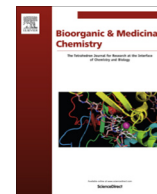


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Synthesis of novel acridine bis-sulfonamides with effective inhibitory activity against the carbonic anhydrase isoforms I, II, IX and XII

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ABSTRACT

By using a multi component reaction system (MCR), nitro acridine sulfonamides were obtained from cyclic-1,3-diketones, 4-aminobenzene sulfonamide and aromatic aldehydes. Some novel acridine bis-sulfonamides **6a–I** were then synthesized by the reaction between sulfonyl chlorides and the novel amino-acridine sulfonamides **5a** and **5b**, obtained by reduction of nitro-acridine sulfonamide derivatives **4a** and **4b**. The newly synthesized compounds were investigated as inhibitors of 4 human carbonic anhydrase isoforms (hCA, EC 4.2.1.1). Several of the compounds showed low micromolar inhibition against the medically relevant isoforms hCA I, II, IX, and XII.

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1. Introduction

Acridines are planar tricyclic aromatic compounds which contain nitrogen in the heterocyclic ring. Even if these heterocycles were discovered the 19th century, some of their derivatives are still under investigation. Acridine shows a similarity in its structure to nicotinamide adenine dinucleotide (NADH),¹ a molecule

possessing important biological functions as a coenzyme/cofactor.² Thus there are many acridine-like compounds showing anti-cancer,^{3,4} or antimicrobial activity,⁵ whereas other such derivatives were reported to act as effective β -channel opener in cardiovascular disease,⁶ in the photodynamic therapy,⁷ or as anti-Alzheimer's agents.⁸ Well-known drugs containing the acridine moiety include Amsacrine, Asulacraine, Acronycine, Acridine carboxamide (DACA), Proflavine and Ascididemin (Chart 1), and they have been used as anti-cancer or anti-bacterial agents.^{12,13} Furthermore, many acridine sulfonamides are known as strong carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs), potentially useful for the treatment glaucoma^{9,10} or other conditions, which the activity of the CA isoforms are deregulated.¹¹

CAs are responsible for the reversible hydration of carbon dioxide to bicarbonate.¹¹ By catalyzing this simple reaction CAs participate in many physiological processes such as regulation of respiration and gas exchange, bone resorption, calcification, pH and CO₂ homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions.^{11–13} This diversity of roles makes different isoforms interesting drug targets for a variety of conditions.¹⁴

The sulfonamides and their isosteres such as the sulfamates and sulfamides, are established CAIs and are in clinical use for almost

Abbreviations: MCR, multi component reaction; CA, carbonic anhydrase; hCA, human carbonic anhydrase; NADH, nicotinamide adenine dinucleotide; Amsacrine, *N*-[4-(acridin-9-ylamino)-3-methoxyphenyl]methanesulfonamide; Asulacraine, 9-[4-(methanesulfonamido)-2-methoxyanilino]-*N*,5-dimethylacridine-4-carboxamide; Acronycine, 6-methoxy-3,3,12-trimethylpyrano[2,3-*c*]acridin-7-one; DACA, *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide; Proflavine, acridine-3,6-diamine; Ascididemin, 9*H*-quino[4,3,2-*de*][1,10]phenanthroline-9-one; CAIs, carbonic anhydrase inhibitors; IR, infrared; NMR, nuclear magnetic resonance; HRMS, high resolution mass spectroscopy; SAR, structure–activity relationship; DMSO, dimethyl sulfoxide; LC, liquid chromatography; DBSA, *p*-dodecylbenzenesulfonic acid; TLC, thin layer chromatography; mp, melting point; Lit, literature; ESI, electron spray ionization; TEA, trimethylamine; μ M, micromolar; nM, nanomolar; *K*_i, inhibition constant.

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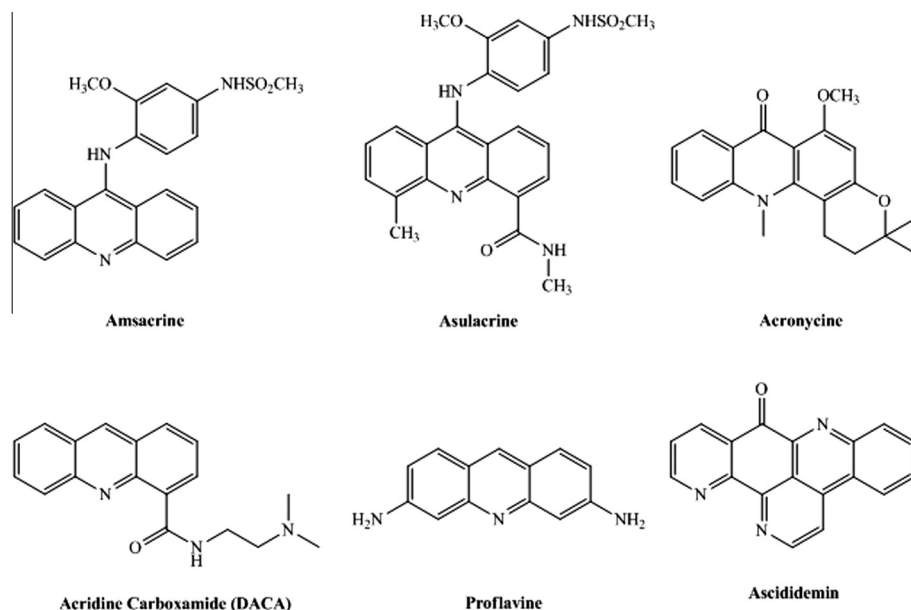


Chart 1. Some drugs incorporating acridine moieties.

70 years for the treatment of glaucoma, obesity, epilepsy and as diuretics.¹⁵ The large use of CAIs for pharmaceutical applications relies on the wide distribution of the 15 human (h) CA isoforms within different tissues as well as on their implication in many physiological/pathological conditions. Antiglaucoma CAI-drugs mainly target CA II, IV and XII; the diuretics CA II, IV, XII and XIV; the antiepileptic CA VII and XIV.^{11,14,16,17} The selective inhibition of the CA IX and XII isoforms results in antitumor and antimetastatic effects.^{13,14} In vivo experiments showed that silencing of hCA IX reduces xenograft tumors to 40% of the volume along with up-regulation response of the gene encoding for hCA XII. Silencing of both hCA IX and hCA XII showed 85% reduction of tumor growth. hCA IX is a 54–58 kDa *trans*-membrane protein consisting of a cytoplasmic fragment, which exhibits phosphorylation sites, a *trans* membrane section and an extracellular region. hCA IX exposes its catalytic domain on the extracellular environment and is further functionalised with glycosidic residues.¹⁸ It should be stressed that currently a sulfonamide CA IX inhibitor (SLC-0111) entered in Phase I clinical studies for the treatment of hypoxic, advanced stage metastatic solid tumors.^{19,20}

