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Synthesis of novel acridine and bis acridine sulfonamides with effective inhibitory activity against the cytosolic carbonic anhydrase isoforms II and VII

Ramazan Ulus [a], İbrahim Yeşildağ [a], Muhammet Tanç [b], Metin Bülbül [a], Muharrem Kaya [a,*], Claudiu T. Supuran [b,*]

[a] Chemistry Department, Faculty of Arts and Science, Dumlupınar University, 43100 Kütahya, Turkey
[b] Università degli Studi di Firenze, NEUROFARBA Dept., Sezione di Scienze Farmaceutiche e Nutraceutiche, 50019 Sesto Fiorentino (Florence), Italy

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ABSTRACT

4-Amino-*N*-(4-sulfamoylphenyl)benzamide was synthesized by reduction of 4-nitro-*N*-(4-sulfamoylphenyl)benzamide and used to synthesize novel acridine sulfonamide compounds, by a coupling reaction with cyclic-1,3-diketones and aromatic aldehydes. The new compounds were investigated as inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1), and more precisely the cytosolic isoforms hCA I, II and VII. hCA I was inhibited in the micromolar range by the new compounds (K_Is of 0.16–9.64 μM) whereas hCA II and VII showed higher affinity for these compounds, with K_Is in the range of 15–96 nM for hCA II, and of 4–498 nM for hCA VII. The structure–activity relationships for the inhibition of these isoforms with the acridine–sulfonamides reported here were also elucidated.

1. Introduction

Sulfonamide/sulfamate represents the main class of inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1).[1–3] CA inhibitors (CAIs) are in clinical use for decades as antiglaucoma,[4] diuretic,[5] antiobesity,[6] and antitumor agents,[7] targeting diverse of the various 16 CA isoforms known to date in humans.[1–3] Recently, targeting CAs from parasites (bacteria, fungi and/or protozoa among others) with specific inhibitors was proposed as an alternative for designing antiinfective agents with a new mechanism of action.[8] There are several clinically used sulfonamides/sulfamates which indiscriminately inhibit most of the mammalian CA isoforms in the nanomolar range, and for this reason such drugs possess significant side effects.[1–3] There is thus a constant search for new types of such derivatives with improved efficacy for the different CA isoforms.[9,10]

2. Results and discussion

2.1. Chemistry

The rationale of this work was to incorporate the polycyclic acridine moiety in the tails[9,10] of aromatic sulfonamides, as it has been reported recently that these are the moieties mainly responsible for effective inhibitory action as well as differentiation between the many CA isoforms, in inducing selectivity.[11–13] The following chemistry has been employed to achieve this goal (Schemes 1 and 2)

The general synthetic method shown in Scheme 1 was used to prepare the sulfonamide containing benzamide derivatives **3**, **4**. The nitro derivative **3** was synthesized in THF at room temperature in the presence of triethylamine (TEA).[14] Compound **4** was then obtained by reduction reaction of the nitro derivative **3** in the presence of aqueous sodium poly-sulfide.[14c]

The general synthetic methods shown in Schemes 1 and 2 were employed to prepare the acridine sulfonamide derivatives **7**–**23** and the bis acridine sulfonamides **24** and **25**, as reported earlier for similar derivatives by one of these groups.[15] The synthesis of acridine sulfonamide compounds were realized in water in a single process through three successive reactions (Aldol condensation, Michael addition and cyclization) and using a phase transfer catalyst-Bronsted acid as *p*-dodecylbenzenesulphonic acid (DBSA).

* Corresponding authors. Tel.: +90 274 2652051; fax: +90 274 2652056 (M.K.); tel.: +39 055 457 3005; fax: +39 055 457 3385 (C.T.S.).
E-mail addresses: mhrkaya@yahoo.com (M. Kaya), claudiu.supuran@unifi.it (C.T. Supuran).

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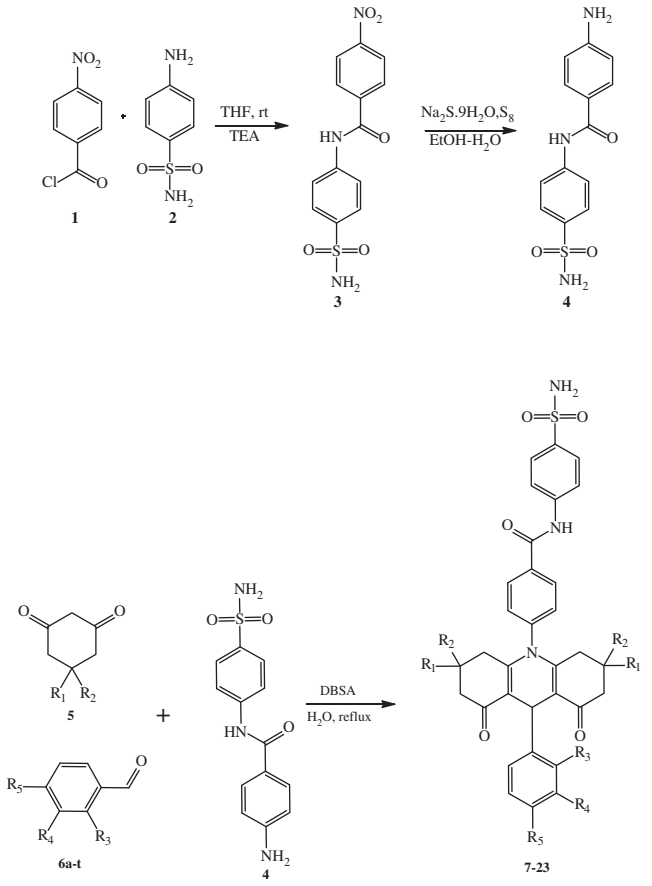


Scheme 1. Synthesis of sulfonamides (containing benzamide derivatives **3**, **4** and acridine moieties incorporating **7**–**23**).

