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Ureidobenzenesulfonamides as efficient inhibitors of carbonic anhydrase II

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ABSTRACT

Sulfonamides represent an important class of drugs because of their inhibitory effect on carbonic anhydrases (CAs). We therefore synthesized several ureidobenzenesulfonamides and evaluated their bCA II inhibition for their potential use as anti-glaucoma gents. Since these compounds must not show cytotoxic effects, their cytotoxic potential against several human tumor cell lines and non-malignant fibroblasts was investigated. Several fluorophenyl substituted sulfonamides were efficient inhibitors of bCA II. Only one benzylphenyl substituted sulfonamide, however, showed a remarkable selectivity for HT29 colorectal carcinoma cells while being significantly less cytotoxic to non-malignant fibroblasts.

1. Introduction

Carbonic anhydrases (CAs; EC 4.2.1.1) are essential for virtually all forms of life since they are involved in the conversion of carbon dioxide and water to bicarbonate and protons. They thereby maintain in tissues and blood an acid-base balance being crucial for the respiratory system. To date 15 different human CAs are known; they are widely distributed in various tissues [1–3]. Therefore carbonic anhydrases represent an interesting drug target to treat pathologies like diuretics [4], epilepsy and glaucoma. For the latter, CA inhibitors (CAIs) are already in use, and their application in treating obesity, bacterial or fungal infections and cancer is presently subject of research [1,2,5–10]. Especially in the last years the development of CAIs was of major interest [6,11–13] as it has been shown that CAIs might be supportive in anti-cancer therapy, and also inhibition of hCA IX and XII which are associated with hypoxic cancers offers therapeutic potential [1,6,14–17]. CA II was found as a novel biomarker for *Pseudomyxoma peritonei* [18], and its altered expression is associated with human carcinogenesis [18]. Quite recently it was shown that the content of CA II activity is related to processes of aging and neuro-degeneration [19]. The main application for CA II inhibitors, however, concerns persons suffering from glaucoma. Glaucoma is among the leading causes of global irreversible blindness. The number of persons suffering from glaucoma will increase to 111.8 million in 2040 [20]. This gives a renewed impetus to the search for and development of CAII inhibitors.

Sulfonamides represent a class of CAIs that are mainly associated with diuretics and anti-glaucoma agents where the sulfonamide moiety is binding to zinc in CAs [5,21]. Several sulfonamides have already been synthesized in this context as they seem to be very promising agents to treat hypoxic tumors. With regard to SLC-0111 - a sulfonamide inhibitor already in phase I clinical trials - the presence of an ureido moiety seems very promising [2,6,22,23]. The cytotoxic of the latter has already been shown several times, and the bioactivity of several sulfonamides is comparable to that of doxorubicin [24]. CAIs intended for use as antiglaucoma gents must not show any cytotoxicity, and we became interested in developing CAII inhibitors based on sulfonamides being devoid of any cytotoxicity.

2. Results and discussion

2.1. Chemistry

The ureidobenzenesulfonamides **1–19** were accessed from the reaction of sulfanilamide with isocyanates [10]. Thereby our focus was the synthesis of benzylphenyl and naphtyl derivatives since preceding docking studies revealed high binding affinities for these compounds and CAII. Thus, compounds **1–19** were obtained in good yields. For comparison, SLC-0111 [25–36] (**8**, being already in phase I clinical trials) was synthesized, too.[23,37] Work-up of these reactions was easy, since most compounds were obtained in analytical pure form (≥99%) by washing the crude product with acetonitrile and ethanol followed by re-crystallization. Interestingly, **19** could not be precipitated in acetonitrile; even the addition of chloroform (all ureidobenzenesulfonamides are insoluble in this solvent) did not lead to a

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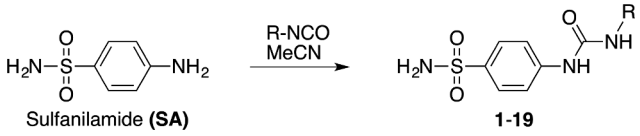