The main drawback associated to the use of CAIs is represented by the lack of selectivity in inhibiting various isoforms by many of the first and second generation CAIs, thus resulting in a plethora of side effects.^{21,22} In this context many efforts have been made for the development of specific CAIs, and some remarkable results have been achieved in the last 15 years since the introduction of the tail approach.^{20–22} Moreover novel CAIs classes such as the polyamines,²³ phenols,²⁴ dithiocarbamates,²⁵ xanthates,²⁶ coumarins, thiocoumarins, 2-thioxo-coumarins and coumarin oximes^{16,27–29} were identified and their inhibition mechanisms of many of these compounds were determined by means of X-ray crystallography CA II adducts.^{21,24,25}

Considering the interest in sulfonamide CAIs, in this study we investigate a new class of acridine bis-sulfonamides which have been designed by an approach of multicomponent reactions. The structure of the novel compounds was confirmed by using spectral analysis (FT-IR, ¹H NMR, ¹³C NMR (APT) and HRMS) and they were investigated the inhibition of human CA isoforms hCA I, II, IX and XII. The structure–activity relationships (SAR) for the inhibition of these isoforms with the acridine bis-sulfonamides are also discussed.

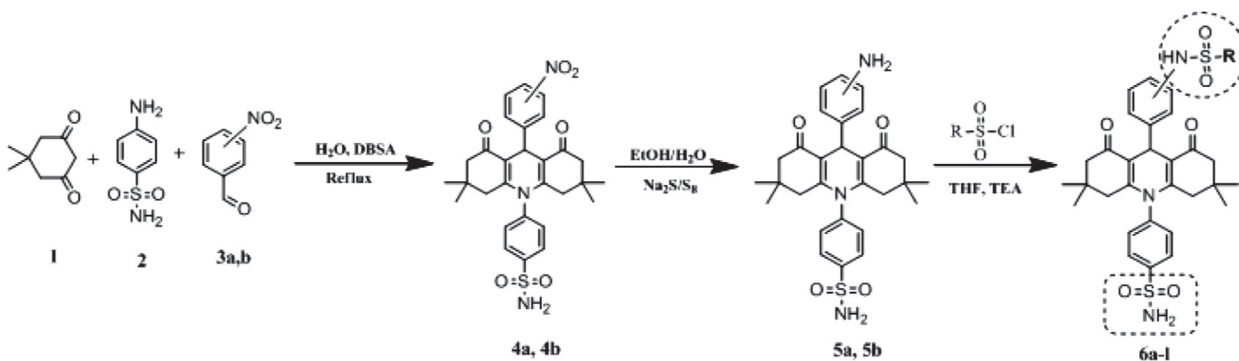
2. Results and discussion

2.1. Chemistry

The general synthetic method, three steps reaction shown in Scheme 1, was used to prepare the nitro acridine derivatives (**4a** and **4b**), novel amino acridine compounds (**5a** and **5b**) and novel acridine bis-sulfonamide compounds (**6a–l**). For the first step MCR method applied, and compounds **4a** and **4b** were obtained by using dimedone (**1**), 4-aminobenzenesulfonamide (**2**), nitro aromatic aldehydes (**3a** and **3b**)—molar ratio of 2:1:1 and DBSA, as phase transfer catalyst, in one-pot reaction in water. For the second step, new amino acridine derivatives (**5a** and **5b**) were synthesized by using excessive reduction reactive—aqueous sodium poly-sulfide—from nitro acridine compounds (**4a** and **4b**) at reflux temperature with high yields (respectively, 87% and 82%). For the final step, the acridine bis-sulfonamide derivatives **6a–l** were obtained from various sulfonyl chlorides and amino acridines (**5a**, **5b**) in THF at room temperature in presence of triethylamine (TEA).^{30,31}

All the products were characterized by melting point detection, IR, NMR and HRMS analyses. In the IR spectra of the compounds **5a**, **5b** and **6a–l**, the $-NH_2$, $-SO_2NH_2$, $-SO_2NHR$ vibrations were observed in the region between 3244 and 3467 cm^{-1} . Aliphatic C–H stretching bands were observed at 2954–2959 cm^{-1} and the aromatic C–H stretching bands were observed at 3032–3098 cm^{-1} . Also, sharp peaks were observed for the carbonyl groups in the region between 1623 and 1634 cm^{-1} for all the acridine bis-sulfonamides.

For the ¹H NMR spectra of compounds **5a**, **5b** and **6a–l** singlet peaks were observed between 0.60 and 0.90 ppm which belong to protons of the methyl groups in position 3 and 6. For the compounds **5a**, **5b** and **6a–l** doublet or multiplet peaks were observed in between 1.62 and 2.22 ppm belong to CH₂ group protons of the cyclohexene ring.³² For the compounds **6b**, **6e**, **6h** and **6k** singlet peaks were observed in between 2.18 and 2.52 ppm belong to protons of the methyl group. For the compounds **6c** and **6i** the signal of the methoxy protons were observed at 3.80 and 3.70 ppm, respectively. Also the signals of the CH protons were observed at 4.90 and 4.98 ppm and the signals for the aromatic protons were observed between 6.30 and 8.36 ppm. For the compounds **5a**, **5b** and **6a–l** $-SO_2NH_2$ group protons were assigned as singlet peaks



	6a	6b	6c	6d	6e	6f	6g	6h	6i	6j	6k	6l
R	C ₆ H ₅	4-CH ₃ C ₆ H ₄	4-CH ₃ OC ₆ H ₄	4-BrC ₆ H ₄	2,4,6-(CH ₃) ₃ C ₆ H ₂	2-Naphthyl	C ₆ H ₅	4-CH ₃ C ₆ H ₄	4-CH ₃ OC ₆ H ₄	4-BrC ₆ H ₄	2,4,6-(CH ₃) ₃ C ₆ H ₂	2-Naphthyl

Scheme 1. Synthesis of novel acridine bis-sulfonamide compounds.

