Bioorganic & Medicinal Chemistry 19 (2011) 3367–3371

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of new chromone containing sulfonamides as potent inhibitors of bovine cytosolic carbonic anhydrase

Mariya al-Rashida [a,*], Muhammad Ashraf [b], Bushra Hussain [b], Saeed Ahmad Nagra [a], Ghulam Abbas [a]

[a] Institute of Chemistry, University of the Punjab, Lahore 54590, Pakistan
[b] Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

ARTICLE INFO

Article history:
Received 7 March 2011
Revised 19 April 2011
Accepted 20 April 2011
Available online 22 April 2011

Keywords:
Carbonic anhydrase
Carbonic anhydrase inhibitors
Formylchromones
Sulfonamides

ABSTRACT

Series of chromone containing sulfonamides were prepared by the reaction of (un)substituted 3-formylchromones with 3-aminobenzenesulfonamide and 4-aminobenzenesulfonamide. Bovine carbonic anhydrase (bCA) inhibitory activity of these newly synthesized compounds was determined. All compounds were active and possessed excellent bCA inhibitory activities with IC_{50} values ranged between 4.31 ± 0.001 and 29.12 ± 0.008 μmol. Compounds derived from 6-fluoro-3-formylchromones were the most active.

1. Introduction

Chromones are a group of naturally occurring oxygen containing heterocyclic compounds. They are widely distributed in plant kingdom and form the basic nucleus of important compounds such as anthocyanin and flavonoids. Chromones are also well known for their antimicrobial,[1,2] antitumor,[3,4] and antiviral[5,6] activities. 3-Formylchromones are important synthons in synthetic chemistry for incorporating chromone moieties into other heterocyclic systems or for creating new heterocyclic systems based on chromone ring.[7,8] They are widely used due to their ease of preparation and modification via Vilsmeier Haack reaction.[9,10] The synthetic utility of 3-formylchromones can be explored by exploiting three electron deficient centers; the (keto) carbonyl carbon, carbon atom C2 and the aldehyde carbon atom C3.[11]

The clinical and medicinal importance of sulfonamides is well documented. The sulfonamide moiety ($-SO_2NH_2$) is an active pharmacophore, exhibiting a wide variety of pharmacological activities such as antimicrobial, antimalarial, insulin-releasing antidiabetic, anti-HIV, high ceiling diuretic, antithyroid, and antitumor.[12–14] Among the broad spectrum of activities exhibited by sulfonamides, their role as inhibitors of the zinc containing metalloenzyme carbonic anhydrase (CA) is probably most widely studied. Many sulfonamide CA inhibitors have been used as therapeutic agents against various diseases including glaucoma, gastro-duodenal ulcers, acid–base disequilibria, and various neurological disorders.[15–18] There are 16 currently known isozymes of CA in humans. It is thus of critical importance to synthesize and assay CA inhibitors from diverse classes of substances, in order to identify compounds with specificity towards specific isozymes.

Over the years, a lot of development has been made in this area and new classes of compounds that can act as CA inhibitors are continuously being emerged in hopes of finding isozyme specific inhibitors. In this regard, the efforts of research group of Supuran are worth mentioning.[19–23] In 2005, Puccetti et al.[24] reported sulfonamides containing chromone moieties as a new class of CA inhibitors for the first time. The compounds reported were Schiff bases obtained by the reaction of 3-formylchromone with various aminobenzene sulfonamides. In the present work, we have expanded this new class of inhibitors by synthesizing novel sulfonamides containing chromone moieties and determined their inhibition activity against bCA.

2. Results

2.1. Chemistry

Two series of sulfonamide containing chromone moieties (**1a–1e** and **2a–2e**) were synthesized by simple and facile condensation reaction of equimolar quantities of 6-(un)substituted 3-formylchromones with 3- and 4-amino benzenesulfonamide using ethanol in the presence of catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH). Reaction mixture was refluxed for 3.5 h and kept overnight. Solid product was obtained by filtration and purified by recrystallization. The substituted 3-formylchromones used

* Corresponding author. Tel.: +92 3432106432; fax: +92 42 99231269.
E-mail address: maria_al_rashida@hotmail.com (M. al-Rashida).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.04.040

were 6-fluoro-3-formylchromone, 6-bromo-3-formylchromone, 6,8-dibromo-3-formylchromone and 6-ethyl-3-formylchromone. The general pathway leading to the synthesis of compounds (**1a–1e** and **2a–2e**) is given in Scheme 1. The structures of newly synthesized compounds were ascertained via IR, MS, ^{1}H NMR and ^{13}C NMR spectroscopic techniques. Single crystal XRD analysis of one of the compounds (**1a**)[25] further confirms the structural elucidation.

