]$^+$: 397.3553, found: 397.3529.

6-Fluoro-2-oxo-*N*-(3-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (16i). The white powder was obtained from 6-fluoro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8i**, 0.2 g, 0.82 mmol) and 3-aminobenzenesulfonamide (**3i**, 0.141 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.291 g, 93% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.43 (s, 2H), 7.59- 7.68 (m, 4H), 7.88- 6.93 (m, 2H), 8.29 (s, 1H), 8.87 (s, 1H), 10.86 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 115.7, 117.4, 118.9, 119.7, 121.8, 123.3, 130.2, 138.7, 145.3, 146.4, 150.8, 157.5, 159.9, 160.3, 160.6. HRMS (ESI): *m/z* calcd for $C_{16}H_{11}FN_2O_5S$ [*M*+Na]$^+$: 385.3238, found: 385.3224.

***N*-(4-(*N*-(5,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-7-hydroxy-2-oxo-2H-chromene-3-carboxamide (17a).** The white powder was obtained from 7-hydroxy-2-oxo-2*H*-chromene-3-carbonyl chloride (**8a**, 0.2 g, 0.96 mmol) and sulfadoxin (**3b**, 0.298 g, 0.96 mmol) by following the experimental conditions described for **9a** (0.17 g, 41% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.71 (s, 3H), 3.91 (s, 3H), 6.85 (d, *J*=7.8 Hz, 1H), 6.91 (dd, *J*=7.6, 2.0 Hz, 1H), 7.85-7.92 (m, 3H), 8.00 (d, *J*=7.8 Hz, 2H), 8.13 (s, 1H), 8.87 (s, 1H), 10.91 (s, 1H), 11.16 (br-s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 54.5, 60.7, 102.4, 111.6, 114.3, 115.1, 119.8, 127.8, 129.3, 132,8, 135.9, 150.8, 156.9, 161.3, 161.5, 162.1, 164.6.HRMS (ESI): *m/z* calcd for $C_{22}H_{18}N_4O_8S$ [*M*+Na]$^+$: 521.4571, found: 521.4541.

***N*-(4-(*N*-(5,6-dimethoxypyrimidin-4-yl)sulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (17e).** The white powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8e**, 0.2 g, 0.96 mmol) and sulfadoxin (**3b**, 0.298 g, 0.96 mmol) by following the experimental conditions described for **9a** (0.299 g, 64% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.71 (s, 3H), 3.91 (s, 3H), 7.49 (t, *J*=7.4 Hz, 1H), 7.57 (d, *J*=8.4 Hz, 1H), 7.79 (t, *J*=7.7 Hz, 1H), 7.93 (d, *J*=8.7 Hz, 2H), 8.01 (d, *J*=8.7 Hz, 3H), 8.13 (s, 1H), 8.91 (s, 1H), 10.93 (s, 1H), 11.11 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 54.5, 60.7, 116.7, 118.8, 119.9, 120.3, 125.8, 127.7, 129.3, 130,8, 134.9, 136.0, 142.2, 148.1, 150.7, 151.1, 154.4, 160.6, 161.1, 162.1. HRMS (ESI): *m/z* calcd for $C_{22}H_{18}N_4O_7S$ [*M*+Na]$^+$: 505.4531, found: 505.4510.

***N*-(2,4-disulfamoyl-5-(trifluoromethyl)phenyl)-6-methoxy-2-oxo-2*H*-chromene-3-carboxamide (18f).** The yellow powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8f**, 0.2 g, 0.84 mmol) and 4-amino-6-(trifluoromethyl)benzene-1,3-disulfonamide (**3j**, 0.268 g, 0.96 mmol) by following the experimental conditions described for **9a** (0.217 g, 49% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.85 (s, 3H), 7.43 (dd, *J*=8.9, 2.6 Hz, 1H), 7.55 (d, *J*=9.0 Hz, 1H), 7.64 (d, *J*=2.6 Hz, 1H), 7.89 (m, 4H), 8.66 (s, 1H), 8.86 (s, 1H), 9.06 (s, 1H) 11.54 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 56.3, 117.1, 118.7, 119.2, 120.4, 121.6, 123.1, 125.9, 130.7, 131.2, 135.1, 139.9, 143.4, 147.1, 156.3, 160.2, 160.9. HRMS (ESI): *m/z* calcd for $C_{18}H_{14}F_3N_3O_8S_2$ [*M*+Na]$^+$: 544.4306, found: 544.4302.

7-Hydroxy-2-oxo-N-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)-2H-chromene-3-carboxamide (19a). The white powder was obtained from 7-hydroxy-2-oxo-2*H*-chromene-3-carbonyl chloride (**8a**, 0.2 g, 0.84 mmol) and Sulfadiazine (**3h**, 0.233 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.19 g, 47%

yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 6.85 (d, *J*=2.5 Hz, 1H), 6.93 (dd, *J*=7.6, 2.1 Hz, 1H), 7.05 (t, *J*=8.6, Hz, 1H), 7.86-7.91 (m, 3H), 7.98 (d, *J*=8.7 Hz, 2H), 8.52 (d, *J*=7.6 Hz, 2H), 8.87 (s, 1H), 10.91 (s, 1H), 11.20 (br-s,1H), 11.74 (s,1H). HRMS (ESI): *m/z* calcd for $C_{20}H_{14}N_4O_6S$ [*M*+Na]$^+$: 461.4051, found: 461.4019.