Table 1

CA inhibition data for compounds (**4a**, **4b**), (**5a**, **5b**), (**6a–l**) and standard inhibitors against human isozymes hCA I, II, IX and XII by a stopped flow CO₂ hydrase assay³²

Entry	-R	K _i (μM)			
		hCA I	hCA II	hCA IX	hCA XII
4a	—	5.1	0.64	1.02	0.35
4b	—	0.69	0.89	0.42	0.50
5a	—	2.5	0.65	1.00	0.61
5b	—	8.87	0.50	0.92	0.03
6a	C ₆ H ₅	2.09	0.10	0.58	0.64
6b	4-CH ₃ C ₆ H ₄	4.55	0.96	0.10	0.53
6c	4-CH ₃ OC ₆ H ₄	6.92	0.37	0.44	0.92
6d	4-BrC ₆ H ₄	6.53	0.60	1.12	0.86
6e	2,4,6-(CH ₃) ₃ C ₆ H ₂	5.62	0.48	0.09	0.26
6f	2-Naphthyl	3.89	0.47	0.28	0.37
6g	C ₆ H ₅	8.91	0.38	0.11	1.02
6h	4-CH ₃ C ₆ H ₄	4.27	0.39	0.10	0.78
6i	4-CH ₃ OC ₆ H ₄	4.31	0.25	0.80	0.92
6j	4-BrC ₆ H ₄	5.38	0.45	0.25	0.56
6k	2,4,6-(CH ₃) ₃ C ₆ H ₂	6.52	0.43	0.09	0.52
6l	2-Naphthyl	6.53	0.50	0.81	0.83
AAZ ^a	—	0.25	0.012	0.025	0.006

^a Acetazolamide (AAZ) was used as a standard inhibitor for all CAs investigated here. K_i-s are means from 3 different assays. Standard errors were in the range of ±5–10% of the reported values (data not shown).

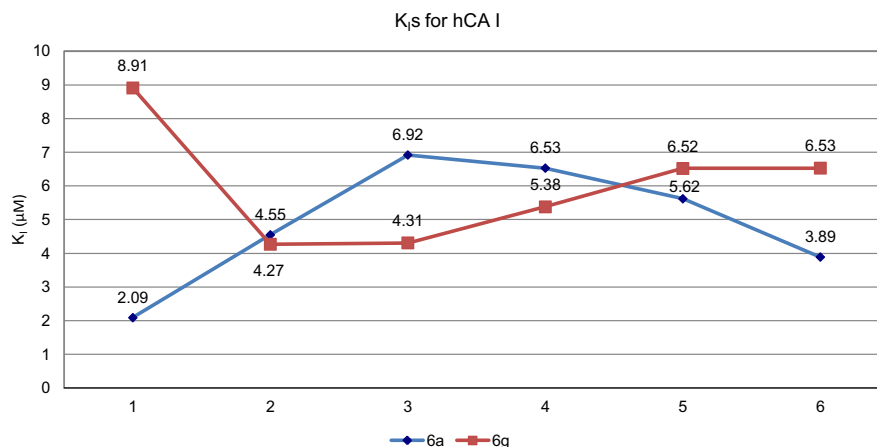
between 7.61 and 7.63 ppm and the compounds **6a–f** and **7a–f** were assigned as singlet peaks between 9.90 and 10.25 ppm. The ¹³C NMR (APT) spectra of the compounds **5a**, **5b** and **6a–l** displayed

a signal for the carbonyl group carbon peak between 195.34 and 195.63 ppm and the signal of carbon–carbon double bonds was observed between 113.29 and 162.67 ppm.³³ Also, aliphatic carbon peaks were observed at 20.82–56.00 ppm. When the HRMS analyses of all the molecules (**5a**, **5b** and **6a–l**) were examined, we observed signals in line with the molecule ion peaks of the proposed structures.

2.2. Carbonic anhydrase inhibition

The new compounds reported here and the standard drug acetazolamide were assayed as inhibitors of four cytosolic human isozymes, hCA I, II, IX and XII (Table 1).³⁴

The new derivatives were rather ineffective inhibitors of the widespread cytosolic isoform hCA I, with inhibition constants in the range of 0.69–8.91 μM (acetazolamide, the standard sulfonamide CAI has a K_i of 250 nM against this isoform). There are few important variations in order to be able to discuss SAR. It was observed that increasing of the bulkiness of the tails for the compound series **4a**, **4b**, and **6a–f** (Chart 2) resulted with slight increasing of K_i values, that is, decreasing on inhibitory effect, in the range of 0.69–6.92 μM. On the contrary the opposite effect of bulkiness was observed that for the compound series **5a**, **5b**, and **6g–l**, (Chart 2), with inhibition constants varying in the range of 2.5–8.91 μM. It should be noted that position of the tails (sulfonyl groups) on the main scaffold (for **6a–f**, are *-para* and for

Chart 2. Change in the K_i values for **6a–f** (red line) and **6g–l** (blue line).

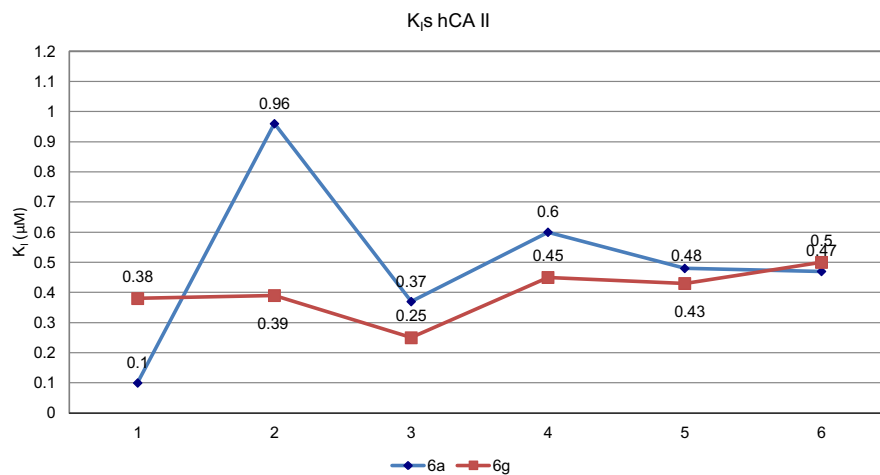


Chart 3. Change in the K_i values for **6a-f** (red line) and **6g-l** (blue line).

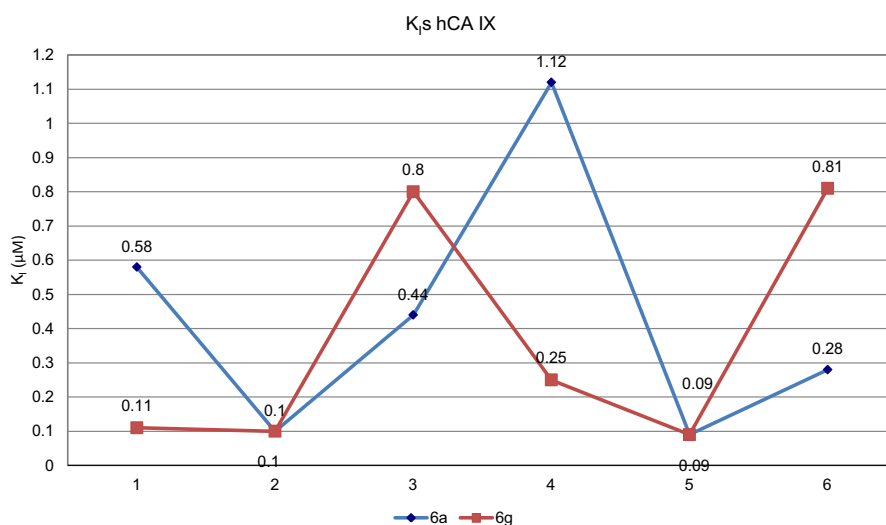


Chart 4. Change in the K_i values for **6a-f** (red line) and **6g-l** (blue line).