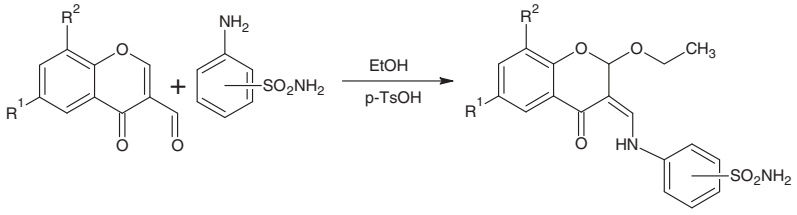
2.2. Carbonic anhydrase assay

CA activity was determined by the method of Pocker and Stone[26] after standardization of reaction conditions like enzyme conc., substrate conc., buffer pH, duration of reaction etc. The method is based on the principle that *p*-nitrophenyl acetate is hydrolyzed by CA to form *p*-nitrophenol which is determined spectrophotometrically. Reaction mixture contained 50 mM Tris–sulfate buffer pH 7.6 containing 0.1 mM $ZnCl_2$, 5 μL (0.5 mM) test compound in DMSO and 10 μL (0.35 U, with activity units of 700 U/g) bovine enzyme per well. Contents were mixed and pre-incubated at 25 °C for 10 min. Plates were pre-read at 348 nm using a 96 well plate reader. Substrate, *p*-nitrophenyl acetate was prepared (3 mM stock using <5% acetonitrile in buffer and used fresh) and 20 μL was added per well to achieve 0.6 mM concentration per well. Total reaction volume was made to 100 μL. After 30 min incubation at 25 °C, contents were mixed and read at 348 nm. Suitable controls with DMSO and known inhibitor acetazolamide were included in the assay. Results reported are mean of three independent experiments (±sem) and expressed as percent inhibitions calculated by the formula, Inhibition (%) = [100−(abs of test comp/abs of control) × 100)]. IC_{50} values of selected compounds exhibiting >50% activity at 0.5 mM were calculated after suitable dilutions.

3. Discussion

A series of structurally related sulfonamide enamines containing chromone moieties (**1a–1e** and **2a–2e**) were prepared according to Scheme 1 by reacting 3-formylchromone, 6-fluoro-3-formylchromone, 6-bromo-3-formylchromone, 6,8-dibromo-3-formylchromone and 6-ethyl-3-formylchromone with 3- and 4-aminobenzene sulfonanmides. All reactions were carried out in ethanol in the presence of catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH). The molecule of 3-formylchromone has a very reactive electron deficient centre at C2 position. Ethanol (solvent) acts as a nucleophile and is added into the C2-C3 olefinic bond of the chromone yielding an enamine product. This enamine product is sufficiently stable and is not converted to Schiff base. Similar additions of ethanol to the molecule of 3-formylchromone have been studied in detail by Stankovicova et al.[27–29] Analogous results were obtained by Dziewulska-Kułaczkowska and Mazur[30] for the preparation of related compound 3-(anilinomethylene)-2-methoxychroman-4-one, in which a molecule of methanol has been added into the C2 position of chromone ring. One of the reasons for the stability of this sulfonamide enamine is the N–H···O intramolecular hydrogen bond. The presence of this hydrogen bond has been confirmed via single crystal X-ray diffraction of one of the compounds **1a** reported here (Fig 1).[25]

All synthesized compounds were tested for their ability to act as CA inhibitors against bovine cytosolic CA containing CA-I and CA-II



1a-1e; 4-SO_2NH_2 1a, 2a; R^1 = H; R^2 = H

2a-2e; 3-SO_2NH_2 1b, 2b; R^1 = F; R^2 = H

1c, 2c; R^1 = Br; R^2 = H

1d, 2d; R^1 = Br; R^2 = Br

1e, 2e; R^1 = CH_2CH_3; R^2 = H

Scheme 1.

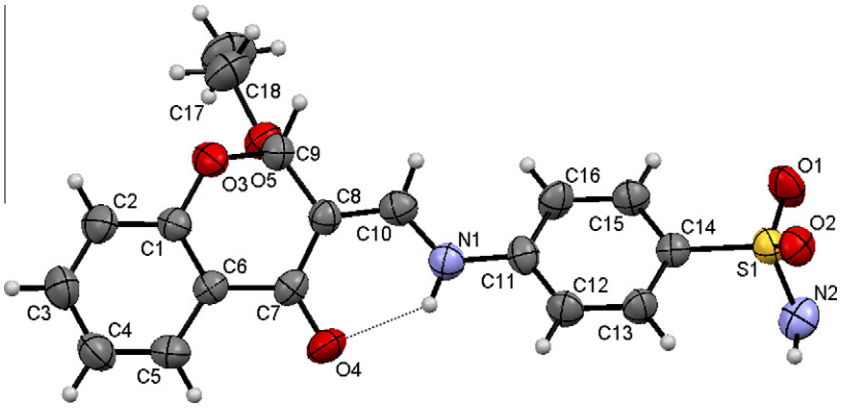


Figure 1. Stabilization of the enamine product via N–H···O intramolecular hydrogen bond in **1a**.