6-Chloro-2-oxo-*N*-(4-sulfamoylphenethyl)-*2H*-chromene-3-carboxamide (20b). The white powder was obtained from 6-chloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8b**, 0.2 g, 0.82 mmol) and 4-(2-aminoethyl)benzenesulfon amide (**3h**, 0.17 g, 0.94 mmol) by following the experimental conditions described for **9a** (0.289 g, 94% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 2.94 (t, *J*=7.6 Hz, 2H), 3.58- 3.63 (q, *J*=7.7 Hz 2H), 7.30 (s, 2H), 7.47 (d, *J*=8.6 Hz, 2H), 7.54 (d, *J*=7.8 Hz, 1H), 7.75- 7.78 (dd, *J*=7.6, 2.1 Hz, 3H), 8.12 (d, *J*=2.4 Hz, 1H), 8.76 (t, *J*=7.6 Hz, 1H), 8.81 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 35.11, 118.2, 120.3, 120.4, 129.2, 129.5, 129.6, 133.9, 142.6, 143.8, 146.7, 152.9, 160.3, 161.2. HRMS (ESI): *m/z* calcd for $C_{18}H_{15}ClN_2O_5S$ [*M*+Na]$^+$: 429.8312, found: 429.8303.

6-Methoxy-2-oxo-*N*-(4-sulfamoylphenethyl)-*2H*-chromene-3-carboxamide (20f). The yellow powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8f**, 0.2 g, 0.84 mmol) and 4-(2-aminoethyl) benzenesulfonamide (**3h**, 0.17 g, 0.94 mmol) by following the experimental conditions described for **9a** (0.212 g, 76% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 2.94 (t, *J*=7.6 Hz, 2H), 3.58- 3.63 (q, *J*=7.6 Hz 2H), 3.38 (s, 3H), 7.30 (s, 2H), 7.36 (dd, *J*=7.6, 2.1 Hz, 1H), 7.44- 7.47 (m, 3H), 7.57 (d, *J*=2.4 Hz, 1H), 7.77 (t, *J*=7.6 Hz, 1H), 8.83 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 35.11, 118.2, 120.3, 120.4, 129.2, 129.5, 129.6, 133.9, 142.6, 143.8, 146.7, 152.9, 160.3, 161.2. HRMS (ESI): *m/z* calcd for $C_{19}H_{18}N_2O_6S$ [*M*+Na]$^+$: 425.4126, found: 425.4112.

6-Chloro-2-oxo-*N*-(2-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (21b). The white powder was obtained from 6-chloro-2-oxo-2*H*-chromene-3-carbonyl chloride (**8b**, 0.2 g, 0.82 mmol) and 2-aminobenzenesulfonamide (**3j**, 0.141 g, 0.84 mmol) by following the experimental conditions described for **9a** (0.255 g, 76% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.37 (t, *J*=6.8, Hz, 1H), 7.52 (s, 2H), 7.60- 7.68 (m, 2H), 7.82 (dd, *J*=8.6, 2.2 Hz, 1H), 7.89 (d, *J*=7.6, 1H), 8.18 (d, *J*=2.1 Hz, 1H), 8.21 (s, 1H), 9.00 (s, 1H), 11.10 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 118.7, 120.3, 120.8, 125.2, 125.6, 127.8, 129.4, 129.8, 132.9, 134.4, 134.7, 147.4, 153.1, 160.1, 160.3. HRMS (ESI): *m/z* calcd for $C_{16}H_{11}ClN_2O_5S$ [*M*+Na]$^+$: 401.7737, found: 401.7716.

6-Bromo-2-oxo-*N*-(3-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (21c). The yellow powder was obtained from 6-bromo-2-oxo-2*H*-chromene-3-carbonyl chloride (**8c**, 0.2 g, 0.70 mmol) and 2-

aminobenzenesulfonamide (**3j**, 0.121 g, 0.70 mmol) by following the experimental conditions described for **9a** (0.219 g, 79% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.37 (t, *J*=6.8, Hz, 1H), 7.52 (s, 2H), 7.56 (d, *J*=2.1, 1H), 7.66 (t, *J*=6.4, Hz, 1H), 7.89- 7.95 (m, 2H), 8.16 (d, *J*=7.6 Hz, 1H), 8.35 (d, *J*=2.4 Hz, 1H), 9.00 (s, 1H), 11.10 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 117.2, 118.9, 120.7, 120.8, 125.2, 125.6, 127.8, 132.8, 132.9, 134.6, 134.7, 137.1, 147.3, 153.5, 160.1, 160.3. HRMS (ESI): *m/z* calcd for $C_{16}H_{11}BrN_2O_5S$ [*M*+Na]$^+$: 446.2247, found: 446.2209.