Sulfonamide compounds were prepared by one pot reaction in processing high yields and simple work-up procedure. Amino-derivative **4** was condensed with substituted 1,3-diketones **5** and substituted benzaldehydes **6**, in the molar range of 1:2:1, at reflux, leading to the acridine-incorporating compounds **7**–**23** (Scheme 1). By changing the nature of the substituents present in the diketone and/or aldehyde components, a rather large chemical diversity can be incorporated in the acridine sulfonamides reported here (Scheme 1 and Table 1). Indeed, 5,5-dimethyl-substituted 1,3-cyclohexanedione and 1,3-cyclohexanedione have been employed in the synthesis, together with substituted benzaldehydes incorporating a range of substituents in the *ortho*-, *meta* and *para* positions with respect to the CHO moiety. These various substituents included cyano, nitro, halogen (F, Cl, Br), methoxy, dimethylamino, methyl, ethyl and formyl moieties (Table 1).

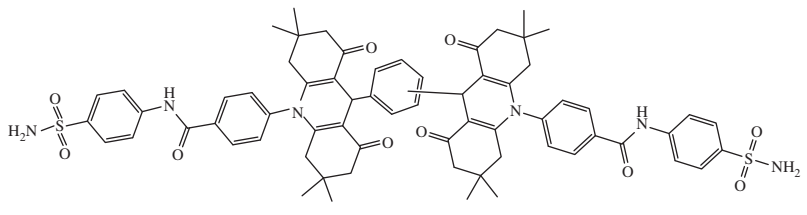
By working at molar ratios of **4**: 1,3-diketone: 1,4-benzene-dialdehyde or 1,3-benzene-dialdehyde of 2:2:1, the bis acridine disulfonamides **24**, **25** were prepared (Scheme 2).

All spectral data of compounds **7**–**25** are in agreement with the assigned structures (see Experimental for details). The infrared (IR) spectra of all the dioxoacridine sulfonamide compounds showed sharp peaks for the carbonyl groups in region between 1690 and 1625 cm^{-1}. The compounds **8**, **9** and **22** of belong to cyano group vibration peak was observed 2228, 2240 and 2228 cm^{-1}, respectively. Besides, in the IR spectra of the compounds, aliphatic C–H stretching bands at 2962–2934 cm^{-1} and aromatic C–H stretching bands at 3097–3036 cm^{-1} were observed. The NH_2 vibrations of acridine sulfonamide compounds were observed in the region between 3354 and 3183 cm^{-1}. The ^1H NMR spectra of compounds **7**–**21** showed singlet peaks that belong to protons of the methyl groups in position 3 and 6 between 0.70 and 0.94 ppm. The CH_2 group protons of the cyclohexene rings of the compounds **7**–**21** showed doublet peaks in the 1.75–2.29 ppm range and compounds **22** and **23** showed multiple peaks in the 1.60–2.30 ppm. The compound **17** showed singlet peak that belongs to protons of the methyl groups 2.87 ppm. The compound **18** showed singlet peak that belongs to protons of the methyl group 2.23 ppm. The compound **19** observed belongs to protons of ethyl group triplet peak (3H) at 1.14 ppm and multiple peaks(2H) at 2.49–2.57 ppm. Signals for the methoxy group protons for compounds **16** and **21** were shown in the range of 3.70–3.71 ppm. The signals for the CH protons at 4.90–5.30 ppm and signals for the aromatic protons in the range of 6.62–8.19 ppm were observed. Hydroxyl group proton of compound **15** and **21** were observed as broad signal at 8.82–9.03 ppm. The broad singlet peaks between 6.62 and 7.34 ppm were assigned to sulfonamide ($-SO_2NH_2$) groups protons of the compounds **7**–**25**. Signals of –NH groups protons for the compounds **7**–**25** showed in the range of 10.73–13.40 ppm.

2.2. Carbonic anhydrase inhibition

The new compounds reported here and the standard drug acetazolamide were assayed as inhibitors of three cytosolic human isoforms, hCA I, II and VII (Table 1).[16] As seen from data of Table 1, these sulfonamides show effective inhibitory activity against all three tested isoforms. The following structure activity relationship (SAR) could be observed:

The slow cytosolic isoform hCA I was inhibited with inhibition constants in the micromolar range by most of the newly synthesized sulfonamides **7**–**25**, which showed KIs of 0.16–9.64 μM (Table 1). Compound **7**, possessing no substituent (except hydrogen) at the part of the molecule originally found in the aldehyde component, was the best hCA I inhibitor (K_Is 0.16 μM) together with the compound possessing OH and methyl moieties in the aromatic part of the molecule (**21**, K_Is of 0.26 μM). The remaining derivatives were less effective as hCA I inhibitors, with KIs of 1.58–9.64 μM. The moiety which seems to influence significantly the enzyme inhibitory activity is the one coming from the aldehyde component in the synthesis of these derivatives. Indeed, both the positions of the substituents R_3–R_4, as well as their nature, influence



Scheme 2. Structures of the bis acridine sulfonamide derivatives (**24** and **25**).

Table 1
Inhibition data of sulfonamides **7**–**25** and acetazolamide (**AAZ**, as standard drug) against hCA I, hCA II and hCA VII isoenzymes by a stopped flow CO_2 hydrase assay