Scheme 1. **1** Naphthalen-1-yl, **2** 4-benzylphenyl, **3** 2-benzylphenyl, **4** 4-methoxy-[1,1′-biphenyl]-3-yl, **5** 4-methoxyphenyl, **6** 3-methoxyphenyl, **7** 2-methoxyphenyl, **8** 4-fluorophenyl, **9** 3-fluorophenyl, **10** 2-fluorophenyl, **11** ethyl, **12** isopropyl, **13** butyl, **14** hexyl, **15** phenyl, **16** benzoyl, **17** ethyl 4-benzoat, **18** 4-benzoic acid, **19** 5-chloro-2-phenoxyphenyl.

precipitation of **19**. Therefore, **19** was purified by column chromatography (see Scheme 1).

2.2. Carbonic anhydrase II inhibition

The synthesized compounds were assayed employing bovine cytosolic carbonic anhydrase bCA II. The results of these assays are compiled in Table 1 and compared to acetazolamide (**AZZ**) and sulfanilamide (**SA**). All compounds showed significantly increased inhibition capabilities as compared to parent sulfanilamide. The inhibition rate of compounds **1** (85%), **3** (84%), **10** (80%) and **17** (75%) can even compete to the inhibition rate of **AAZ** (83%). Interestingly, compound **4** did not show any inhibition at all despite having a 2-substituted methoxy group, which might be considered to be an ideal functional group to inhibit CA II (as can be seen from compound **7**). The biphenyl moiety seems to be too rigid to fit into the cavity - besides having a good calculated binding energy of −8.2 kcal/mol. The lowest IC_{50} values were measured for compounds **1** (0.47 µM), **3** (0.50 µM), **7** (0.45 µM), **10** (0.45 µM) and **17** (0.53 µM), and their K_i values were determined, too. Unfortunately, compound **2** (despite showing the best results in the docking studies) was falling off - compared to **AAZ** and compounds **1** and **3**. Especially alkyl substituted ureidobenzenesulfonamides **11–13** failed to be good inhibitors. All sulfonamides were mixed type inhibitors. Our results are – at least in part – in agreement with the results from previous studies: Thus, for a 2-methyl-4-methoxy analog of **5** a K_i = 3300 nm [34], for a 3-isopropoxy analog of **6** a K_i = 3.9 nm [38], for **7** a K_i = 4700 nm [9,34], for **8** a K_i = 96 nm [9,34], for **10** a K_i = 33 nm [6], and for **15** a K_i = 240 nm [39] have been reported. All other compounds of this study are either unknown so far (**2–4**, **14**, **16**, **19**) or have not been tested for their ability to inhibit bCAII (**5**, **6**, **9**, **11–13**, **17**, **18**).

2.3. Cytotoxicity activity

Since a low cytotoxicity is mandatory for anti-glaucoma active agents, compounds **1–19** were also screened in colorimetric sulforhodamine B (SRD) assays to examine their *in vitro* cytotoxicity. The EC_{50} values were determined for the nonmalignant mouse fibroblasts NIH 3T3 and six human tumor cell lines. The results are summarized in Table 2.

By and large, the ureidobenzenesulfonamides were not cytotoxic for the tested cell lines. Compound **19**, however, showed weak cytotoxicity for all evaluated cell lines. Rather outstanding results can be observed with compound **2** being a moderate bCA II inhibitor. Compound **2** showed a surprisingly high selectivity for the HT29 colorectal carcinoma cells with a selectivity factor $Si_{(Ni3\text{-}HT3/HT29)} \geq 14$. Although an EC_{50} value of 2.1 µM is not as low as those measured for commercial chemotherapeutics (for example, paclitaxel or *cis*-platinum), our results show the potential of CA II inhibitors having been neglected for many years (see Fig. 1).