2-Oxo-*N*-(3-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (21e). The white powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8e**, 0.2 g, 0.96 mmol) and 2-aminobenzenesulfonamide (**3j**, 0.17 g, 0.96 mmol) by following the experimental conditions described for **9a** (0.265 g, 81% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.36 (t, *J*=6.8, Hz, 1H), 7.46- 7.58 (m, 4H), 7.67 (t, *J*=6.7, Hz, 1H), 7.78 (dd, *J*=7.6, 2.1 Hz, 1H), 7.92 (d, *J*=7.6, 1H), 8.07 (d, *J*=2.1 Hz, 1H), 8.19 (d, *J*=2.4 Hz, 1H), 9.03 (s, 1H), 11.14 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 116.6, 118.9, 119.6, 125.1, 125.6, 125.7, 127.8, 131.0, 134.6, 134.7, 135.0, 148.7, 154.5, 160.6, 160.7. HRMS (ESI): *m/z* calcd for $C_{16}H_{12}N_2O_5S$ [*M*+Na]$^+$: 367.3292, found: 367.3244.

6-Methoxy-2-oxo-*N*-(3-sulfamoylphenyl)-2*H*-chromene-3-carboxamide (21f). The yellow powder was obtained from 2-oxo-2*H*-chromene-3-carbonyl chloride (**8f**, 0.2 g, 0.84 mmol) and 2-aminobenzenesulfonamide (**3j**, 0.145 g, 0.96 mmol) by following the experimental conditions described for **9a** (0.247 g, 74% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 3.85 (s, 3H), 7.34- 7.41 (m, 2H), 7.51 (d, *J*= 6.4 Hz, 3H), 7.64- 7.68 (m, 2H), 7.90 (dd, *J*=7.6, 2.1 Hz, 1H), 8.17 (d, *J*= 7.4 Hz, 1H), 8.99 (s, 1H), 11.17 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 56.3, 112.5, 117.8, 119.4, 119.8, 122.8, 125.1, 125.7, 127.8, 132.9, 134.7, 148.5, 149.0, 156.5, 160.7. HRMS (ESI): *m/z* calcd for $C_{17}H_{14}N_2O_6S$ [*M*+Na]$^+$: 397.3553, found: 397.3539.

2-Oxo-2-((4-sulfamoylphenyl)amino)ethyl-7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylate (22). To a solution of ethyl 7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylate (**2g**, 0.2 g, 0.77 mmol) in dry DMF (15 mL), anhydrous K_2CO_3 (0.18 g, 1.28 mmol) was added. The mixture was stirred for 15 mins at 70-80 °C and 2-chloro-*N*-(4-sulfamoylphenyl) acetamide (**4a**, 0.21 g, 0.85 mmol) was added, followed by a pinch of KI, and heated overnight. Water (10 mL) was added to the reaction mixture, followed by 1 mL 6N HCl. The resulting solid was filtered, washed with water and air dried, and purification by flash column chromatography (hexane/EtOAc 20:1) afforded the title compound as a yellow powder (0.198 g, 54% yield): ^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.15 (t,

J=6.8 Hz, 6H), 3.50- 3. (q, *J*=6.8 Hz 4H), 4.88 (s, 2H), 6.58 (s, 1H), 6.82 (d, *J*=8.3 Hz, 1H), 7.27 (s, 1H), 7.70 (d, *J*=9.0 Hz, 1H), 7.79 (s, 4H), 8.19 (br-s, 1H), 8.68 (s, 1H), 10.53 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ= 12.8, 44.8, 63.2, 96.3, 106.6, 107.5, 110.5, 119.2, 127.2, 132.5, 139.2, 141.8, 146.8, 153.6, 157.8, 162.8, 163.4, 165.6. HRMS (ESI): *m/z* calcd for $C_{22}H_{23}N_3O_7S$ [*M*+Na]$^+$: 496.4863, found: 496.4835.

2.2 Carbonic Anhydrase Assay

Catalyzed CO_2 hydration activity was evaluated using a photophysics stopped-flow instrument using phenol red (0.2 mM) as an indicator and UV detection at 557 nm as previously described.(Ewies et al., 2022) 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (20 mM, pH 7.5) was used as a buffer, and 20 mM Na_2SO_4 to keep its ionic strength constant. Initial rates of the CA-catalyzed CO_2 hydration reaction were applied for 10–100 seconds. Then, CO_2 concentrations (1.7–17 mM) were applied for kinetic parameters and inhibition constant calculation. The initial velocity for each inhibitor was determined by using a minimum of six traces of the initial 5%–10% of the reaction. Simultaneously, the uncatalyzed rates were likewise measured to be subtracted from the total observed rates.