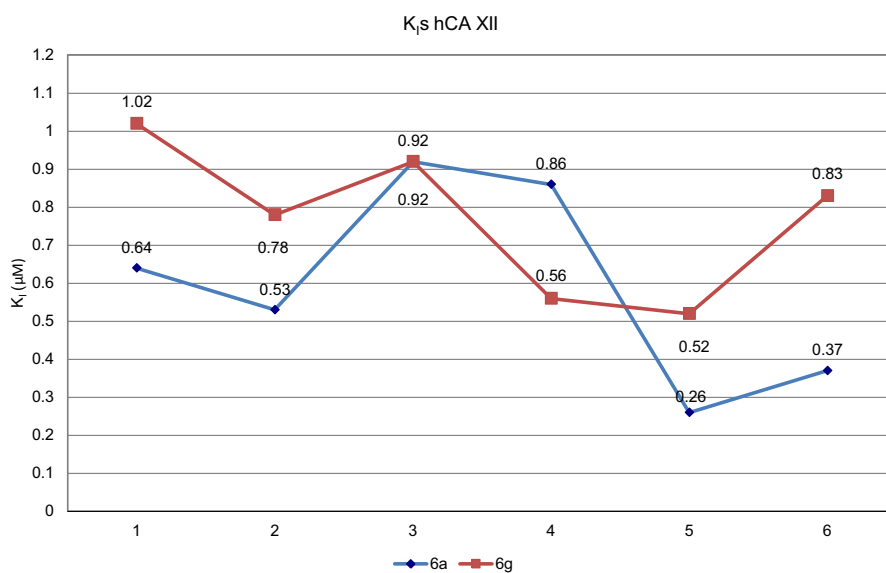


Chart 5. Change in the K_i values for **6a-f** (red line) and **6g-l** (blue line).

6g–1 are *-meta*) also influence the K_i values. However, a salient feature of all these sulfonamides is their rather flat SAR, probably due to the extreme bulkiness of the tails that they incorporate and which probably extend out of the active site.

For the dominant cytosolic isoform hCA II, as for isoform CA I, it was observed that the new derivatives were rather ineffective inhibitors, showing a limited range of inhibitory power, with a variation of K_i between 0.10 and 0.96 μM . However, the K_i values were slightly lower for on hCA II when compared with other CA isoforms and any important effect of bulkiness of tail was not observed for this isoform (Chart 3). It is suggested that due to the fact hCA II has a wide entrance to active site, probably the hydrophobic and hydrophilic interactions of these tails with the active site were not highly effective, and leading in fact to the flat SAR mentioned above also for isoform hCA I.

The transmembrane isoform hCA IX was inhibited better than the cytosolic ones, with K_i s ranging between 90 nM and 1.12 μM (Table 1). However the isoform which was better inhibited was hCA XII (a transmembrane one as hCA IX), with K_i s ranging between 30 nM and 1.02 μM . The small range of inhibitory power of these compounds against the two transmembrane isoforms may be due to the fact that the variations in the structure are at rather distant parts of the tail from the primary sulfonamide, and we hypothesize that these parts of the molecules lay outside the active site, affording thus for less specific interactions with amino acid residues crucial for the binding of inhibitors. It is thus probable that the bulky scaffold of these sulfonamides does not make a lot of favorable contacts with the enzyme active site, whereas the secondary sulfonamide moiety is too far away for assuring the right interactions with the residues at the entrance of the cavity, which would lead to isoform-selective CAIs (Charts 4 and 5).^{13–15}

3. Conclusion

There are still many important drug design aspect to be addressed for obtaining isoform-selective and more effective sulfonamide CAIs. Considering our interest in sulfonamides, herein we investigate a new series of acridine bis-sulfonamides which have been obtained by using multicomponent reaction techniques. The new series of acridine bis-sulfonamides was synthesized with effective yields. The compounds were characterized by physico-chemical methods and tested for their in vitro inhibition activity against the CA isoforms I, II, IX and XII. Several compounds showed low micromolar inhibition against the pharmacologically relevant isoforms hCA I, II, IX, and XII. The prepared compounds containing both acridine ring and sulfonamide group are thought to be of interest because sulfonamides are used in the treatment of many diseases, possessing antimicrobial, antimalarial, antiglaucoma, and anticancer properties.

4. Experimental

4.1. Chemistry

The chemicals used in the synthesis of acridine bis-sulfonamide derivatives were obtained from Merck and Aldrich Chemical Company. All chemicals and solvents used for the synthesis were of spectroscopic reagent grade.

Melting points were measured on a Bibby Scientific Stuart Digital, Advanced, and SMP30. Fourier Transform Infrared (FT-IR) spectra were recorded on Bruker Optics, ALPHA FT-IR spectrometer. The ^1H NMR and ^{13}C NMR spectra were obtained in DMSO- d_6 with Bruker DPX-300 as solvents with tetramethylsilane as the internal reference. The mass analyses were performed on an Agilent Technologies 6530 Accurate-Mass Q-TOF LC/HRMS at the

advanced technology research center of Dumlupinar University (ILTEM).

4.2. General procedure for preparation of nitro-acridine sulfonamide compounds (4a, 4b)

A mixture of a dimedone **1** (0.280 g, 2 mmol), 4-aminobenzene-sulfonamide (0.172 g, 1 mmol) **2**, *-meta* and *-para* substitute nitrobenzaldehydes (**3a** and **3b**) (0.106 g, 1 mmol), and *p*-dodecylbenzenesulfonic acid (DBSA) (0.033 g, 10 mmol %) in 40 mL H_2O was stirred and refluxing for 24 h. The progress of the reaction was monitored by TLC. After, the reaction is completed, the mixture was cooled to room temperature solid filtered off and washed with H_2O . The products (**4a** and **4b**) were purified and recrystallized from ethanol.¹⁰

4.2.1. 4-(3,3,6,6-Tetramethyl-9-(4-nitrophenyl)-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9H)-yl)benzenesulfonamide (4a)

As yellow solid (ethanol), mp 298–300 °C (Lit. >250),³⁰ yield 93%.