Table 1
Percent inhibition and IC_{50} values (μmol) of compounds

Compounds	Bovine carbonic anhydrase	
	Inhibition at 0.5 mM (%)	IC_{50} (μmol)
1a	64.00 ± 0.01	19.21 ± 0.002
1b	71.22 ± 0.03	4.31 ± 0.001
1c	65.16 ± 0.07	16.12 ± 0.002
1d	67.34 ± 0.03	21.12 ± 0.006
1e	69.65 ± 0.02	16.63 ± 0.002
2a	67.60 ± 0.01	17.72 ± 0.001
2b	66.83 ± 0.05	11.37 ± 0.003
2c	68.75 ± 0.01	18.27 ± 0.005
2d	61.18 ± 0.05	29.12 ± 0.008
2e	65.29 ± 0.02	11.71 ± 0.003
Acetazolamide	91.32 ± 0.01	1.13 ± 0.001

Results are mean of 3-independent determinations (mean ± sem, $n = 3$)

isozymes. CA inhibition data for these compounds is given in Table 1. CA activities of these compounds were tested against the standard clinically used inhibitor acetazolamide. The compounds **1a–1e** and **2a–2e** differ only in the relative position of the sulfonamide group on the benzene ring. In general, compounds containing sulfonamide group at *p*-position of the benzene ring (**1a–1e**) were more active than compounds having sulfonamide group at the *m*-position (**2a–2e**). Compound **1b** was the most active having IC_{50} value of 4.31 ± 0.001 μmol, whereas compound **2d** was the least active with IC_{50} value of 29.12 ± 0.008 μmol. 6-Fluoro substituted compounds were the most active, followed by 6-bromo substituted and un-substituted compounds (which had almost comparable activity 16.12 ± 0.002 and 19.21 ± 0.002 μmol). The lowest CA inhibition was shown by 6,8-dibromo substituted compounds with IC_{50} values of 21.12 ± 0.006 and 29.12 ± 0.008 μmol (Table 1). It has been demonstrated[31] that C-6 substitution of chromone ring with electron withdrawing groups enhances the biological activity. The difference in activity of these compounds can be explained, in part, due to the difference in nature of halogen substituent attached at 6 position of the chromone ring. In general, fluorine containing compounds have been found to be very active biologically. For instance, methanesulfonamide ($CH_3SO_2NH_2$) is a weak inhibitor of CA ($K_I = 10^{-4}$ M), whereas its fluoro analog, trifluoromethanesulfonamide ($CF_3SO_2NH_2$) is an excellent inhibitor of CA ($K_I = 2 \times 10^{-9}$ M)[32], due to the strong electron withdrawing effect of fluorine. Similar trend in activity has been observed here (F > Br > H ≈ Et). However, when 6 and 8 positions of chromone ring are substituted simultaneously, as in 6,8-dibromo compounds (**1d** and **2d**), a significant decrease in activity is observed.

4. Conclusions

Chromone containing sulfonamide enamines were prepared via reaction of 3-formylchromone, 6-fluoro-3-formylchromone, 6-bromo-3-formylchromone, 6,8-dibromo-3-formylchromone and 6-ethyl-3-formylchromone with 3- and 4-aminobenzene sulfonanmides. Keeping in view CA inhibitory role of sulfonamides, the compounds were assayed as inhibitors of bCA and all compounds exhibited excellent CA inhibition activity (IC_{50} values 4.31 ± 0.001 to 29.12 ± 0.008 μmol). Compounds containing fluorine showed the most activity, whereas the lowest activity was exhibited by 6,8-dibromo substituted compounds. Compounds derived from 4-aminobenzene sulfonanmides were more active than those derived from 3-aminobenzene sulfonanmides. Based on the results we propose that there is a need for these compounds to be tested against a wide range of CA isozymes, that is, CA-IV, CA-IX and CA-XII etc. which are important drug targets in drug development program.