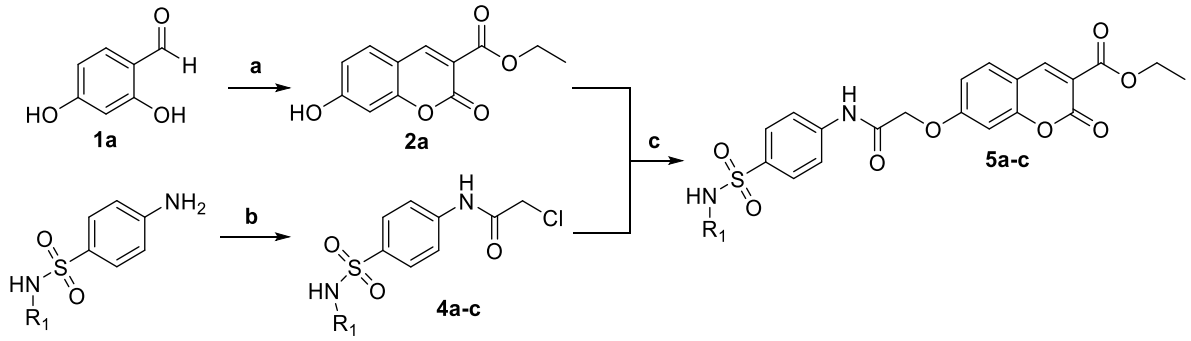
Stock solutions (0.1 mM) of the new compounds and the reference compound, AAZ, were prepared and subsequently diluted with distilled-deionized water to obtain 0.01 nM concentration. Prior incubation of the mixture of the compounds and enzyme solutions for 15 minutes at room temperature was carried out until the formation of enzyme–inhibitor complex. Nonlinear least-squares methods were used to calculate the inhibition constants using the Cheng–Prusoff equation.

3. Results and Discussion

3.1. Chemistry

Coumarin derivatives (*2H*-chromen-2-one derivatives) can be accessed by a number of reported syntheses.(Chimenti et al., 2009; He et al., 2014; Yang et al., 2019) Herein, coumarin ethyl ester was synthesized according to the route depicted in **Scheme 1**.(Ahmed et al., 2019; Saglik et al., 2019) Commercially available 2,4-dihydroxybenzaldehyde (**1a**) was reacted with diethyl malonate in the presence of piperidine to afford ethyl

7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**2a**). The synthesis of sulfonamide derivatives with the chloroacetamide linker were obtained from commercially available sulfonamides (**3a-c**), avoiding the need for Pd/C reduction of the respective nitro intermediates.(Kinarivala & Trippier, 2014) These compounds were reacted with 2-chloroacetyl chloride at 0 °C in the presence of anhydrous potassium carbonate and a catalytic amount of potassium iodide to afford the corresponding chloroacetamide derivatives (**4a-c**) in good yield.(Angeli et al., 2019) Williamson ether synthesis employing **2a** with **4a-c** afforded coumarin sulfonamides (**5a-c**) in moderate yields (**Table 2**).(Jonnalagadda, Huwaimel, Jonnalagadda, Garrison, & Trippier, 2022; Yang et al., 2019)



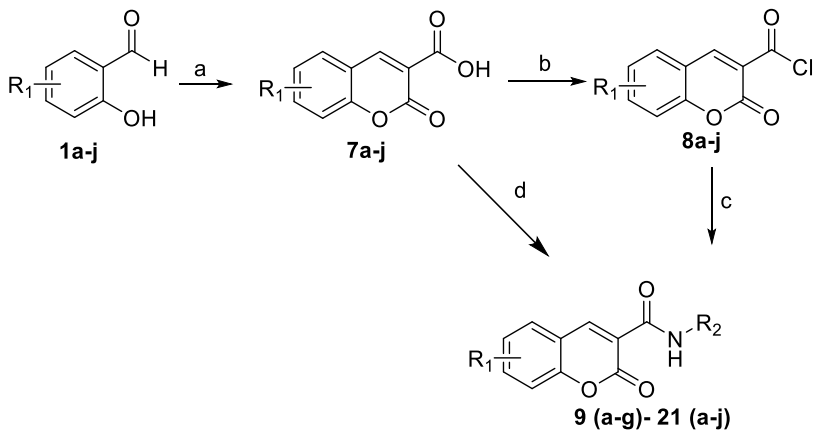
3a R_1= H

3b R_1=

3c R_1= $COCH_3$

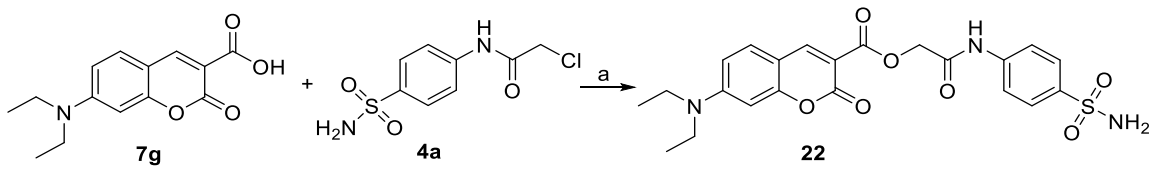
Scheme 1. Synthesis of carbonic anhydrase inhibitors 5a-c. *Reagents and Conditions*: **a)** Diethyl malonate, piperidine, 3 h, 60 °C, 87%; **b)** Chloroacetyl chloride, K_2CO_3, KI, THF, 2 h, 0 °C, 76-93%; **c)** Na_2CO_3, DMF, 155 °C, 12 h, 49-61%

Further coumarin derivatives (**9a-j**) were accessed through hydrolysis of the respective substituted ester of type **2a**. The respective acid chlorides (**8a-j**) were afforded using refluxing thionyl chloride. The desired coumarin sulphonamide derivatives were obtained via reacting commercially available sulphonamides with either acid **7a-j** through HOBt mediated amide formation or direct reaction with acid chlorides **8a-j** (**Scheme 2**) (**Table 3**).(Endo et al., 2017; Mincione et al., 2001; Nishikawa et al., 2019)



Scheme 2. Synthesis carbonic anhydrase inhibitors 9 (a-g)-18 (a-g). *Reagents and Conditions*: **a)** i) Diethyl malonate, piperidine, 3 h, 60 °C, 70-92%; ii) NaOH, methanol, RT, 84-99%; **c)** $SOCl_2$, 75 °C, 5 h, 71-96%; **d)** Appropriate sulfonamide amines, pyridine, DMF, 12 h, 80 °C, 49-96%; **e)** Appropriate sulfonamide, EDC, HOBt•H_2O, Et_3N, DMF, RT, 5 h, 73%.