Inhibitor	R_1	R_2	R_3	R_4	R_5	*Ki* values[a] (μM)		
						CA I	CA II	CA VII
AAZ						0.25	0.012	0.028
7	CH_3	CH_3	H	H	H	0.16	0.021	0.004
8	CH_3	CH_3	H	H	CN	4.26	0.047	0.055
9	CH_3	CH_3	H	CN	H	1.58	0.015	0.065
10	CH_3	CH_3	H	H	NO_2	7.34	0.029	0.199
11	CH_3	CH_3	H	NO_2	H	3.51	0.023	0.041
12	CH_3	CH_3	H	H	F	6.22	0.041	0.245
13	CH_3	CH_3	H	H	Cl	8.85	0.023	0.368
14	CH_3	CH_3	H	H	Br	8.26	0.046	0.264
15	CH_3	CH_3	H	H	OH	2.13	0.049	0.152
16	CH_3	CH_3	H	H	OCH_3	9.64	0.065	0.127
17	CH_3	CH_3	H	H	$N(CH_3)_2$	9.49	0.096	0.498
18	CH_3	CH_3	H	H	CH_3	5.33	0.059	0.334
19	CH_3	CH_3	H	H	C_2H_5	1.89	0.051	0.225
20	CH_3	CH_3	Cl	H	Cl	9.34	0.023	0.375
21	CH_3	CH_3	OH	OCH_3	H	0.26	0.033	0.005
22	H	H	H	H	CN	2.14	0.038	0.014
23	H	H	Cl	H	Cl	3.47	0.067	0.022
24	1,4-Phenylene bis acridine sulfonamide					4.33	0.053	0.142
25	1,3-Phenylene bis acridine sulfonamide					0.85	0.030	0.046

[a] Mean from 3 different assays. Errors were in the range of ±10% of the reported data (not shown).

significantly the inhibition of hCA I. For example compounds possessing such a group in the meta position (**9**, **11** and **21**) have a good inhibitory activity, whereas such a group in the para position, such as in **8** as in **12**, **13**, **14**, **16** and **17**, led to less effective inhibitors. However some p-substituted compounds, such as **15**, **19** and **22** showed effective hCA I inhibition, as the meta-substituted ones mentioned above (Table 1).

hCA II, the physiologically dominant CA isoform was also effectively inhibited by the new sulfonamides **7**–**25**, with KIs in the range of 15–96 nM. In this case the SAR shows a more compact behavior, of potent inhibitor for most of the synthesized compounds. The best hCA II inhibitors in this series were derivatives **7**, **9**, **11**, **13**, and **20** which showed K_Is of 15–23 nM. They incorporate methyl moiety in the part of the molecule coming from the 1,3-diketone, as well as meta- and para- substituents (e.g., no substituent at all apart H in **7**, and cyano, nitro and chloro in the remaining compounds) in the part of the molecule originary present in the aldehyde component for the synthesis of Schemes 1 (Table 1). However, there are no important differences of activity between these compounds with the least effective ones (**16**, **17** and **23**) possessing anyhow K_Is of 67–96 nM. Interestingly, the very bulky bis sulfonamides **24** and **25** was also a rather effective hCA II inhibitor.

The brain-associated cytosolic isoform CA VII was inhibited by sulfonamides **7**–**25** with KIs in the range of 4–498 nM (Table 1). SAR is more complicated for the inhibition of this isoform with respect to the other two discussed above. Indeed, compound **7** was a very effective hCA VII inhibitor (K_I of 4 nM) being much more effective compared to acetazolamide, a clinically used drug.[1–3] Other effective hCA VII inhibitors were **11**, **21**, **22**, and **23**, with inhibition constants in the range of 5–41 nM. These compounds incorporate 3-nitro, 3-methoxy, 4-cyano and 4-chloro moieties in the fragment of the molecule coming from the aldehyde component. Other substitution patterns, as those found in the remaining derivatives, led to a loss of activity, with these derivatives having K_Is in the range of 55–498 nM. The bis-sulfonamide **25** was also a rather effective hCA VII inhibitor.

3. Conclusions

A series of novel sulfonamides incorporating acridine and bis acridine moieties were synthesized and their structural features were identified. As sulfonamide CAIs have applications in treatment of some diseases, such as glaucoma, obesity and as diuretics, exploring diverse sulfonamide chemotypes targeting various such enzymes is of interest for many medicinal chemists. Furthermore, antimicrobial, antimalarial, antitrypanosomal and anticancer effects were also recently reported for many CAIs targeting parasite or human (hCA IX and XII) enzymes.[17,18] As shown in a recent special issue of this journal,[19] at the 80th anniversary of the discovery of this enzyme, there are still many important drug design aspect to be addressed for obtaining isoform-selective and more effective sulfonamide inhibitors.

4. Materials and methods

4.1. Materials

All chemicals and solvents used for the synthesis were spectroscopic reagent grade. Melting points were measured on a Bibby Stuart Scientific apparatus. FT-IR spectra were recorded from a Bruker Optics, Andrtex 70 FT-IR spectrometer with an ATR diamond crystal. ^{1}H NMR, and ^{13}C NMR spectra were obtained with a Bruker DPX-300 FT-NMR and Bruker DPX-400 FT-NMR instrument in $CDCl_3$ or DMSO-d_6 as a solvent, at 300 MHz and 400 MHz, respectively. Chemical shifts are expressed in δ units (ppm). The mass analyses were performed on Waters 2695 Alliance Micromass ZQ instrument LC/MS.

4.1.1. General procedure for preparation of 4-nitro-*N*-(4-sulfamoylphenyl)benzamide compound (3)

4-Aminobenzenesulfonamide (1.739 g, 10.1 mmol), *p*-nitro benzoyl chloride (1.856 g, 10 mmol), 3 mL dry triethylamine (TEA) and in dry 30 mL THF were stirred for 5 h at room temperature. After the solvent was removed in vacuo and washed with H_2O. The raw product was purified by recrystallization from ethanol.

4.1.2. General procedure for preparation of 4-amino-*N*-(4-sulfamoylphenyl)benzamide compound (4)

$Na_2S{\cdot}9H_2O$ (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 and warm solution of compound **3** (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. The sulfonamide product was purified and recrystallized from the ethanol (90%).

As white crystals, (0.25 g, 86%), mp 313 °C (ethanol–water). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 5.85 (s, 2H, –NH_2), 6.63 (d, 2H, J = 8.3 Hz, Ar-H), 7.26 (s, 2H, –NH_2), 7.77 (t, 4H, J = 8.3 Hz, Ar-H), 7.95 (d, 2H, J = 8.6 Hz, Ar-H), 10.08 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 112.54, 119.39, 120.41, 126.40, 129.58, 137.83, 142.84, 152.50, 165.56; IR (cm^{-1}): 3448 w (–NH) 3382 and 3328 w (–NH_2), 3035 w (Ar-H), 2953 w (C–H), 1650 s (C=O), 1589 and 1500 m (C=C), 1285 s and 1144 s (SO_2); MS(CI) m/z 292 $[M+1]^+$ (100%).