2.4. Molecular docking

As shown by Santos-Martins et al. [40], Autodock [41] seems well suited for docking of ligands with zinc-metallo-enzymes, and therefore we applied the $Autodock4_{Zn}$ force field using the X-ray crystallographic structure of 1V9E (from www.rcsb.org) as it matches the bovine carbonic anhydrase II used in the inhibition assays. All synthesized compounds docked with reasonable good scores (cf. Supplementary material). The sulfonamide moiety is fitted well next to the zinc ion making ionic interactions. Most of the docked compounds yielded similar top poses in the active site. Compound **1**, **3** and **4** are outliers compared to the other sulfonamides (compound **2** and **7** are depicted as examples). We herein identified benzylphenyl and biphenyl residues as interesting inhibitors. However, it is not possible to see a direct correlation of the $Autodock4_{Zn}$ scores to the measured inhibitory potential. This might be due either to the small sample size, the considerably large chemical difference in the chosen residues or intrinsic limitations of Autodock for calculations of metallo-enzymes.

3. Conclusion

Most of the synthesized ureidobenzenesulfonamides were good inhibitors for CAII but not cytotoxic for several human tumor cell lines

Table 1
Inhibition of bCA II with compounds **1–19** and comparison to the standard inhibitor acetazolamide (**AAZ**) and the starting material sulfanilamide (**SA**). All experiments were performed at least in triplicate; reported are means ± SD.

Compound	Inhibition [%]	IC_{50} [µM]	K_i [µM]	K_i' [µM]	$AD4_{Zn}$ [kcal/mol]
AAZ	82.92 ± 0.37	0.42 ± 0.06	0.37 ± 0.01	> 0.40	− / −
SA	6.28 ± 0.15	9.33 ± 0.64	− / −	− / −	− / −
1	85.37 ± 0.65	0.47 ± 0.06	0.40 ± 0.04	> 0.30	−8.28
2	72.06 ± 0.59	0.75 ± 0.13	2.95 ± 0.10	> 6.00	−8.95
3	83.82 ± 0.69	0.50 ± 0.06	0.58 ± 0.03	> 0.40	−8.51
4	1.55 ± 0.56	− / −	− / −	− / −	−8.2
5	66.71 ± 0.37	0.56 ± 0.08	− / −	− / −	−7.38
6	67.76 ± 0.74	0.57 ± 0.09	0.80 ± 0.19	0.31 ± 0.14	−7.93
7	75.19 ± 0.25	0.45 ± 0.04	0.39 ± 0.01	> 2.50	−7.12
8	66.86 ± 0.03	0.53 ± 0.02	− / −	− / −	−7.44
9	75.13 ± 0.49	0.45 ± 0.07	0.54 ± 0.07	0.35 ± 0.07	−7.4
10	80.42 ± 0.56	0.45 ± 0.02	0.37 ± 0.01	> 3.00	−7.33
11	36.4 ± 1.14	1.48 ± 0.13	− / −	− / −	−6.88
12	41.70 ± 0.46	1.31 ± 0.11	− / −	− / −	−6.87
13	56.5 ± 1.27	0.78 ± 0.08	− / −	− / −	−7.5
14	74.90 ± 0.13	0.52 ± 0.05	0.53 ± 0.01	0.33 ± 0.01	−7.1
15	70.52 ± 0.40	0.52 ± 0.03	0.49 ± 0.03	> 0.50	−7.66
16	33.46 ± 0.13	1.22 ± 0.48	− / −	− / −	−7.51
17	75.36 ± 0.33	0.53 ± 0.04	0.49 ± 0.06	> 0.20	−7.89
18	58.4 ± 0.23	0.69 ± 0.03	− / −	− / −	−8.03
19	60.52 ± 0.80	0.72 ± 0.07	− / −	− / −	−7.69

Table 2
Cytotoxicity of compounds **1–19**. EC_{50} values in μM from SRB assay after 96 h of treatment; nonmalignant mouse fibroblasts (NIH 3T3) and human cancer cell lines: A375 (melanoma), 518A2 (melanoma), HT29 (colorectal carcinoma), A549 (adenocarcinoma), FaDu (squamous carcinoma), SW1736 (thyroid carcinoma); independent experiments were at least performed in triplicate; standard errors are given).