A third derivative class was designed to incorporate a linker between the coumarin acid and the sulfonamide moiety. This derivative was synthesized based on the rationale of a higher rate of hydrolysis and the potential to release subunits of sulfonamide and coumarin which could individually inhibit the activity of CA. 2-Oxo-2-((4-sulfamoylphenyl)amino)ethyl7-(diethylamino)-2-oxo-*2H*-chromene-3-carboxylate (**22**) was obtained by reaction of 7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylic acid (**7g**) with 2-chloro-*N*-(4-sulfamoylphenyl) acetamide (**4a**) in the presence of anhydrous potassium carbonate and a catalytic amount of potassium iodide to afford a moderate yield of **22** (**Scheme 3**).(Yang et al., 2019)

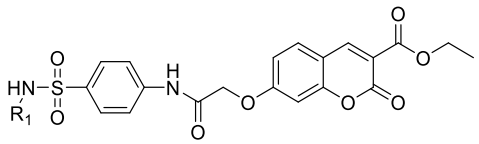


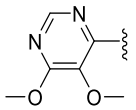
Scheme 3. Synthesis of carbonic anhydrase inhibitors 22. *Reagents and Conditions*: **a)** K_2CO_3, KI, DMF, 155 °C, 12 h, 54%

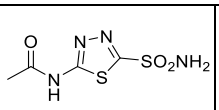
3.2. Structure-Activity Relationship

Many reports describe that sulfonamide or coumarin-based molecules are able to inhibit the carbonic anhydrase enzymes IX and XII and effect tumor pH, leading to inhibition of growth in both primary tumors and metastatic sites.(Emami & Dadashpour, 2015; Lomelino et al., 2016; Neri & Supuran, 2011) Herein we report a number of synthesized compounds show inhibition of tumor associated human carbonic anhydrases IX and XII with nanomolar activity. Twelve of these compounds proved to be more potent compared with acetazolamide (**AAZ**) control to inhibit CA IX (**9g**, **9i**, **16b-i**, **18f**, **22**), while only one was more potent than AAZ to inhibit CA XII (**18f**). These compounds possess significant selectivity for CA IX and CA XII over CA I and/or CAII.

Ethyl 2-oxo-7-(2-oxo-2-((4-sulfamoylphenyl)amino)ethoxy)-2*H*-chromene-3-carboxylate (**5a**) possessing a primary sulphonamide with a hydroxyl group in position 7 of the coumarin ring provides selective nanomolar inhibition of CA II, IX, and XII with Ki values of 9.1 nM, 26.9 nM and 20.3 nM, respectively. Conversion of the primary sulfonamide to secondary such as adding Sulfadoxine (**5b)** and *N*-((4-aminophenyl)sulfonyl)acetamide (**5c**) lead to decrease in this activity in agreement with the literature (**Table 2**).(Wang et al., 2013)



	R_1	Mw	cLogP[a]	PSA[b]	K_I (μM, unless stated)[c]			
					CA I	CA II	CA IX	CA XII
5a	H	446.43	1.37	151.09	738.1 nM	9.1 nM	26.9 nM	20.3 nM
5b		584.56	3.17	180.28	>100	>100	>100	>100
5c		488.47	0.97	154.17	>100	>100	6.1	1.9

AAZ		222.24	-0.98	113.99	250 nM	12.5 nM	25.0 nM	5.7 nM

[a]Calculated by ChemDraw Professional 16.0.

[b]Polar surface area (pH 7.4), calculated by ChemDraw Professional 16.0.

[c]Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values).

Table 2. Structure, molecular weight, cLogP, polar surface area and CA I, II, IX and XII isoform inhibition actvity of synthesized carbonic anhydrase inhibitors from scheme 1.

A range of coumarin side chains featuring various sulfonamide substitutions were synthesized. 7-Hydroxy-2-oxo-*N*-(4-sulfamoylphenyl)-*2H*-chromene-3-carboxamide (**9a**) is a more potent and selective CA XII inhibitor than the similar compounds **9b-j** with Ki value of 6.2 nM (**Table 3**). Addition of halide substitution to the coumarin ring results in decreased activity to inhibit CA II, IX and XII (compounds **9b**, **9c**, **9d** and **9i**) compared to hydroxyl substitution.(Huwaimel et al., 2021; Huwaimel et al., 2022) Converting the 7-hydroxy in the coumarin ring to 7-diethylamino (**9g**) increases activity to inhibit CA IX with Ki = 13.9 nM compared to **AAZ** which possesses a Ki of 25 nM. Addition of a second chlorine to the coumarin ring (**9d**) lead to a decrease in activity to inhibit CA II, IX and XII compared with a single chlorine (**9b**). Homologation of the linker between coumarin and sulphonamide from zero (**9b** and **9f**) to ethyl (**20b** and **20f**) lead to a approximate two-fold decrease in the activity to inhibit CA XII (Ki's of (**9b**) 28 nM to (**20b**) 46 nM and (**9f**) 29 nM to (**20f**) 46 nM) while increasing activity to inhibit CA II and IX (Ki's of (**9b**) 64 and 52 nM to (**20b**) 9 and 36 nM and (**9f**) 100 and 60 nM to (**20f**) 57 and 19 nM respectively). 6-Chloro-2-oxo-*N*-(4-sulfamoylphenethyl)-2*H*-chromene-3-carboxamide (**20b**) is more potent and selective to CA II than other synthesized compounds with a Ki of 8.6 nM compared to **AAZ** with a Ki of 12.5 nM.