4.1.3. General procedure for preparation of acridine sulfonamide derivatives (7–23)

A mixture of a 4-amino-*N*-(4-sulfamoylphenyl)benzamide **4** (0.291 g, 1 mmol), 5,5-dimethylcyclohexane-1,3-dione **5** (0.280 g, 2 mmol), benzaldehyde **6a** (0.106 g, 1 mmol) and DBSA (0.420 g, 10 mol %) in 40 mL H_2O was stirred and refluxed for 24 h. 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. The acridine sulfonamide products were purified and recrystallized from the following solvent mixture for each compound (42–96%).

4.1.4. *N*-(4-sulfamoylphenyl)-4-(3,3,6,6-tetramethyl-1,8-dioxo-9-phenyl-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)benzamide (7)

As yellow crystal, (0.45 g, 72%), mp 216 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.75 (s, 6H, 2×–CH_3), 0.90 (s, 6H, 2×–CH_3), 1.80 (d, 2H, –CH_2), 2.02 (d, 2H, –CH_2), 2.20 (d, 2H, –CH_2), 2.25 (d, 2H, –CH_2), 5.10 (s, 1H, –CH), 7.12 (s, 2H, –NH_2), 7.24–7.36 (m, 5H, Ar-H), 7.62 (d, 2H, J = 8.0 Hz, Ar-H) 7.84 (d, 2H, J = 8.0 Hz, Ar-H), 8.00 (d, 2H, J = 8.0 Hz, Ar-H), 8.17 (d, 2H, J = 8.0 Hz, Ar-H), 10.75 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.56, 29.73, 32.47, 32.51, 41.50, 50.04, 113.60, 120.43, 126.30, 127.07, 128.06, 128.40, 130.11, 130.43, 135.66, 139.48, 141.87, 142.40, 146.61, 150.36, 165.52, 195.56; IR (cm^{-1}): 3229 w (–NH_2), 3036 w (Ar-H), 2953 w (C–H), 1666 and 1641 s (C=O), 1534 m (C=C), 1319 s and 1154 s (SO_2); MS(CI) m/z 622 $[M-1]^+$ (100%).

4.1.5. 4-(9-(4-Cyanophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (8)

As yellow crystal, (0.61 g, 94%), mp 228 °C (decomposition) (ethanol–water). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.73 (s, 6H, 2×–CH_3), 0.90 (s, 6H, 2×–CH_3), 1.84 (d, 2H, –CH_2), 2.02–2.10 (m, 2H, –CH_2), 2.21–2.28 (m, 4H, 2×–CH_2), 5.10 (s, 1H, –CH), 7.55 (d, 2H, J = 8.3 Hz, Ar-H), 7.69 (d, 2H, J = 8.6 Hz, Ar-H), 7.34 (s, 2H, –NH_2), 7.75 (d, 2H, J = 8.3 Hz, Ar-H), 7.86 (d, 2H, J = 9.0 Hz, Ar-H), 8.01 (d, 2H, J = 9.0 Hz, Ar-H), 8.19 (d, 2H, J = 8.8 Hz, Ar-H), 13.40 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 26.12, 29.13, 32.05, 33.07, 41.02, 49.37, 108.65, 112.11, 118.97, 119.95, 126.59, 128.82, 129.63, 129.95, 132.00, 135.28, 138.99, 141.13, 141.91, 150.51, 151.46, 165.00, 195.06; IR (cm^{-1}): 3229 w (–NH_2), 3095 w (Ar-H), 2954 w (C–H), 2228 s (C≡N), 1677 and 1633 s (C=O), 1524 m (C=C), 1362 s and 1150 s (SO_2); MS(CI) m/z 647 $[M-1]^+$ (65%).

4.1.6. 4-(9-(3-Cyanophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (9)

As yellow crystal, (0.58 g, 89%), mp 294 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.73 (s, 6H, 2×–CH_3), 0.89 (s, 6H, 2×–CH_3), 1.85 (d, 2H, –CH_2), 2.04 (d, 2H, –CH_2), 2.22 (d, 4H, 2×–CH_2), 5.03 (s, 1H, –CH), 7.32 (s, 2H, –NH_2), 7.52 (t, 3H, J = 7.5 Hz, Ar-H), 7.61 (d, 2H, J = 8.0 Hz, Ar-H), 7.68 (s, 1H, Ar-H), 7.84 (d, 2H, J = 8.0 Hz, Ar-H), 7.99 (d, 2H, J = 8.0 Hz, Ar-H), 8.18 (d, 2H, J = 8.0 Hz, Ar-H), 10.79 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.64, 29.51, 32.57, 33.18, 41.47, 49.81, 111.04, 112.65, 119.54, 120.41, 127.06, 127.20, 130.01, 130.25, 130.35, 131.80, 133.11, 135.72, 139.44, 141.60, 142.38, 148.00, 150.99, 165.48, 195.64; IR (cm^{-1}): 3272 w (–NH_2), 2959 w (C–H), 2240 (C≡N), 1678 and 1642 s (C=O), 1595 m (C=C), 1361 s and 1222 s (SO_2); MS(CI) m/z 648 $[M]^+$ (65%).