5. Experimental

5.1. General methods

All reactants were purchased from either Sigma or Aldrich and were used without further purification. Commercially available solvents were used. Ethanol was distilled and dried using standard methods and stored over molecular sieves. Reaction progress and product purity was checked by precoated TLC silica gel plates (0.2 mm, 60 HF_{254}, Merck). Spots were visualized under short and long wavelength UV light. Melting points were taken on a Gallenkamp melting point apparatus and are not corrected. IR spectra were recorded on Perkin Elmer Spectrum BX-II. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker (300, 400 and 500 MHz) AMX Spectrometer. Chemical shift values are reference against TMS. DMSO-d_6 was used as solvent for NMR spectroscopy. Mass Spectra were recorded on Finnigan MAT 312 Spectrometer. Bovine enzyme, carbonic anhydrase (bCA), was purchased from Calzyme Lab Inc. USA, Cat No 147 A2000 (700 U/g). The enzyme was 98.9% pure as given by the suppliers. A 96 well plate reader from Synergy HT Bio-Tek, USA was used. For the calculation of IC_{50} values, data was computed using EZ-Fit5 Perrella Scientific Inc. Amherst USA software.

5.2. General method for the synthesis of compounds (1a–1e and 2a–2e)

A solution containing 0.001 mol 6-(un)substituted 3-formylchromone in 5–7 mL ethanol was stirred with heating until dissolved. Then catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) was added followed by the addition of 0.001 mol of 3- and 4-aminobenzene sulfonamide in equal volumes of ethanol. Reaction mixture was refluxed for 3.5 h and kept overnight. Solid product was obtained by filtration and purified by recrystallization from a mixture of hot ethanol and acetone (1:1).

5.2.1. 4-{[(2-Ethoxy-4-oxo-2H-chromen-3(4H)-ylidene)methyl]amino}benzenesulfonamide (1a)

Yield: 85%; Mp 158–160 °C. IR (ν, cm^{-1}): 1646 (C=O), 3400 (NH_2), 1152 (SO_2^{sym}), 1376 (SO_2^{asym}). EI–MS *m*/*z* (rel. int.%): 328.0 (23), 171.9 (93), 156.0 (57), 92.0 (90), 65 (100). ^{1}H NMR (400 MHz, DMSO) δ: 11.81 (1H, d, J = 12.4 Hz, N*H*), 8.17 (1H, d, J = 12.3 Hz, C*H* enamine), 7.85–7.80 (3H, m, H-5, ArH), 7.56–7.51 (3H, m, H-7, ArH benzene), 7.28 (2H, br s, SO_2NH_2), 7.16–7.06 (2H, m, ArH chromome), 5.94 (1H, s, H-2), 3.74 (2H, q, J = 7.1 Hz, CH_2), 1.10 (3H, t, J = 7.1 Hz, CH_3). ^{13}C NMR (300 MHz, DMSO) δ: 14.94 (CH_3), 63.07 (CH_2), 180.56 (C-4), 155.69 (C-9), 142.41 (C-1′), 138.78 (C-4′), 127.56 (C-10), 104.84 (C-3), 144.08 (C-11), 127.41 (C-3′, C-5′), 116.39 (C-2′, C-6′), 99.92 (C-2), 134.78 (C-7), 118.09 (C-8), 125.67 (C-5), 121.96 (C-6).

5.2.2. 4-{[(2-Ethoxy-6-fluoro-4-oxo-2H-chromen-3(4H)-ylidene)methyl]amino}benzenesulfonamide (1b)

Yield: 80%. Mp 158–160 °C. IR (ν, cm^{-1}): 1654 (C=O), 3319 (NH_2), 1154 (SO_2^{sym}). EI–MS *m*/*z* (rel. int.%): 346.1 (100), 265.1 (15), 190.1 (78), 164.1 (62), 138.1 (23), 106.1 (58), 78.1 (56). ^{1}H NMR (500 MHz, DMSO), δ: 11.78 (1H, d, J = 12.5 Hz, NH), 8.20 (1H, d, J = 12.5 Hz, C*H* enamine), 7.81 (2H, d, J = 8.5 Hz, H-3′, H-5′), 7.55 (2H, d, J = 8.6 Hz, H-2′, H-6′), 7.52 (1H, dd, J = 3.0 Hz, J = 8.3 Hz, H-5), 7.43 (1H, ddd, J = 3 Hz, J = 8.6 Hz, H-7), 7.29 (2H, br s, SO_2NH_2), 7.15 (1H, dd, J = 3 Hz, J = 8.8 Hz, H-8), 5.95 (1H, s, H-2), 3.74 (2H, q, J = 7 Hz, CH_2), 1.09 (3H, t, J = 7.0 Hz, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 63.15 (CH_2), 14.90 (CH_3), 179.42 (C-4), 158.23 (C-9), 155.86 (C-6), 151.85 (C-1′), 142.34 (C-4′), 139.04 (C-10), 104.39 (C-3), 144.77 (C-11), 127.38 (C-3′, C-5′), 116.62 (C-2′, C-6′), 99.94 (C-2), 121.88 (C-7), 120.03 (C-8), 110.90 (C-5).