4.2.2. 4-(3,3,6,6-Tetramethyl-9-(3-nitrophenyl)-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9H)-yl)benzenesulfonamide (4b)

As yellow solid (ethanol), mp 256–257 °C (Lit. >250)³⁰ yield 89%.

4.3. General procedure for preparation of amino-acridine sulfonamide compounds (5a, 5b)

$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ (1 mmol) and sulfur (2 mmol) were dissolved by boiling in 20 ml of water. This solution (sodium poly-sulfur) was then added dropwise to a stirred solution of warm nitro acridine compounds (**4a**, **4b**) (1 mmol) in ethanol–water. The progress of the reaction was monitored by TLC. Once the reaction is completed, the mixture was cooled to room temperature and solid filtered off and washed with H_2O .¹⁰

4.3.1. 4-(9-(4-Aminophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9H)-yl)benzenesulfonamide (5a)

As white solid (ethanol), mp 257–258 °C, yield 87%. IR (cm^{-1}): 3458 and 3371 w ($-\text{NH}_2$), 3040 w (Ar–H), 2954 w (C–H), 1626 s (C=O), 1577 s (C=C), 1363 and 1152 s (SO_2); ^1H NMR (300 MHz, DMSO- d_6) δ (ppm): 0.70 (s, 6H, 2 \times $-\text{CH}_3$), 0.90 (s, 6H, 2 \times $-\text{CH}_3$), 1.71 (d, 2H, J = 17.2 Hz, $-\text{CH}_2$), 2.00 (d, 2H, J = 16.1 Hz, $-\text{CH}_2$), 2.18 (d, 4H, J = 16.5 Hz, 2 \times $-\text{CH}_2$), 4.80 (s, 2H, $-\text{NH}_2$), 4.90 (s, 1H, $-\text{CH}$), 6.43 (d, 2H, J = 8.4 Hz, Ar–H), 6.95 (d, 2H, J = 8.4 Hz, Ar–H), 7.62–7.68 (m, 4H, Ar–H and $-\text{SO}_2\text{NH}_2$), 8.03 (d, 2H, J = 8.7 Hz, Ar–H); ^{13}C NMR (75 MHz, DMSO- d_6) δ (ppm): 26.53, 29.79, 30.92, 32.44, 41.33, 50.17, 114.07, 114.23, 122.88, 128.35, 132.46, 133.49, 134.46, 138.42, 146.87, 149.81, 195.57; HRMS (QTOF-ESI): m/z calcd for $\text{C}_{29}\text{H}_{33}\text{N}_3\text{O}_4\text{S}$: 519.2192; found: 518.2321 [M–H] $^-$.

4.3.2. 4-(9-(3-Aminophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9H)-yl)benzenesulfonamide (5b)

As white solid (ethanol), mp 218–219 °C, yield 82%. IR (cm^{-1}): 3368 and 3272 w ($-\text{NH}_2$), 3066 w (Ar–H), 2957 w (C–H), 1628 s (C=O), 1574 s (C=C), 1334 and 1155 s (SO_2); ^1H NMR (300 MHz, DMSO- d_6) δ (ppm): 0.70 (s, 6H, 2 \times $-\text{CH}_3$), 0.90 (s, 6H, 2 \times $-\text{CH}_3$), 1.72 (d, 2H, J = 17.2 Hz, $-\text{CH}_2$), 2.02 (d, 2H, J = 16.0 Hz, $-\text{CH}_2$), 2.16–2.22 (m, 4H, 2 \times $-\text{CH}_2$), 4.90 (s, 1H, $-\text{CH}$), 5.10 (s, 2H, $-\text{NH}_2$), 6.30–6.33 (m, 1H, Ar–H), 6.50 (d, 2H, J = 7.7 Hz, Ar–H), 6.89 (t, J = 7.7 Hz, 1H, Ar–H), 7.61 (s, 2H, $-\text{SO}_2\text{NH}_2$), 7.64 (d, 2H, J = 8.7 Hz, Ar–H), 8.03 (d, 2H, J = 8.7 Hz, Ar–H); ^{13}C NMR (75 MHz, DMSO- d_6) δ (ppm): 26.56, 29.56, 32.59, 32.97, 41.41, 49.80, 112.84, 121.53, 122.67, 127.92, 130.23, 131.04, 134.73,

141.34, 145.34, 147.88, 148.51, 150.99, 195.63; HRMS (QTOF-ESI): m/z calcd for $C_{29}H_{33}N_3O_4S$: 519.2192; found: 518.2271 [M–H][–].

4.4. General procedure for preparation of acridine bis-sulfonamide derivatives (6a–l)

4-Amino-acridine sulfonamide **5a** (0.520 g, 1 mmol), benzene sulfonyl chloride (0.177 g, 1 mmol), and 0.5 mL dry triethylamine (TEA) were stirred in dry 5 mL THF for 5 h at room temperature. After the solvent was removed under in vacuo and washed with H_2O .³¹

4.4.1. *N*-(4-(3,3,6,6-Tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzenesulfonamide (6a)

As white solid (ethanol), mp 208–210 °C, yield 90%. IR (cm^{-1}): 3375 w (–NH₂), 3075 w (Ar–H), 2959 w (C–H), 1623 s (C=O), 1589 s (C=C), 1362 and 1153 s (SO₂); ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm): 0.70 (s, 6H, 2 \times –CH₃), 0.90 (s, 6H, 2 \times –CH₃), 1.70 (d, 2H, J = 17.2 Hz, –CH₂), 1.98 (d, 2H, J = 16.0 Hz, –CH₂), 2.16 (d, 4H, J = 16.5 Hz, 2 \times –CH₂), 4.93 (s, 1H, –CH), 6.95 (d, 2H, J = 8.5 Hz, Ar–H), 7.15 (d, 2H, J = 8.5 Hz, Ar–H), 7.46–7.70 (m, 7H, Ar–H), 7.61 (s, 2H, –SO₂NH₂), 8.01 (d, 2H, J = 8.6 Hz, Ar–H), 10.10 (s, 1H, –NH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ (ppm): 26.46, 29.65, 31.72, 32.50, 41.41, 49.95, 113.49, 120.60, 126.96, 127.80, 128.62, 129.55, 131.13, 133.21, 135.74, 140.14, 141.57, 142.56, 145.15, 150.08, 195.50; HRMS (QTOF-ESI): m/z calcd for $C_{35}H_{37}N_3O_6S_2$: 659.2124; found: 682.2021 [M+Na]⁺.