4.1.7. *N*-(4-Sulfamoylphenyl)-4-(3,3,6,6-tetramethyl-9-(4-nitrophenyl)-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)benzamide (10)

As brown crystal, (0.44 g, 66%), mp 237 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.75 (s, 6H, 2×–CH_3), 0.90 (s, 6H, 2×–CH_3), 1.82 (d, 2H, –CH_2), 2.03 (d, 2H, –CH_2), 2.22 (d, 2H, –CH_2), 2.27 (d, 2H, –CH_2), 5.14 (s, 1H, –CH), 7.31 (s, 2H, –NH_2), 7.62 (d, 2H, J = 8.0 Hz, Ar-H), 7.70 (d, 2H, J = 8.0 Hz, Ar-H) 7.85 (d, 2H, J = 8.0 Hz, Ar-H), 8.00 (d, 2H, J = 8.0 Hz, Ar-H), 8.17 (t, 4H, Ar-H), 10.76 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.60, 29.63, 32.52, 33.49, 41.52, 49.86, 112.57, 120.46, 123.77, 127.08, 129.49, 130.12, 130.49, 135.79, 139.49, 141.61, 142.40, 146.18, 151.10, 154.04, 165.49, 195.53; IR (cm^{-1}): 3236 w (–NH_2), 3069 (Ar-H), 2954 (C–H), 1633 s (C=O), 1515 m (C=C), 1339 s and 1149 s (SO_2); MS(CI) m/z 667 $[M-1]^+$ (100%).

4.1.8. *N*-(4-Sulfamoylphenyl)-4-(3,3,6,6-tetramethyl-9-(3-nitrophenyl)-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)benzamide (11)

As yellow crystal, (0.51 g, 76%), mp 240 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.70 (s, 6H, 2×–CH_3), 0.89 (s, 6H, 2×–CH_3), 1.84 (d, 2H, –CH_2), 2.04 (d, 2H, –CH_2), 2.23 (d, 2H, –CH_2), 2.29 (d, 2H, –CH_2), 5.16 (s, 1H, –CH), 7.31 (s, 2H, –NH_2), 7.59–7.64 (m, 4H, Ar-H), 7.83 (t, 2H, J = 7.5 Hz, Ar-H) 8.01 (t, 2H, J = 8.6 Hz, Ar-H), 8.19 (t, 4H, J = 7.5 Hz, Ar-H), 10.78 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.53, 29.62, 32.59, 32.98, 41.45, 49.83, 112.79, 120.42, 120.46, 121.54, 122.58, 127.07, 130.22, 130.26, 134.80, 135.81, 139.49, 141.56, 142.38, 147.93, 148.60, 151.17, 165.44, 195.63; IR (cm^{-1}): 3205 w (–NH_2), 3092 w (Ar-H), 2962 w (C–H), 1631 s (C=O), 1523 m (C=C), 1365 s and 1153 s (SO_2); MS(CI) m/z 667 $[M-1]^+$ (100%).

4.1.9. 4-(9-(4-Fluorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (12)

As yellow crystal, (0.61 g, 95%), mp 218 °C (ethanol-water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.75 (s, 6H, 2×–CH_3), 0.89 (s, 6H, 2×–CH_3), 1.80 (d, 2H, –CH_2), 2.03 (d, 2H, –CH_2), 2.19 (d, 2H, –CH_2), 2.25 (d, 2H, –CH_2), 5.04 (s, 1H, –CH), 7.08 (t, 2H, J = 8.8 Hz, Ar-H), 7.31 (s, 2H, –NH_2), 7.33–7.38 (m, 2H, Ar-H), 7.63 (d, 2H, J = 8.1 Hz, Ar-H) 7.84 (d, 2H, J = 8.7 Hz, Ar-H), 7.98 (d, 2H, J = 8.7 Hz, Ar-H), 8.17 (d, 2H, J = 8.3 Hz, Ar-H), 10.77 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.58, 29.69, 31.98, 32.51, 41.49, 50.00, 113.49, 114.87, 120.44, 127.06, 129.76, 130.42, 135.69, 139.50, 141.79, 142.40, 142.87, 150.41, 159.33, 162.52, 165.49, 195.55; IR (cm^{-1}): 3183 w (–NH_2), 3096 w (Ar-H), 2955 w (C–H), 1681 s (C=O), 1594 m (C=C), 1362 s (C–N), 1362 s and 1151 s (SO_2); MS(CI) m/z 642 $[M+1]^+$ (100%).

4.1.10. 4-(9-(4-Chlorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (13)

As yellow crystal, (0.59 g, 90%), mp 230 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.76 (s, 6H, 2×–CH_3), 0.91 (s, 6H, 2×–CH_3), 1.80 (d, 2H, –CH_2), 2.02 (d, 2H, –CH_2), 2.19

(d, 2H, –CH_2), 2.25 (d, 2H, –CH_2), 5.00 (s, 1H, –CH), 7.31 (s, 2H, –NH_2), 7.33–7.37 (m, 4H, Ar-H), 7.64 (d, 2H, J = 8.6 Hz, Ar-H), 7.84 (d, 2H, J = 8.6 Hz, Ar-H) 7.98 (d, 2H, J = 8.6 Hz, Ar-H), 8.16 (d, 2H, J = 8.6 Hz, Ar-H), 10.76 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.59, 29.67, 32.30, 32.51, 41.49, 49.96, 113.19, 120.43, 127.06, 128.36, 129.97, 130.44, 130.78, 130.60, 135.70, 139.48, 141.74, 142.39, 145.57, 150.57, 165.49, 195.54; IR (cm^{-1}): 3250 w (–NH_2), 3096 w (Ar-H), 2955 w (C–H), 1680 and 1630 s (C═O), 1595 m (C═C), 1362 s and 1152 s (SO_2); MS(CI) m/z 658 $[M]^+$ (100%).