5.2.3. 4-{[(6-Bromo-2-ethoxy-4-oxo-2H-chromen-3(4H)-ylidene)methyl]amino}benzenesulfonamide (1c)

Yield: 87%. Mp 174–176 °C. IR (ν, cm^{-1}): 1660 (C=O), 3324 (NH_2), 1159 (SO_2^{sym}), 1340 (SO_2^{asym}). EI–MS *m/z* (rel. int.%): 406.0 (32), 327.0 (39), 251.9 (60), 224.0 (100), 172.1 (36), 156.0 (51), 92.0 (48), 64.1 (50). ^{1}H NMR (400 MHz, DMSO) δ: 11.78 (1H, d, J = 12.7 Hz, N*H*), 8.23 (1H, d, J = 12.8 Hz, C*H* enamine), 7.90 (1H, s, H-5), 7.83 (2H, d, J = 8.6 Hz, H-3′, H-5′), 7.72 (1H, dd, J = 2.4 Hz, J = 8.7 Hz, H-7), 7.58 (2H, d, J = 8.7 Hz, H-2′, H-6′), 7.31 (2H, br s, SO_2NH_2), 7.11 (1H, d, J = 8.6 Hz, H-8), 5.99 (1H, s, H-2), 3.69 (2H, q, J = 7.1 Hz, CH_2), 1.11 (3H, t, J = 7.1 Hz, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 178.88 (C-4), 154.74 (C-9), 142.30 (C-1′), 139.12 (C-4′), 124.10 (C-10), 113.69 (C-6), 104.26 (C-3), 144.94 (C-11), 137.02 (C-7), 128.13 (C-5), 127.38 (C-3′, C-5′), 121.54 (C-8), 116.69 (C-2′, C-6′), 100.09 (C-2), 63.27 (CH_2), 14.90 (CH_3).

5.2.4. 4-{[(6,8-Dibromo-2-ethoxy-4-oxo-2H-chromen-3(4H)-ylidene)methyl]amino}benzenesulfonamide (1d)

Yield: 87%. Mp 215–217 °C. IR (ν, cm^{-1}): 1651 (C=O), 3321 (NH_2), 1155 (SO_2^{sym}), 1336 (SO_2^{asym}). EI–MS *m/z* (rel. int.%): 420.8 (20), 404.8 (53), 329.8 (69), 303.8 (100), 172 (38), 92 (51), 156 (59). ^{1}H NMR (400 MHz, DMSO) δ: 1.11 (3H, t, J = 7.2 Hz, CH_3), 3.78 (2H, q, J = 7.2 Hz, CH_2), 6.11 (1H, s, H-2), 7.30 (2H, br s, SO_2NH_2), 7.58 (2H, d, J = 8.4 Hz, H-2′, H-6′), 7.82 (2H, d, J = 8.4 Hz, H-3′, H-5′), 7.88 (1H, s, H-7), 8.0 (1H, s, H-5), 8.27 (1H, d, J = 12.8 Hz, C*H* enamine), 11.76 (1H, d, J = 12.8 Hz, NH). ^{13}C NMR (300 MHz, DMSO) δ: 177.85 (C-4), 151.45 (C-9), 142.14 (C-8), 139.41 (C-1′), 124.96 (C-4′), 113.86 (C-10), 113.01 (C-6), 103.58 (C-3), 145.70 (C-7), 138.86 (C-11), 138.74 (C-5), 127.53 (C-3′, C-5′), 116.95 (C-2′, C-6′), 100.63 (C-2), 63.57 (CH_2), 14.74 (CH_3).

5.2.5. 4-{[(6-Ethyl-2-ethoxy-4-oxo-2H-chromen-3(4H)-ylidene)methyl]amino}benzenesulfonamide (1e)

Yield: 77%. Mp 225–227 °C. IR (ν, cm^{-1}): 1647 (C=O), 3341 (NH_2), 1158 (SO_2^{sym}), 1345 (SO_2^{asym}). EI–MS *m/z* (rel. int.%): 355.8 (13), 274.9 (78), 199.9 (100), 158.9 (48), 77.0 (62), 52.0 (24). ^{1}H NMR (400 MHz, DMSO) δ: 1.09 (3H, t, J = 7.6 Hz, -CH_2CH_3), 1.20 (3H, t, J = 7.2 Hz, -OCH_2CH_3), 2.64 (2H, q, J = 7.6 Hz, -CH_2CH_3), 3.72 (2H, q, J = 7.2 Hz, -OCH_2CH_3), 5.91 (1H, s, H-2), 7.28 (2H, s, SO_2NH_2), 7.00 (1H, d, J = 8.4 Hz, H-8), 7.40 (1H, dd, J = 2.0 Hz, J = 8.4 Hz, H-7), 7.53 (2H, d, J = 8.4 Hz, H-2′, H-6′), 7.64 (1H, s, H-5), 7.80 (2H, d, J = 8.8 Hz, H-3′, H-5′), 8.15 (1H, d, J = 12.0 Hz, H-11), 11.80 (1H, d, J = 12.4 Hz, N*H*). ^{13}C NMR (400 MHz, DMSO) δ: 14.94 (CH_3 ethyl), 15.55 (CH_3 ethoxy), 64.57 (–OCH_2), 27.23 (CH_2), 180.77 (C-4), 146.99 (C-9), 142.21 (C-1′), 137.21 (C-4′), 127.36 (C-10), 105.00 (C-3), 143.89 (C-11), 127.42 (C-3′, C-5′), 116.31 (C-2′, C-6′), 99.83 (C-2), 134.47 (C-7), 117.98 (C-8), 125.17 (C-5), 132.26 (C-6).