4.4.2. 4-Methyl-*N*-(4-(3,3,6,6-tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzenesulfonamide (6b)

As white solid (ethanol), mp 230–231 °C, yield 92%. IR (cm^{-1}): 3357 w (–NH₂), 3033 w (Ar–H), 2959 w (C–H), 1631 s (C=O), 1590 s (C=C), 1365 and 1159 s (SO₂); ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm): 0.70 (s, 6H, 2 \times –CH₃), 0.90 (s, 6H, 2 \times –CH₃), 1.70 (d, 2H, J = 17.3 Hz, –CH₂), 1.98 (d, 2H, J = 16.0 Hz, –CH₂), 2.16 (d, 4H, J = 16.5 Hz, 2 \times –CH₂), 2.31 (s, 3H, –CH₃), 4.92 (s, 1H, –CH), 6.95 (d, 2H, J = 8.5 Hz, Ar–H), 7.15 (d, 2H, J = 8.5 Hz, Ar–H), 7.28 (d, 2H, J = 8.1 Hz, Ar–H), 7.58 (d, 2H, J = 8.3 Hz, Ar–H), 7.61 (s, 2H, –SO₂NH₂), 7.66 (d, 2H, J = 8.5 Hz, Ar–H), 8.01 (d, 2H, J = 8.5 Hz, Ar–H), 10.05 (s, 1H, –NH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ (ppm): 21.41, 26.46, 29.65, 31.72, 32.51, 41.41, 49.95, 113.51, 120.34, 127.03, 127.81, 128.61, 130.01, 131.14, 135.89, 137.35, 141.58, 142.38, 143.53, 145.15, 150.08, 195.52; HRMS (QTOF-ESI): m/z calcd for $C_{36}H_{39}N_3O_6S_2$: 673.2280; found: 696.2194 [M+Na]⁺.

4.4.3. 4-Methoxy-*N*-(4-(3,3,6,6-tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzenesulfonamide (6c)

As white solid (ethanol), mp 215–216 °C, yield 94%. IR (cm^{-1}): 3393 w (–NH₂), 3064 w (Ar–H), 2958 w (C–H), 1626 s (C=O), 1584 s (C=C), 1361 and 1151 s (SO₂); ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm): 0.65 (s, 6H, 2 \times –CH₃), 0.85 (s, 6H, 2 \times –CH₃), 1.70 (d, 2H, J = 17.2 Hz, –CH₂), 1.99 (d, 2H, J = 16.0 Hz, –CH₂), 2.17 (d, 4H, J = 16.5 Hz, 2 \times –CH₂), 3.80 (s, 3H, –OCH₃), 4.93 (s, 1H, –CH), 6.95 (d, 2H, J = 8.5 Hz, Ar–H), 6.99 (d, 2H, J = 8.9 Hz, Ar–H), 7.15 (d, 2H, J = 8.5 Hz, Ar–H), 7.61 (s, 2H, –SO₂NH₂), 7.63–7.66 (m, 4H, Ar–H), 8.02 (d, 2H, J = 8.5 Hz, Ar–H), 9.98 (s, 1H, –NH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ (ppm): 26.46, 29.65, 31.69, 32.50, 41.41, 49.95, 56.00, 113.51, 114.68, 120.28, 127.80, 128.58, 129.20, 131.12, 131.80, 136.01, 141.58, 142.28, 145.15, 150.07, 162.75, 195.51; HRMS (QTOF-ESI): m/z calcd for $C_{36}H_{39}N_3O_7S_2$: 689.2229; found: 712.2138 [M+Na]⁺.

4.4.4. 4-Bromo-*N*-(4-(3,3,6,6-tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzenesulfonamide (6d)

As white solid (ethanol), mp 235–236 °C, yield 97%. IR (cm^{-1}): 3377 w (–NH₂), 3095 w (Ar–H), 2957 w (C–H), 1627 s (C=O), 1579 s (C=C), 1363 and 1159 s (SO₂); ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm): 0.70 (s, 6H, 2 \times –CH₃), 0.90 (s, 6H, 2 \times –CH₃), 1.70 (d, 2H, J = 17.2 Hz, –CH₂), 1.99 (d, 2H, J = 15.9 Hz, –CH₂), 2.17 (d, 4H, J = 16.5 Hz, 2 \times –CH₂), 4.92 (s, 1H, –CH), 6.95 (d, 2H, J = 8.5 Hz, Ar–H), 7.17 (d, 2H, J = 8.5 Hz, Ar–H), 7.61 (s, 2H, –SO₂NH₂), 7.58–7.71 (m, 6H, Ar–H), 8.02 (d, 2H, J = 8.5 Hz, Ar–H), 10.20 (s, 1H, –NH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ (ppm): 26.42, 29.67, 31.78, 32.50, 41.41, 49.94, 113.45, 120.85, 127.10, 127.82, 128.73, 129.03, 131.14, 132.67, 135.40, 139.30, 141.57, 142.87, 145.15, 150.10, 195.50; HRMS (QTOF-ESI): m/z calcd for $C_{35}H_{36}BrN_3O_6S_2$: 737.1229; found: 760.1125 [M+Na]⁺.

4.4.5. 2,4,6-Trimethyl-*N*-(4-(3,3,6,6-tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzenesulfonamide (6e)

As white solid (ethanol), mp 239–240 °C, yield 93%. IR (cm^{-1}): 3467 and 3388 w (–NH₂), 3172 w (–NH), 3035 w (Ar–H), 2958 w (C–H), 1623 s (C=O), 1582 s (C=C), 1360 and 1146 s (SO₂); ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm): 0.70 (s, 6H, 2 \times –CH₃), 0.90 (s, 6H, 2 \times –CH₃), 1.70 (d, 2H, J = 17.2 Hz, –CH₂), 1.98 (d, 2H, J = 15.9 Hz, –CH₂), 2.16 (d, 4H, J = 16.6 Hz, 2 \times –CH₂), 2.21 (s, 3H, –CH₃), 2.47 (s, 6H, 2 \times –CH₃), 4.90 (s, 1H, –CH), 6.85 (d, 2H, J = 8.5 Hz, Ar–H), 6.95 (s, 2H, Ar–H), 7.14 (d, 2H, J = 8.5 Hz, Ar–H), 7.61 (s, 2H, –SO₂NH₂), 7.64 (d, 2H, J = 8.6 Hz, Ar–H), 8.01 (d, 2H, J = 8.6 Hz, Ar–H), 9.90 (s, 1H, –NH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ (ppm): 20.84, 22.91, 26.50, 29.64, 31.79, 32.50, 41.40, 49.95, 113.52, 120.65, 127.79, 128.62, 131.11, 132.16, 134.58, 135.50, 139.00, 141.59, 142.27, 142.44, 145.15, 150.07, 195.50; HRMS (QTOF-ESI): m/z calcd for $C_{38}H_{43}N_3O_6S_2$: 701.2593; found: 724.2482 [M+Na]⁺.