4.1.11. 4-(9-(4-Bromophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (14)

As brown crystal, (0.60 g, 85%), mp 233 °C (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.73 (s, 6H, 2×–CH_3), 0.89 (s, 6H, 2×–CH_3), 1.80 (d, 2H, –CH_2), 2.02 (d, 2H, –CH_2), 2.19 (d, 2H, –CH_2), 2.25 (d, 2H, –CH_2), 5.00 (s, 1H, –CH), 7.28 (s, 2H, Ar-H), 7.30 (s, 2H, –NH_2), 7.45 (d, 2H, J = 8.4 Hz, Ar-H), 7.64 (d, 2H, J = 8.4 Hz, Ar-H) 7.84 (d, 2H, J = 8.4 Hz, Ar-H), 7.98 (d, 2H, J = 8.4 Hz, Ar-H), 8.17 (d, 2H, J = 8.4 Hz, Ar-H), 10.78 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.61, 29.67, 32.39, 32.52, 41.49, 49.97, 113.14, 119.29, 120.44, 127.06, 130.07, 130.21, 130.40, 131.28, 135.71, 139.49, 141.74, 142.39, 145.99, 150.58, 165.49, 195.54; IR (cm^{-1}): 3263 w (–NH_2), 3066 w (Ar-H), 2956 w (C–H), 1631 s (C═O), 1596 m (C═C), 1363 s and 1152 s (SO_2); MS(CI) m/z 702 $[M]^+$ (100%).

4.1.12. 4-(9-(4-Hydroxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (15)

As yellow crystal, (0.60 g, 94%), mp 308 °C (decomposition) (ethanol-water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.71 (s, 6H, 2×–CH_3), 0.86 (s, 6H, 2×–CH_3), 1.75 (d, 2H, –CH_2), 2.01 (s, 2H, –CH_2), 2.17 (s, 4H, 2×–CH_2), 4.90 (s, 1H, –CH), 6.62 (s, 2H, –NH_2), 7.00–7.40 (m, 4H, Ar-H), 7.50–7.65 (m, 2H, Ar-H) 7.75–8.30 (m, 6H, Ar-H), 9.03 (s, 1H, –OH) 10.74 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.57, 29.76, 31.30, 32.50, 41.48, 50.11, 114.03, 115.13, 120.42, 127.06, 128.91, 130.10, 130.44, 135.60, 137.33, 139.46, 141.97, 142.40, 149.92, 155.76, 165.53, 195.58; IR (cm^{-1}): 3260 br (Ar-OH), 3259 w (–NH_2), 2957 w (C–H), 1667 and 1624 s (C═O), 1574 m (C═C), 1365 s and 1151 s (SO_2); MS(CI) m/z 640 $[M]^+$ (100%).

4.1.13. 4-(9-(4-Methoxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (16)

As yellow crystal, (0.61 g, 93%), mp 244 °C (decomposition) (ethanol-water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.79 (s, 6H, 2×–CH_3), 0.88 (s, 6H, 2×–CH_3), 1.79 (d, 2H, –CH_2), 2.02 (d, 2H, –CH_2), 2.19 (d, 2H, –CH_2), 2.24 (d, 2H, –CH_2), 3.70 (s, 3H, –OCH_3), 4.98 (s, 1H, –CH), 6.79 (d, 2H, J = 8.0 Hz, Ar-H), 7.24 (d, 2H, J = 8.0 Hz, Ar-H) 7.06 (s, 2H, –NH_2), 7.61 (d, 2H, J = 8.0 Hz, Ar-H), 7.84 (d, 2H, J = 8.0 Hz, Ar-H), 7.98 (d, 2H, J = 8.0 Hz, Ar-H), 8.17 (d, 2H, J = 8.0 Hz, Ar-H) 10.78 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.60, 29.75, 31.53, 32.50, 41.49, 50.07, 55.33, 113.73, 113.87, 120.43, 127.07, 129.02, 130.09, 130.43, 135.64, 138.97, 139.48, 141.93, 142.40, 150.06, 157.79, 165.51, 195.51; IR (cm^{-1}): 3189 w (–NH_2), 3067 w (Ar-H), 2957 w (C–H), 1691 and 1627 s (C═O), 1529 m (C═C), 1367 s and 1150 s (SO_2); MS(CI) m/z 652 $[M-1]^+$ (100%).

4.1.14. 4-(9-(4-(Dimethylamino)phenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (17)

As brown crystal, (0.60 g, 90%), mp 197 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.75 (s, 6H, 2×–CH_3), 0.88 (s, 6H, 2×–CH_3), 1.77 (d, 2H, –CH_2), 2.01 (d, 2H, –CH_2), 2.18 (d, 2H, –CH_2), 2.24 (d, 2H, –CH_2), 2.87 (s, 6H, 2×–CH_3), 4.92 (s, 1H, –CH), 6.62 (d, 2H, J = 8.7 Hz, Ar-H), 7.14 (d, 2H, J = 8.6 Hz, Ar-H), 7.31 (s, 2H, –NH_2) 7.55–7.65 (m, 2H, Ar-H), 7.84 (d, 2H, J = 8.8 Hz, Ar-H), 7.98 (d, 2H, J = 8.8 Hz, Ar-H), 8.16 (d, 2H, J = 8.6 Hz, Ar-H), 10.76 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.63, 29.79, 31.14, 32.50, 40.78, 41.47, 50.11, 112.57, 114.13, 120.41, 127.06, 128.57, 130.07, 131.05 134.94, 135.59, 139.44, 142.04, 142.41, 149.10, 149.75, 165.53, 195.60; IR (cm^{-1}): 3206 w (–NH_2), 3040 w (Ar-H) 2959 w (C–H), 1634 s (C═O), 1534 m (C═C), 1366 s and 1155 s (SO_2); MS(CI) m/z 667 $[M+1]^+$ (100%).