5.2.6. 3-{[(2-Ethoxy-4-oxo-2H-chromen-3(4H)-ylidene)methyl]amino}benzenesulfonamide (2a)

Yield: 87%. Mp 150–152 °C. IR (ν, cm^{-1}): 1642 (C=O), 3429 (NH_2), 1157 (SO_2^{sym}), 1377 (SO_2^{asym}). EI–MS *m/z* (rel. int.%): 328.0 (16), 247.1 (14), 172.0 (95), 146.0 (20), 92.0 (91), 65.0 (100). ^{1}H NMR (400 MHz, DMSO) δ: 1.08 (3H, t, J = 7.2 Hz, CH_3), 3.76 (2H, q, J = 7.1 Hz, CH_2), 5.98 (1H, s, H-2), 7.08 (1H, d, J = 8.0 Hz, H-8), 7.15 (1H, t, J = 7.6 Hz, H-7), 7.39 (2H, br s, SO_2NH_2), 7.51–7.59 (4H, m, ArH benzene, H-6), 7.79 (1H, s, H-2′), 7.84 (1H, dd, J = 1.2 Hz, J = 7.8 Hz, H-5), 8.15 (1H, d, J = 12.4 Hz, H-11), 11.90 (1H, d, J = 12.0 Hz, NH). ^{13}C NMR (300 MHz, DMSO) δ: 15.16 (CH_3), 63.16 (CH_2), 180.44 (C-4), 155.65 (C-9), 145.58 (C-1′), 140.20 (C-3′), 121.88 (C-10), 104.41 (C-3), 144.44 (C-11), 134.64 (C-7), 130.44 (C-5), 125.64 (C-5′), 121.89 (C-6), 120.58 (C-6′), 119.99 (C-4′), 118.04 (C-8), 113.30 (C-2′), 99.89 (C-2).

5.2.7. 3-{[(6-Fluoro-2-ethoxy-4-oxo-2H-chromen-3(4H)-ylidene)methyl]amino}benzenesulfonamide (2b)

Yield: 88%. Mp 168–170 °C. IR (ν, cm^{-1}): 1645 (C=O), 3300 (NH_2), 1157 (SO_2^{sym}), 1377 (SO_2^{asym}). EI–MS *m/z* (rel. int.%): 346.1 (8), 164.1 (16), 265.1 (100), 190.1 (42), 76.1 (23). ^{1}H NMR (500 MHz, DMSO) δ: 1.08 (3H, t, J = 7.08 Hz, CH_3), 3.75–3.66 (2H, m, CH_2), 5.98 (1H, s, H-2), 7.15 (1H, dd, J = 4.2 Hz, J = 9.0 Hz, H-8), 7.42–7.38 (3H, m, H-7, SO_2NH_2), 7.50–7.61 (4H, m, H-5, ArH benzene), 7.81 (1H, s, H-2′), 8.19 (1H, d, J = 12.5 Hz, H-11), 11.88 (1H, d, J = 12.5 Hz, NH). ^{13}C NMR (400 MHz, DMSO) δ: 15.16 (CH_3), 63.14 (CH_2), 179.29 (C-4), 158.25 (C-9), 155.87 (C-6), 151.81 (C-1′), 145.59 (C-3′), 110.68 (C-10).

5.2.8. 3-{[(6-Bromo-2-ethoxy-4-oxo-2H-chromen-3(4H)-ylidene)methyl]amino}benzenesulfonamide (2c)