Compound	NIH-3 T3	A375	518A2	HT29	A549	FaDu	SW1736
SA	> 30	> 30	> 30	> 30	> 30	> 30	> 30
1; 3–8	> 30	> 30	> 30	> 30	> 30	> 30	> 30
2	> 30	> 30	7.4 ± 0.8	2.1 ± 0.5	> 30	> 30	> 30
9	> 30	> 30	26.6 ± 2.1	> 30	> 30	> 30	> 30
10–18	> 30	> 30	> 30	> 30	> 30	> 30	> 30
19	14.6 ± 1.9	19.85 ± 1.4	11.1 ± 0.9	9.5 ± 0.8	16.8 ± 1.9	21.1 ± 2.9	19.5 ± 3.0

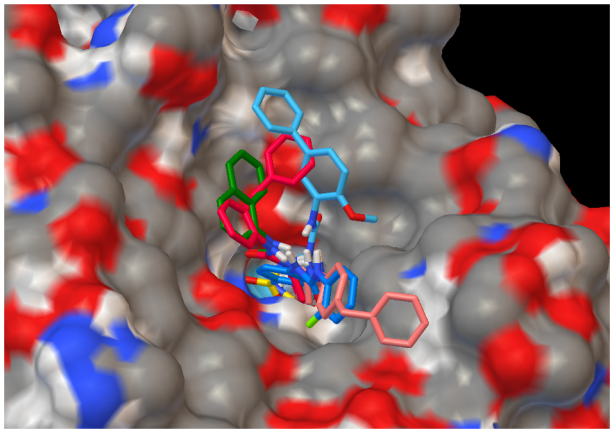

Fig. 1. Active site of hCAII 1v9e with compounds **1** (green), **2** (rose), **3** (red), **4** (light blue), **7** (dark blue).

and non-malignat fibroblasts (NIH 3T3. The benzylphenyl substituted ureidobenzenesulfonamide **2**, however**,** seems to be an exception of the rule, inasmuch as this compound showed remarkable results in the SRB-assay as it was highly selective and cytotoxic for human colorectal adenoma carcinoma HT29 cells; this compound was only a very moderate inhibitor for bCAII. A comparison of the most similar benzylphenyl compounds **2** and **3** with **1** and **4** showed ureidobenzenesulfonamides as an interesting class of highly selective CAIs with promising therapeutic potential as anti-glaucoma agents.

4. Experimental section

4.1. General

Reagents were bought from commercial suppliers and used without further purification. The solvents were dried according to usual procedures. TLC was performed on silica gel (Macherey-Nagel, detection with UV absorption). Melting points are uncorrected (Büchi M-565). NMR spectra were recorded using the VARIAN spectrometers Gemini 2000 or Unity 500 at 27 °C (δgiven in ppm; *J* in Hz, typical experiments: ^{13}C-APT, HMBC, HSQC). ASAP-MS spectra were taken on an Advion expression CMS-L with an ASAP/APCI Ion source (capillary voltage 150 V, capillary temperature 220 °C, and voltage of the ion source: 15 V; APCI source temperature 300 °C with 5 μA). IR spectra were recorded on a Perkin-Elmer Spectrum Two (UATR Two Unit). The SRB assays were performed as previously reported.

4.2. Molecular docking

Crystal structure of the Carbonic anhydrase (PDB = 1V9E) was retrieved from the protein databank. The enzyme was prepared according to usual procedures. Hydrogen atoms were added and water molecules were removed. Zinc was assigned a positive charge 2 as reported for Autodock4$_{Zn}$. Ligand minimizations were performed with MMFF94 force field in Datawarrior. Openbabel was used to create the pdbqt files. Ligands were prepared using MGLTools 1.5.6. Calculations were done with Lamarckian Genetic algorithm; the grid center was placed at the zinc ion with a box size of 20 Å.

4.3. Carbonic anhydrase II inhibition assay

4.3.1. Spectrometer and chemicals

A TECAN SpectraFluorPlus working on the kinetic mode and measuring the absorbance at λ = 415 nm was used for the enzymatic studies. Carbonic anhydrase II (bCAII, from bovine erythrocytes) as well as 4-nitrophenyl acetate (4-NA) and acetazolamide were purchased from Sigma.