4.1.15. *N*-(4-Sulfamoylphenyl)-4-(3,3,6,6-tetramethyl-1,8-dioxo-9-(*p*-tolyl)-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)benzamide (18)

As yellow crystal, (0.61 g, 96%), mp 225 °C (ethanol-water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.72 (s, 6H, 2×–CH_3), 0.86 (s, 6H, 2×–CH_3), 1.79 (d, 2H, –CH_2), 2.01 (d, 2H, –CH_2), 2.17-2.26 (m, 4H, 2×–CH_2), 2.23 (s, 3H, –CH_3), 5.00 (s, 1H, –CH), 7.06 (d, 2H, J = 7.9 Hz, Ar-H), 7.14 (d, 2H, J = 7.9 Hz, Ar-H), 7.31 (s, 2H, –NH_2) 7.58-7.63 (m, 2H, Ar-H), 7.84 (d, 2H, J = 8.8 Hz, Ar-H), 7.98 (d, 2H, J = 8.8 Hz, Ar-H), 8.17 (d, 2H, J = 8.6 Hz, Ar-H), 10.73 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 21.08, 26.59, 29.75, 31.97, 32.50, 50.06, 56.50, 113.75, 120.43, 127.06, 127.96, 128.98, 129.40, 130.10, 135.13, 135.64, 139.48, 141.91, 142.40, 143.74, 150.19, 165.51, 195.52; IR (cm^{-1}): 3198 w (–NH_2), 3066 w (Ar-H), 2958 w (C–H), 1679 and 1626 s (C═O), 1596 m (C═C), 1365 s and 1156 s (SO_2); MS(CI) m/z 638 $[M+1]^+$ (100%).

4.1.16. 4-(9-(4-Ethylphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (19)

As yellow crystal, (0.60 g, 92%), mp 231 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.72 (s, 6H, 2×–CH_3), 0.94 (s, 6H, 2×–CH_3), 1.14 (t, 3H, –CH_3), 1.79 (d, 2H, –CH_2), 2.02 (d, 2H, –CH_2), 2.19 (d, 2H, –CH_2), 2.24 (d, 2H, –CH_2), 2.49–2.57 (m, 2H, –CH_2), 5.00 (s, 1H, –CH), 7.09 (d, 2H, J = 8.1 Hz, Ar-H), 7.24 (d, 2H, J = 8.1 Hz, Ar-H), 7.31 (s, 2H, –NH_2) 7.57–7.65 (m, 2H, Ar-H), 7.84 (d, 2H, J = 8.9 Hz, Ar-H), 7.98 (d, 2H, J = 8.9 Hz, Ar-H), 8.17 (d, 2H, J = 8.7 Hz, Ar-H), 10.80 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 15.90, 26.65, 28.17, 29.71, 32.00, 32.50, 41.49, 50.07, 113.76, 120.42, 127.06, 127.74, 127.99, 130.10, 130.44, 135.64, 139.48, 141.43, 141.91, 142.40, 143.99, 150.20, 165.50, 195.54; IR (cm^{-1}): 3251 and 3209 w (–NH_2), 3068 w (Ar-H), 2958 w (C–H), 1679 and 1629 s (C═O), 1596 m (C═C), 1363 s and 1153 s (SO_2); MS(CI) m/z 652 $[M+1]^+$ (100%).

4.1.17. 4-(9-(2,4-Dichlorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (20)

As brown crystal, (0.46 g, 71%), mp 310 °C (decomposition) (ethanol–water). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.77 (s, 6H, 2×–CH_3), 0.88 (s, 6H, 2×–CH_3), 1.79 (d, 2H, –CH_2), 1.98 (d, 2H, –CH_2), 2.19 (d, 4H, 2×–CH_2), 5.26 (s, 1H, –CH), 7.34 (s, 2H, –NH_2), 7.37 (d, 1H, J = 4.0 Hz, Ar-H), 7.43 (d, 1H, J = 4.0 Hz, Ar-H), 7.55 (d, 1H, J = 8.0 Hz, Ar-H), 7.66 (s, 2H, Ar-H), 7.86 (d, 2H, J = 8.0 Hz, Ar-H), 8.00 (d, 2H, J = 8.0 Hz, Ar-H), 8.19 (d, 2H, J = 4.0 Hz, Ar-H) 10.82 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 26.03, 29.21, 31.87, 32.88, 41.21, 49.38, 111.49, 119.92, 126.59, 128.63, 129.47, 129.89, 130.29, 130.63, 130.92, 133.48, 135.23, 138.96, 141.33, 141.92, 142.08, 150.67, 165.02, 194.94; IR (cm^{-1}): 3225 w (–NH_2), 3068 w (Ar-H), 2960 w (C–H), 1638 s (C═O), 1537 m (C═C), 1362 s and 1154 s (SO_2); MS(CI) m/z 692 $[M]^+$ (100%).

4.1.18. 4-(9-(2-Hydroxy-3-methoxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (21)

As brown crystal, (0.59 g, 88%), mp 235 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.75 (s, 6H, 2×–CH_3), 0.89 (s, 6H, 2×–CH_3), 1.76 (d, 2H, –CH_2), 2.00 (d, 2H, –CH_2), 2.21 (d, 4H, 2×–CH_2), 3.74 (s, 3H, –OCH_3), 5.01 (s, 1H, –CH), 6.65–6.73 (m, 2H, Ar-H), 6.83 (dd, 1H, J_1 = 7.2 Hz, J_2 = 2.1 Hz, Ar-H), 7.31 (s, 2H, –NH_2) 7.71 (s, 2H, Ar-H), 7.84 (d, 2H, J = 8.8 Hz, Ar-H), 7.98 (d, 2H, J = 8.8 Hz, Ar-H), 8.15 (d, 2H, J = 8.7 Hz, Ar-H), 8.82 (s, 1H, –OH) 10.79 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.49, 29.34, 29.78, 32.41, 41.59, 49.89, 56.00, 110.16, 112.15, 118.80, 120.41, 122.80, 127.06, 129.92, 130.63, 132.67, 135.61, 139.43, 142.17, 142.42, 144.61, 148.69, 151.50, 165.1, 196.43; IR (cm^{-1}): 3200 br (Ar-OH), 3200 w (–NH_2), 3097 w (Ar-H) 2954 w (C–H), 1671 s (C═O), 1591 m (C═C), 1367 s and 1151 s (SO_2); MS(CI) m/z 670 $[M+1]^+$ (100%).