Yield: 88%. Mp 168–170 °C. IR (ν, cm^{-1}): 1643 (C=O), 3231 (NH_2), 1150 (SO_2^{sym}), 1336 (SO_2^{asym}). EI–MS *m/z* (rel. int.%): 406.0 (16), 325.0 (14), 250.0 (25), 172.1 (74), 92.1 (100), 65.1 (93). ^{1}H NMR (300 MHz, DMSO) δ: 1.10 (3H, t, J = 7.1 Hz, CH_3), 3.77–3.65 (2H, m, CH_2), 6.01 (1H, s, H-2), 7.09 (1H, d, J = 8.7 Hz, H-8), 7.40 (2H, br s, SO_2NH_2), 7.54–7.60 (3H, m, ArH benzene), 7.66 (1H, dd, J = 2.4 Hz, J = 8.7 Hz, H-7), 7.81 (1H, s, H-2′), 7.88 (1H, s, H-5), 8.21 (1H, d, J = 12.6 Hz, H-11), 11.86 (1H, d, J = 12.6 Hz, NH). ^{13}C NMR (300 MHz, DMSO) δ: 63.25 (CH_2), 14.95 (CH_3), 178.77 (C-4), 154.74 (C-9), 145.61 (C-1′), 128.15 (C-3′), 124.18 (C-10), 119.59 (C-3), 103.89 (C-6), 145.33 (C-11), 140.04 (C-7), 136.93 (C-5), 130.42 (C-5′), 127.73 (C-6′), 120.73 (C-4′), 120.17 (C-8), 113.65 (C-2′), 100.10 (C-2).

5.2.9. 3-{[(6,8-Dibromo-2-ethoxy-4-oxo-2H-chromen-3(4H)-ylidene)methyl]amino}benzenesulfonamide (2d)

Yield: 85%. Mp 196–198 °C. IR (ν, cm^{-1}): 1641 (C=O), 3301 (NH_2), 1158 (SO_2^{sym}), 1343 (SO_2^{asym}). EI–MS *m/z* (rel. int.%): 329.8 (17), 172 (100), 92 (31), 65 (27), 485.9 (15), 404.9 (14), 329.8 (17). ^{1}H NMR (400 MHz, DMSO) δ: 1.11 (3H, t, J = 7.2 Hz, CH_3), 3.84–3.70 (2H, m, CH_2), 6.14 (1H, s, H-2), 7.42 (2H, s, SO_2NH_2), 7.57–7.62 (3H, m, ArH benzene), 7.83 (1H, s, H-7), 7.85 (1H, s, H-5), 8.06 (1H, s, H-5), 8.26 (1H, d, J = 12.8 Hz, H-11), 11.86 (1H, d, J = 12.8 Hz, NH). ^{13}C NMR (300 MHz, DMSO) δ: 14.77 (CH_3), 63.53 (CH_2), 177.70 (C-4), 145.58 (C-9), 139.86 (C-1′), 127.84 (C-3′), 125.02 (C-10), 114.12 (C-6), 112.97 (C-3), 103.19 (C-8), 146.05 (C-7), 138.76 (C-11), 130.41 (C-5), 127.41 (C-5′), 121.21 (C-6′), 120.42 (C-4′), 113.82 (C-2′), 100.61 (C-2).

5.2.10. 3-{[(6-Ethyl-2-ethoxy-4-oxo-2H-chromen-3(4H)-ylidene)methyl]amino}benzenesulfonamide (2e)

Yield: 73%. Mp 215–217 °C. IR (ν, cm^{-1}): 1643 (C=O), 3325 (NH_2), 1156 (SO_2^{sym}), 1343 (SO_2^{asym}). EI–MS *m/z* (rel. int.%): 356.0 (3), 308.8 (2), 275.0 (100), 200 (40), 132.9 (20), 91.0 (10), 75.9 (31). ^{1}H NMR (400 MHz, DMSO) δ: 1.09 (3H, t, J = 7.2 Hz, -CH_2CH_3), 1.20 (3H, t, J = 7.6 Hz, -OCH_2CH_3), 2.62 (2H, q, J = 7.6 Hz, -CH_2CH_3), 3.78–3.63 (2H, m, -OCH_2CH_3), 5.94 (1H, s, H-2), 7.58 = 7.51 (4H, m, H-7, ArH benzene), 7.43–7.37 (2H, m, SO_2NH_2), 7.00 (1H, d, J = 8.4 Hz, H-8), 7.64 (1H, S, H-5), 7.77 (1H, s, H-2′), 8.14 (1H, d, J = 12.0 Hz, H-11), 11.89 (1H, d, J = 12.4 Hz, N*H*). ^{13}C NMR (400 MHz, DMSO) δ: 15.16 (CH_3 ethoxy), 15.00 (CH_3 ethyl), 62.94 (–OCH_2), 27.27 (CH_2), 180.44 (C-4), 155.65 (C-9), 145.58 (C-1′), 140.20 (C-3′), 121.89 (C-6), 121.88 (C-10), 104.41 (C-3), 144.25 (C-11), 134.35 (C-7), 130.40 (C-5), 124.13 (C-5′), 120.50 (C-6′), 119.79 (C-4′), 117.92 (C-8), 113.21 (C-2′), 99.78 (C-2).

Acknowledgments

Authors acknowledge HEJ Research Institute of Chemistry, University of Karachi, Karachi, Pakistan for spectroscopic analyses.