4.4.6. *N*-(4-(3,3,6,6-Tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)naphthalene-2-sulfonamide (6f)

As white solid (ethanol), mp 243–245 °C, yield 95%. IR (cm^{-1}): 3382 w (–NH₂), 3249 w (–NH), 3032 w (Ar–H), 2957 w (C–H), 1625 s (C=O), 1582 s (C=C), 1363 and 1153 s (SO₂); ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm): 0.60 (s, 6H, 2 \times –CH₃), 0.80 (s, 6H, 2 \times –CH₃), 1.66 (d, 2H, J = 17.2 Hz, –CH₂), 1.93 (d, 2H, J = 15.9 Hz, –CH₂), 2.13 (d, 4H, J = 16.5 Hz, 2 \times –CH₂), 4.90 (s, 1H, –CH), 6.99 (d, 2H, J = 8.5 Hz, Ar–H), 7.13 (d, 2H, J = 8.5 Hz, Ar–H), 7.61 (s, 2H, –SO₂NH₂), 7.60–7.71 (m, 5H, Ar–H), 7.97–8.08 (m, 5H, Ar–H), 8.36 (s, 1H, Ar–H), 10.20 (s, 1H, –NH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ (ppm): 26.37, 29.61, 31.74, 32.45, 41.36, 49.90, 113.45, 120.66, 122.41, 127.78, 128.03, 128.22, 128.27, 128.64, 129.29, 129.62, 129.73, 131.10, 131.96, 134.64, 135.71, 137.16, 141.55, 142.58, 145.13, 150.03, 195.45; HRMS (QTOF-ESI): m/z calcd for $C_{39}H_{39}N_3O_6S_2$: 709.2280; found: 732.2168 [M+Na]⁺.

4.4.7. *N*-(3-(3,3,6,6-Tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzenesulfonamide (6g)

As white solid (ethanol), mp 182–184 °C, yield 84%. IR (cm^{-1}): 3384 w (–NH₂), 3266 w (–NH), 3068 w (Ar–H), 2956 w (C–H), 1633 s (C=O), 1584 s (C=C), 1364 and 1161 s (SO₂); ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm): 0.70 (s, 6H, 2 \times –CH₃), 0.90 (s, 6H, 2 \times –CH₃), 1.68 (d, 2H, J = 17.3 Hz, –CH₂), 1.97 (d, 2H, J = 16.0 Hz, –CH₂), 2.15–2.22 (m, 4H, 2 \times –CH₂), 4.98 (s, 1H, –CH), 6.81–6.83 (m, 1H, Ar–H), 7.00–7.09 (m, 2H, Ar–H), 7.20 (s, 1H, Ar–H), 7.61 (s, 2H, –SO₂NH₂), 7.43–7.72 (m, 7H, Ar–H), 8.05 (d, 2H, J = 8.5 Hz, Ar–H), 10.20 (s, 1H, –NH); ¹³C NMR (75 MHz, DMSO-*d*₆)

δ (ppm): 26.65, 29.67, 32.31, 32.44, 41.45, 49.96, 113.30, 118.27, 119.67, 124.49, 126.90, 127.75, 128.87, 129.53, 131.06, 133.17, 138.05, 140.08, 141.60, 145.24, 147.57, 150.16, 195.38; HRMS (QTOF-ESI): m/z calcd for $C_{35}H_{37}N_3O_6S_2$: 659.2124; found: 658.2239 $[M-H]^-$.

4.4.8. 4-Methyl-*N*-(3-(3,3,6,6-tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzenesulfonamide (6h)

As white solid (ethanol), mp 190–192 °C, yield 86%. IR (cm^{-1}): 3396 w ($-NH_2$), 3252 w ($-NH$), 3098 w (Ar–H), 2959 w (C–H), 1632 s (C=O), 1585 s (C=C), 1363 and 1156 s (SO_2); 1H NMR (300 MHz, DMSO- d_6) δ (ppm): 0.70 (s, 6H, $2 \times -CH_3$), 0.90 (s, 6H, $2 \times -CH_3$), 1.68 (d, 2H, $J = 17.4$ Hz, $-CH_2$), 1.97 (d, 2H, $J = 16.0$ Hz, $-CH_2$), 2.15–2.22 (m, 4H, $2 \times -CH_2$), 2.28 (s, 3H, $-CH_3$), 4.98 (s, 1H, $-CH$), 6.81 (d, 1H, $J = 7.6$ Hz, Ar–H), 6.99–7.08 (m, 2H, Ar–H), 7.19 (s, 1H, Ar–H), 7.25 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.58 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.62 (s, 2H, $-SO_2NH_2$), 7.66 (d, 2H, $J = 8.5$ Hz, Ar–H), 8.04 (d, 2H, $J = 8.5$ Hz, Ar–H), 10.10 (s, 1H, $-NH$); ^{13}C NMR (75 MHz, DMSO- d_6) δ (ppm): 22.85, 26.60, 29.69, 32.29, 32.43, 41.46, 49.96, 113.32, 118.12, 119.56, 124.37, 126.96, 127.75, 128.84, 129.98, 131.10, 137.27, 138.19, 141.60, 143.42, 145.24, 147.53, 150.16, 195.39; HRMS (QTOF-ESI): m/z calcd for $C_{36}H_{39}N_3O_6S_2$: 673.2280; found: 672.2375 $[M-H]^-$.

4.4.9. 4-Methoxy-*N*-(3-(3,3,6,6-tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzenesulfonamide (6i)

As white solid (ethanol), mp 187–189 °C, yield 87%. IR (cm^{-1}): 3386 w ($-NH_2$), 3247 w ($-NH$), 3088 w (Ar–H), 2956 w (C–H), 1634 s (C=O), 1587 s (C=C), 1363 and 1155 s (SO_2); 1H NMR (300 MHz, DMSO- d_6) δ (ppm): 0.65 (s, 6H, $2 \times -CH_3$), 0.85 (s, 6H, $2 \times -CH_3$), 1.68 (d, 2H, $J = 17.2$ Hz, $-CH_2$), 1.97 (d, 2H, $J = 16.0$ Hz, $-CH_2$), 2.18–2.22 (m, 4H, $2 \times -CH_2$), 3.70 (s, 3H, $-OCH_3$), 4.98 (s, 1H, $-CH$), 6.82–7.09 (m, 5H, Ar–H), 7.16 (s, 1H, Ar–H), 7.62–7.68 (m, 6H, Ar–H and $-SO_2NH_2$), 8.05 (d, 2H, $J = 8.5$ Hz, Ar–H), 10.00 (s, 1H, $-NH$); ^{13}C NMR (75 MHz, DMSO- d_6) δ (ppm): 26.61, 29.66, 32.28, 32.44, 41.45, 49.97, 55.98, 113.32, 114.65, 118.21, 119.66, 124.33, 127.74, 128.81, 129.13, 131.11, 131.74, 138.26, 141.59, 145.24, 147.51, 150.14, 162.67, 195.38; HRMS (QTOF-ESI): m/z calcd for $C_{36}H_{39}N_3O_7S_2$: 689.2229; found: 688.2352 $[M-H]^-$.