4.1.19. 4-(9-(4-Cyanophenyl)-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (22)

As brown crystal, (0.40 g, 67%), mp 329 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 1.60–2.30 (m, 12H, 6×–CH_2), 5.20 (s, 1H, –CH), 7.30 (s, 2H, –NH_2), 7.52 (d, 2H, J = 8.3 Hz, Ar-H), 7.68 (s, 2H, Ar-H), 7.72 (d, 2H, J = 8.3 Hz, Ar-H), 7.83 (d, 2H, J = 8.8 Hz, Ar-H), 7.97 (d, 2H, J = 8.8 Hz, Ar-H), 8.15 (d, 2H, J = 8.8 Hz, Ar-H), 10.72 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 21.09, 28.31, 32.79, 36.60, 109.10, 113.43, 119.52, 120.44, 127.06, 129.18, 129.73, 130.49, 132.60, 135.70, 139.46, 141.66, 142.36, 152.40, 153.10, 165.46, 195.94; IR (cm^{-1}): 3354 and 3204 w (–NH_2), 3092 w (Ar-H), 2952 w (C–H), 2228 s (C≡N), 1667 and 1630 s (C═O), 1599 m (C═C), 1363 s and 1153 s (SO_2); MS(CI) m/z 593 $[M+1]^+$ (100%).

4.1.20. 4-(9-(2,4-Dichlorophenyl)-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl)-*N*-(4-sulfamoylphenyl)benzamide (23)

As brown crystal, (0.46 g, 72%), mp 240 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 1.63–2.16 (m, 12H, 6×–CH_2), 5.30 (s, 1H, –CH), 7.31 (s, 2H, –NH_2), 7.33–7.34 (m, 1H, Ar-H), 7.37–7.38 (m, 1H, Ar-H), 7.53 (d, 1H, J = 8.3 Hz, Ar-H), 7.67–7.78 (br, 2H, Ar-H), 7.84 (d, 2H, J = 8.7 Hz, Ar-H), 7.97 (d, 2H, J = 8.7 Hz, Ar-H), 8.16 (d, 2H, J = 7.8 Hz, Ar-H), 10.72 (s, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 21.19, 28.46, 32.94, 36.64, 113.34, 120.43, 127.05, 128.93, 129.65, 130.29, 130.82, 131.36, 134.04, 134.24, 135.68, 139.46, 141.87, 142.37, 143.24, 152.96, 165.47, 195.59; IR (cm^{-1}): 3252 w (–NH_2), 3091 (Ar-H), 2950 w (C–H), 1665 and 1621 s (C═O), 1592 m (C═C), 1358 s and 1155 s (SO_2); MS(CI) m/z 636 $[M]^+$ (100%).

4.1.21. 4,4′-(9,9′-(1,4-Phenylene)bis(3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridine-10,9(9*H*)-diyl))bis(*N*-(4-sulfamoylphenyl)benzamide) (24)

As yellow crystal, (0.650 g, 56%), mp 307 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.68 (s, 12H, 4×–CH_3), 0.89 (s, 12H, 4×–CH_3), 1.80 (d, 4H, 2×–CH_2), 2.01 (d, 4H, 2×–CH_2), 2.17 (d, 4H, 2×–CH_2), 2.23 (d, 4H, 2×–CH_2), 5.04 (s, 2H, 2×–CH), 7.21 (s, 4H, Ar-H), 7.30 (s, 4H, 2×–NH_2) 7.61 (d, 4H, J = 7.6 Hz, Ar-H), 7.84 (d, 4H, J = 8.7 Hz, Ar-H), 7.98 (d, 4H, J = 8.7 Hz, Ar-H), 8.17 (d, 4H, J = 8.0 Hz, Ar-H), 10.76 (s, 2H, 2×–NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.44, 29.70, 31.63, 32.52, 41.49, 50.04, 113.61, 120.43, 127.07, 127.48, 130.09, 130.44, 135.63, 139.44, 141.85, 142.40, 143.97, 150.32, 165.54, 195.64; IR (cm^{-1}): 3313 and 3258 w (–NH_2), 3096 w (Ar-H), 2959 w (C–H), 1627 s (C═O), 1596 m (C═C), 1364 s and 1149 s (SO_2); MS(CI) m/z 1169 $[M]^+$ (100%).

4.1.22. 4,4′-(9,9′-(1,3-Phenylene)bis(3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridine-10,9(9*H*)-diyl))bis(*N*-(4-sulfamoylphenyl)benzamide) (25)

As yellow crystal, (0.500 g, 42%), mp 295 °C (decomposition) (ethanol–water). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 0.71 (s, 12H, 4×–CH_3), 0.86 (s, 12H, 4×–CH_3), 1.80 (d, 4H, 2×–CH_2), 1.96 (d, 4H, 2×–CH_2), 2.24 (t, 8H, 4×–CH_2), 5.04 (s, 2H, 2×–CH), 7.07–7.17 (m, 3H, Ar-H), 7.30 (s, 4H, 2×–NH_2) 7.49 (s, 1H, Ar-H), 7.84 (d, 8H, J = 8.8 Hz, Ar-H), 8.00 (d, 4H, J = 8.8 Hz, Ar-H), 8.19 (d, 4H, J = 8.5 Hz, Ar-H), 10.77 (s, 2H, 2×–NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.52, 30.07, 32.14, 32.43, 41.57, 50.14, 113.58, 120.40, 120.45, 125.38, 127.06, 127.95, 127.97, 130.07, 130.60, 135.58, 139.42, 142.12, 142.30, 142.43, 146.18, 150.22, 165.54, 195.42; IR (cm^{-1}): 3311 and 3262 w (–NH_2), 3060 w (Ar-H), 2956 w (C–H), 1631 s (C═O), 1596 m (C═C), 1363 s and 1151 s (SO_2); MS(CI) m/z 1169 $[M]^+$ (100%).

4.2. CA inhibition assay

An applied photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity.[16] 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 Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 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 nonlinear least-squares methods using PRISM 3, as reported earlier,[8] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.[11–13]

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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.2013.07.014.

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