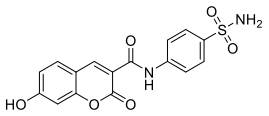
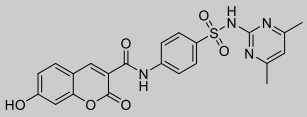
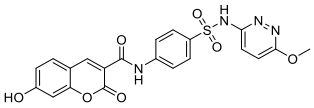
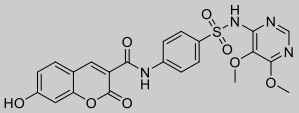
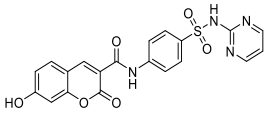
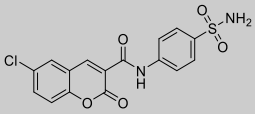
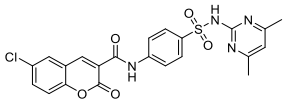
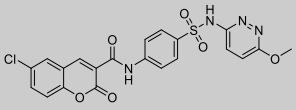
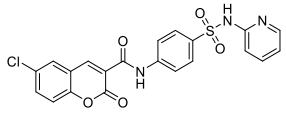
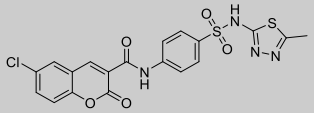
A substituent screen of the terminal sulphonamide of 6-chloro substituted coumarins revealed a preference of *meta* (**16b**) > *para* (**9b**) > *ortho* (**21b**) in terms of activity for CA I, II and IX, with Ki's of 8.4, 52 and

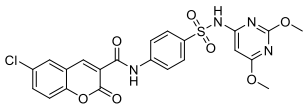
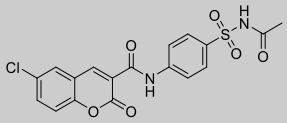
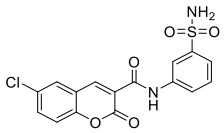
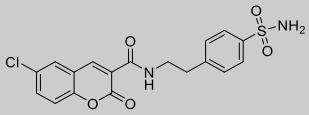
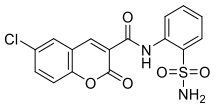
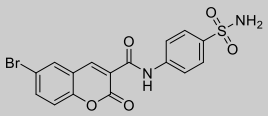
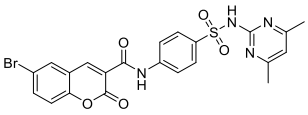
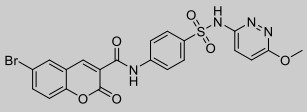
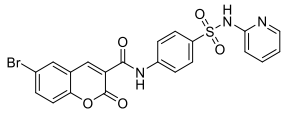
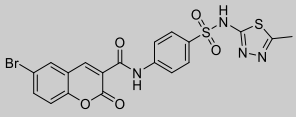
68 nM respectively for CA IX. For CA XII *ortho* > *para* = *meta* with Ki's of 16, 28 and 30 nM for **16b**, **9b** and **21b** respectively. The greater activity of the *meta* position for CA IX inhibition was confirmed when the 6-chloro substituent of the coumarin ring was converted to 6-methoxy with compound **16f** possessing a Ki of 5 nM for CA IX which was greater than *ortho* (**21f**) and *para* (**9f**). *Meta*-sulphonamides **16b** and **16f** represent the most potent CA IX inhibitors identified, at least three-fold more potent than **AAZ** control. Changing the methoxy group in the coumarin ring from position 6 (**16f**) to position 7 (**16h**) lead to decreased CA IX inhibition with Ki values 5.2 nM to 34.2 nM respectively (**Table 3**).