4.4.10. 4-Bromo-*N*-(3-(3,3,6,6-tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzenesulfonamide (6j)

As white solid (ethanol), mp 196–198 °C, yield 94%. IR (cm^{-1}): 3383 w ($-NH_2$), 3248 w ($-NH$), 3078 w (Ar–H), 2959 w (C–H), 1633 s (C=O), 1583 s (C=C), 1338 and 1159 s (SO_2); 1H NMR (300 MHz, DMSO- d_6) δ (ppm): 0.65 (s, 6H, $2 \times -CH_3$), 0.85 (s, 6H, $2 \times -CH_3$), 1.67 (d, 2H, $J = 17.2$ Hz, $-CH_2$), 1.97 (d, 2H, $J = 15.9$ Hz, $-CH_2$), 2.15–2.22 (m, 4H, $2 \times -CH_2$), 4.95 (s, 1H, $-CH$), 6.86 (d, 1H, $J = 7.9$ Hz, Ar–H), 7.02–7.15 (m, 3H, Ar–H), 7.62 (s, 2H, $-SO_2NH_2$), 7.60–7.70 (m, 6H, Ar–H), 8.05 (d, 2H, $J = 8.5$ Hz, Ar–H), 10.25 (s, 1H, $-NH$); ^{13}C NMR (75 MHz, DMSO- d_6) δ (ppm): 26.52, 29.69, 32.33, 32.43, 41.45, 49.93, 113.95, 118.67, 120.10, 124.59, 127.06, 127.78, 128.99, 129.05, 131.04, 132.65, 137.62, 139.17, 141.55, 145.24, 147.64, 149.80, 195.36; HRMS (QTOF-ESI): m/z calcd for $C_{35}H_{36}BrN_3O_6S_2$: 737.1229; found: 736.1343 $[M-H]^-$.

4.4.11. 2,4,6-Trimethyl-*N*-(3-(3,3,6,6-tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzenesulfonamide (6k)

As white solid (ethanol), mp 203–204 °C, yield 88%. IR (cm^{-1}): 3239 w ($-NH$), 3077 w (Ar–H), 2957 w (C–H), 1632 s (C=O), 1584 s (C=C), 1333 and 1151 s (SO_2); 1H NMR (300 MHz,

DMSO- d_6) δ (ppm): 0.70 (s, 6H, $2 \times -CH_3$), 0.90 (s, 6H, $2 \times -CH_3$), 1.68 (d, 2H, $J = 17.4$ Hz, $-CH_2$), 1.96 (d, 2H, $J = 16.1$ Hz, $-CH_2$), 2.18 (s, 3H, $-CH_3$), 2.14–2.22 (m, 4H, $2 \times -CH_2$), 2.52 (s, 6H, $2 \times -CH_3$), 4.95 (s, 1H, $-CH$), 6.73 (d, 1H, $J = 7.9$ Hz, Ar–H), 6.94–7.07 (m, 5H, Ar–H), 7.62 (s, 2H, $-SO_2NH_2$), 7.68 (d, 2H, $J = 8.5$ Hz, Ar–H), 8.04 (d, 2H, $J = 8.5$ Hz, Ar–H), 10.10 (s, 1H, $-NH$); ^{13}C NMR (75 MHz, DMSO- d_6) δ (ppm): 20.82, 22.96, 26.72, 29.65, 32.22, 32.43, 41.46, 49.93, 113.29, 116.98, 119.47, 124.02, 127.73, 128.79, 131.14, 132.17, 134.41, 137.87, 138.97, 141.59, 142.20, 145.26, 147.58, 150.12, 195.35; HRMS (QTOF-ESI): m/z calcd for $C_{38}H_{43}N_3O_6S_2$: 701.2593; found: 700.2733 $[M-H]^-$.

4.4.12. *N*-(3-(3,3,6,6-Tetramethyl-1,8-dioxo-10-(4-sulfamoylphenyl)-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)naphthalene-2-sulfonamide (6l)

As white solid (ethanol), mp 207–208 °C, yield 90%. IR (cm^{-1}): 3387 w ($-NH_2$), 3244 w ($-NH$), 3064 w (Ar–H), 2958 w (C–H), 1632 s (C=O), 1585 s (C=C), 1334 and 1156 s (SO_2); 1H NMR (300 MHz, DMSO- d_6) δ (ppm): 0.60 (s, 6H, $2 \times -CH_3$), 0.80 (s, 6H, $2 \times -CH_3$), 1.62 (d, 2H, $J = 17.3$ Hz, $-CH_2$), 1.88 (d, 2H, $J = 16.0$ Hz, $-CH_2$), 2.10–2.18 (m, 4H, $2 \times -CH_2$), 4.95 (s, 1H, $-CH$), 6.86 (d, 1H, $J = 7.9$ Hz, Ar–H), 6.96–7.07 (m, 2H, Ar–H), 7.23 (s, 1H, Ar–H), 7.63 (s, 2H, $-SO_2NH_2$), 7.58–7.75 (m, 5H, Ar–H), 7.95–8.06 (m, 5H, Ar–H), 8.33 (s, 1H, Ar–H), 10.25 (s, 1H, $-NH$); ^{13}C NMR (75 MHz, DMSO- d_6) δ (ppm): 26.41, 29.61, 32.30, 32.37, 41.42, 49.88, 113.29, 118.40, 119.93, 122.39, 124.33, 127.76, 127.99, 128.11, 128.25, 128.92, 129.27, 129.68, 131.10, 131.96, 134.65, 137.12, 137.94, 141.56, 145.23, 147.56, 150.10, 195.34; HRMS (QTOF-ESI): m/z calcd for $C_{39}H_{39}N_3O_6S_2$: 709.2280; found: 708.2390 $[M-H]^-$.

4.5. CA inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO₂ hydration activity by using method of Khalifah.³³ Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na₂SO₄ (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO₂ hydration reaction for a period of 10–100 s. The CO₂ concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM (www.graphpad.com), and non-linear least squares methods, values representing the mean of at least three different determinations, as described earlier by us.^{35,36} All enzymes used were recombinant ones, produced in *Escherichia coli* as reported earlier.^{37,38} CA IX and XII were the catalytic domain only. The concentrations of the enzymes in the assay system were: 11.4 nM for hCA I; 7.5 nM for hCA II, 8.6 nM for hCA IX and 10.6 nM for hCA XII, respectively.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.bmc.2015.09.022>.

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