Mariya-al-Rashida gratefully acknowledges Higher Education Commission, Pakistan, for the award of Indigenous Scholarship for PhD.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.04.040.

References and notes

1. el-Shaaer, H. M.; Foltinova, P.; Lacova, M.; Chovancova, J.; Stankovicova, H. *Farmaco* **1998**, *53*, 224.
2. Chohan, Z. H.; Rauf, A.; Naseer, M. M.; Somra, M. A.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2006**, *21*, 173.
3. Nam, D. H.; Lee, K. Y.; Moon, C. S.; Lee, Y. S. *Eur. J. Med. Chem.* **2010**, *45*, 4288.
4. Huang, W.; Ding, Y.; Miao, Y.; Liu, M. Z.; Li, Y.; Yang, G. F. *Eur. J. Med. Chem.* **2009**, *44*, 3687.
5. Desideri, N.; Mastromarino, P.; Conti, C. *Antiviral Chem. Chemother.* **2003**, *14*, 195.
6. Houghton, P. J.; Woldemariam, T. Z.; Khan, A. I.; Burke, A.; Mahmood, N. *Antiviral Res.* **1994**, *25*, 235.
7. Kaspentakis, G. C.; Tsoleridis, C. A.; Stephanatou, J. S. *J. Heterocycl. Chem.* **2007**, *44*, 425.
8. Sosnovskikh, V.; Moshkin, V. *Russ. Chem. Bull.* **2010**, *59*, 1056.
9. Nohara, A.; Umetani, T.; Sanno, Y. *Tetrahedron* **1974**, *30*, 3553.
10. Nohara, A.; Umetani, T.; Sanno, Y. *Tetrahedron Lett.* **1973**, *22*, 1995.
11. Ishiki, H. M.; Donate, P. M.; Galembeck, S. E. *J. Mol. Struc-Theochem* **1998**, *423*, 235.
12. Stokstad, E. L. R.; Jukes, T. H. *J. Nutr.* **1987**, *117*, 1335.
13. Hong, Y. L.; Hossler, P. A.; Calhoun, D. H.; Meshnic, S. R. *Antimicrob. Agents Chemother.* **1995**, *39*, 1756.
14. Nalam, M. N. L.; Peeters, A.; Jonckers, T. H. M.; Dierynck, I.; Schiffer, C. A. *J. Virol.* **2007**, *81*, 9512.
15. Santos, M. A.; Marques, S.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1538.
16. Nishimori, I.; Minakuchi, T.; Kohsaki, T.; Onishi, S.; Takeuchi, H.; Vullo, D.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3585.
17. Ilies, M. A.; Scozzafava, A.; Supuran, C. T. *Met. Based Drugs* **2000**, *7*, 57.
18. Zareef, M.; Iqbal, R.; De Dominguez, N. G.; Rodrigues, J.; Zaidi, J. H.; Arfan, M.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2007**, *22*, 301.
19. Supuran, C. T.; Scozzafava, A. *Bioorg. Med. Chem.* **2007**, *15*, 4336.
20. Supuran, C. T. *Therapy* **2007**, *4*, 355.
21. Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *12*, 3467.
22. Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168.
23. Ozensoy, O.; Puccetti, L.; Fasolis, G.; Arslan, O.; Scozzafava, A.; Supuran, C. T. *Med. Chem. Lett.* **2005**, *15*, 4862.
24. Puccetti, L.; Fasolis, G.; Vullo, D.; Chohan, Z. H.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3096.
25. al-Rashida, M.; Tahir, M. N.; Nagra, S. A.; Imran, M.; Iqbal, J. *Acta Crystallogr.* **2009**, *E65*, 01818.
26. Pocker, Y.; Stone, J. T. *Biochemistry* **1967**, *6*, 668.
27. Stankovicova, H.; Fabian, W. M. F.; Lacova, M. *Molecules* **1996**, *1*, 223.
28. Stankovicova, H.; Gasparova, R.; Lacova, M.; Chovancova, J. *Collect. Czech. Chem. Commun.* **1997**, *62*, 781.
29. Stankovicova, H.; Lacova, M.; Gaplovsky, A.; Chovancova, J.; Pronayova, N., *II-International Electronic Conference on Synthetic Organic Chemistry (ECSOC-2)*, 1998 http://www.mdpi.org/ecsoc.
30. Dziewulska-Kułaczkowska, A.; Mazur, L. *J. Mol. Struct.* **2011**, *985*, 233.
31. Gajbhiye, A.; Mallareddy, V.; Achaiah, G. *Indian J. Pharm. Sci.* **2008**, *70*, 118.
32. Maren, T. H.; Conroy, C. W. *J. Biol. Chem.* **1993**, *268*, 26233.