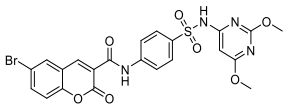
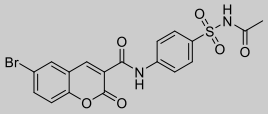
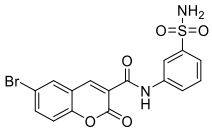
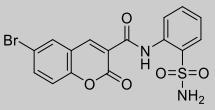
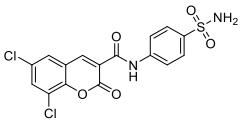
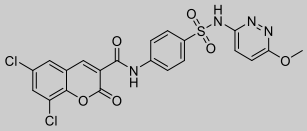
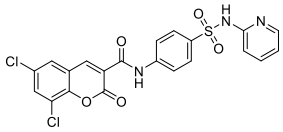
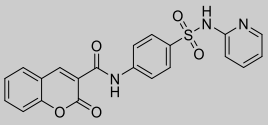
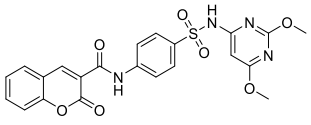
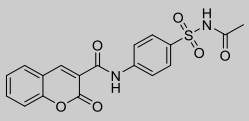
Increasing the number of sulfonamides to two groups on the terminal phenyl ring with 2,4-disubtitution and addition of a CF_3 moiety at the 5-position (**18f**) lead to further increased activity and selectivity with Ki's = 955, 515, 21 and 5 nM for CA's I, II, IX and XII respectively. Compound **18f** represents the most activity CA XII inhibition synthesized, equipotent with **AAZ** control (5.7 nM). Interestingly, addition of an ester linkage which would be expected to metabolically labile in *in vivo* systems results in a pan CA I, II, IX and XII inhibitor with Ki's of 74, 23, 20 and 66 nM respectively.

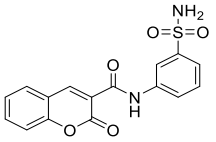
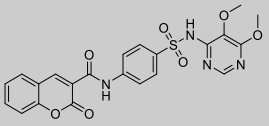
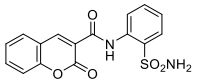
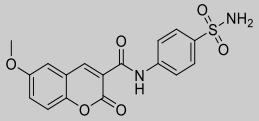
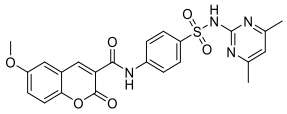
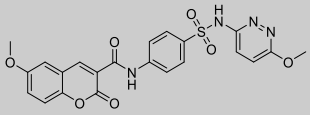
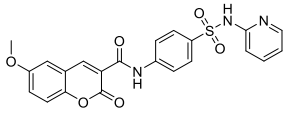
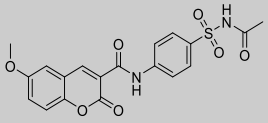
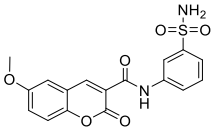
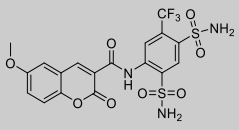
As expected, most compounds possessing secondary sulfonamides possessed generally lower activity in inhibiting CA enzymes than their primary sulphonamide counterparts. As shown in **Table 3**, most of the secondary sulfonamides, especially those with functionalized aromatic heterocycles, proved inactive to inhibit CA, with Ki values >100 μM (**10-14a-e**, **17a-e**, **19a**). *N*-acetyl functionalized secondary sulphonamides **15b-f** possess some activity to inhibit CA's I, II, IX and XII. *N*-(4-(*N*-acetylsulfamoyl)phenyl)-6-bromo-2-oxo-2*H*-chromene-3-carboxamide (**15c**) and *N*-(4-(*N*-acetylsulfamoyl)phenyl)-2-oxo-2*H*-chromene-3-carboxamide (**15e**) possess Ki's of 910 nM and 790 nM respectively, to inhibit CA XI.

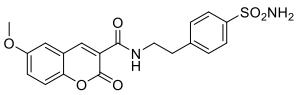
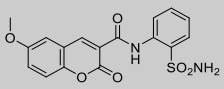
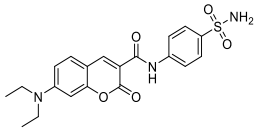
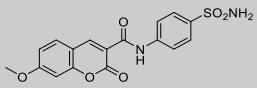
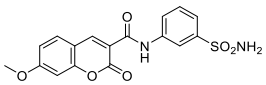
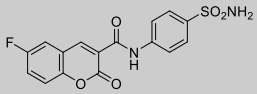
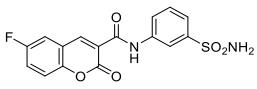
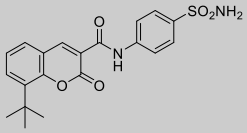
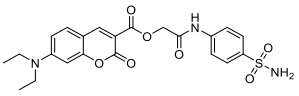
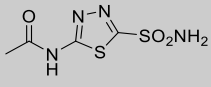
	Structure	**Mw**	**cLogP[a]**	**PSA[b]**	**K_I (μM, unless stated)[c]**			
					CA I	**CA II**	**CA IX**	**CA XII**

9a		360.34	0.44	135.79	265.4 nM	12.4 nM	29.2 nM	6.2 nM
10a		466.47	2.11	146.52	64.8	29.1	17.4	8.3
11a		468.44	1.57	155.75	68.9	24.0	8.1	4.7
17a		498.47	2.24	165	>100	>100	>100	>100
19a		438.41	1.11	147	>100	>100	25.8	50.1
9b		378.78	1.64	115.56	818.4 nM	63.5 nM	52.3 nM	28.4 nM
10b		484.91	3.31	126.29	>100	>100	>100	>100
11b		486.88	2.77	135.52	>100	>100	>100	>100
12b		455.87	3.05	113.93	>100	>100	28.4	9.1
13b		476.91	2.63	126.29	>100	>100	58.8	>100

14b		516.91	4.19	144.75	>100	>100	>100	>100
15b		420.82	1.32	118.64	>100	37.2	6.1	3.4
16b		378.78	1.64	115.56	639.2 nM	44.9 nM	8.4 nM	29.6 nM
20b		406.84	1.92	115.56	23.5 nM	8.6 nM	35.8 nM	46.0 nM
21b		378.78	1.64	115.56	726.5 nM	139.4 nM	56.2 nM	66.6 nM
9c		423.24	1.79	115.56	1422 nM	85.4 nM	67.6 nM	16.2 nM
10c		529.37	3.46	126.29	>100	>100	>100	>100
11c		531.34	2.92	135.52	>100	>100	>100	>100
12c		500.32	3.20	113.93	>100	>100	39.1	>100
13c		521.36	2.78	126.29	>100	>100	>100	>100

14c		561.36	4.34	144.75	>100	>100	>100	>100
15c		465.27	1.47	118.64	>100	46.8	0.91	10.6
16c		423.24	1.79	115.56	1961 nM	76.3 nM	14.4 nM	54.2 nM
21c		423.24	1.79	115.56	1569 nM	436.8 nM	79.5 nM	156.7 nM
9d		413.23	2.35	115.56	2322 nM	238.4 nM	104.9 nM	46.3 nM
11d		521.33	3.48	135.52	>100	>100	>100	>100
12d		490.31	3.76	113.93	>100	>100	>100	>100
12e		421.43	2.32	113.93	>100	>100	39.1	>100
14e		482.47	3.47	144.75	>100	>100	>100	>100
15e		386.38	0.60	118.64	>100	10.3	0.79	6.3

16e		344.34	0.92	115.56	192.8 nM	53.5 nM	18.9 nM	69.8 nM
17e		482.47	2.72	144.75	>100	>100	>100	>100
21e		344.34	0.92	115.56	552.4 nM	126.5 nM	75.2 nM	42.0 nM
9f		374.37	0.89	179.24	1571 nM	99.9 nM	60.1 nM	29.0 nM
10f		480.50	2.56	135.52	>100	>100	>100	>100
11f		482.47	2.02	144.75	>100	>100	>100	>100
12f		451.45	2.30	123.16	>100	>100	23.2	>100
15f		416.40	0.58	127.87	90.1	28.7	14.5	3.7
16f		3.74.37	0.89	124.79	774.1 nM	212.3 nM	5.2 nM	52.1 nM
18f		521.44	-0.30	184.95	954.6 nM	514.5 nM	21.1 nM	5.1 nM

20f		402.42	1.17	124.79	654.4 nM	56.8 nM	18.7 nM	45.8 nM
21f		3.74.37	0.89	124.79	832.1 nM	110.9 nM	35.7 nM	61.2 nM
9g		415.46	2.20	118.80	590.1 nM	26.8 nM	13.9 nM	22.1 nM
9h		374.37	0.89	124.79	575.3 nM	42.7 nM	31.8 nM	22.9 nM
16h		374.37	0.89	124.79	185.3 nM	68.7 nM	34.2 nM	18.3 nM
9i		362.33	1.07	115.56	665.2 nM	51.2 nM	25.0 nM	31.4 nM
16i		362.33	1.07	115.56	225.4 nM	75.2 nM	15.2 nM	49.5 nM
9j		400.45	2.74	115.56	52.0 nM	16.4 nM	28.7 nM	22.9 nM
22		473.50	2.22	145.10	74.4 nM	23.0 nM	19.6 nM	65.7 nM
AAZ		222.24	-0.98	113.99	250 nM	12.5 nM	25.0 nM	5.7 nM

[a]Calculated by ChemDraw Professional 16.0.

[b]Polar surface area (pH 7.4), calculated by ChemDraw Professional 16.0.

[c]Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values).

Table 3. Inhibition data of human CA isoforms I, II, IX and XII, structure, molecular weight, calculated logP and polar surface area of synthesized carbonic anhydrase inhibitors from schemes 2-3.

4. Conclusion

In summary, a library of sulphonamide functionalized coumarins has been synthesized that revealed several compounds with high activity to inhibit human carbonic anhydrases I, II, IX and XII with many displaying selectivity to the tumor-associated CA IX and CA XII. These CA inhibitors are exemplified by compound **16b** possessing Ki's of 8.4, 30, 45 and 639 nM to CA IX, XII, II and I respectively with 5- to 76-fold selectivity for the cancer-associated CAs and compound **16f** possessing Ki's of 5, 52, 212 and 774 nM for CA IX, XII, II and I respectively with 42- to 155-fold selectivity for cancer associated CAs. Both compounds are more potent than the clinical CA inhibitor acetazolamide. Compound **18f** (Ki's = 5, 21, 515 and 955 nM for CA's XII, IX, II and I respectively) is representative of a novel CA IX and XII inhibitor. All three compounds represent novel structures for further development.

Conflict of Interest

The authors declare no conflict of interest

Data Availability Statement

^{1}H and ^{13}C NMR spectra of the designed compounds are available in the supporting information material of this article.

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