4.3.2. Standard solutions preparation

Preparation of 50 mM Tris-HCl buffer solutions: Tris(hydroxymethyl)-aminomethane (606 mg) was dissolved in bidestilled water (100 ml) and adjusted with HCl to a pH of 8.0 ± 0.1. Buffer was freshly prepared and stored in the refrigerator. bCA II solution: the enzyme (≥3000 W-A units/mg, 4.37 mg) was dissolved in freshly prepared buffer pH 8.0 (20 ml). 4-NA solution 6 mM: 4-NA (21.6 mg) was dissolved in methanol (2.1 ml) and bi-destilled water (17.9 ml). All solutions were stored in Eppendorf caps in the refrigerator or freezer, if necessary. The pure compounds were initially dissolved in DMSO. The final concentrations for the enzymatic assay were obtained by diluting the stock solution with bi-distilled water. No inhibition was detected by residual DMSO.

4.3.3. bCA II assays

A mixture of buffer solution pH 8.0 (125 μL), enzyme (25 μL) and compounds solutions (25 μL) was prepared and incubated at 37 °C for 20 min in well plates. 4-NA (25 μL) was added to start the enzymatic reaction. The relative inhibition was determined as the quotient of the slopes (compound divided by blank) of the linear ranges. The concentration of each compound was 1 μM. The used substrate concentration was [4-NA] = 0.75 mM. The absorbance data (λ = 415 nm) was recorded under a controlled temperature of 37 °C for 10 min at 1 min intervals. The IC_{50} values were calculated using GraphPad Prism 5 software. The final inhibitor concentrations were as follows: [inhibitor] = 0.05 μM, 0.1 μM, 0.2 μM, 0.4 μM, 0.5 μM, 0.8 μM, 1.0 μM, 2.0 μM, 5.0 μM, 6.0 μM. A mixture of various compounds solutions (25 μL, 3 different concentrations and once blank), buffer solution (125 μL) and enzyme solution (25 μL) was prepared and incubated at 37 °C for 20 min. The substrate (25 μL, 4 different concentrations) was added to start the enzymatic reaction. The substrate concentrations in the test were as follows: [4-NA] = 0.75 mM, 0.50 mM, 0.25 mM, 0.15 mM. The mode of inhibition as well as Ki and Ki′ were determined using Lineweaver-Burk plots, Dixon plots and Cornish Bowden plots, respectively.

4.4. General procedure for the synthesis of compounds *1–18*

Sulfanilamide (2.5 mmol, 431 mg) was dissolved in dry acetonitrile

(10 ml) and the corresponding isocyanate (1 eq.) was added. The reaction was stirred for 12 h at room temperature. The precipitate was filtered off and washed subsequently with acetonitrile (5 ml), ethanol (5 ml) and diethyl ether (5 ml) to yield compounds **1–18** each as colorless crystalline solid.

4.5. Syntheses

4.5.1. 4-(3-(Naphthalen-1-yl)ureido)benzensulfonamide (1) [42]

73%; R_F = 0.53 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 254 °C; IR (ATR): ν = 1686*m*, 1491*m*, 1300*m*, 1245*m*, 1212*m*, 1147*s*, 794*m*, 769*m*, 614*s*, 542*s* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 9.40 (s, 1H, 8-H), 8.85 (s, 1H, 6-H), 8.10 (d, J = 8.4 Hz, 1H, 10-H), 7.97 (d, J = 7.5 Hz, 1H, 14-H), 7.93 (d, J = 7.9 Hz, 1H, 17-H), 7.79–7.72 (*m*, 2H, 3-H), 7.69–7.62 (*m*, 2H, 4-H), 7.69–7.64 (*m*, 1H, 12-H), 7.60–7.55 (*m*, 1H, 16-H), 7.55–7.48 (*m*, 1H, 11-H), 7.47 (*m*, 1H, 15-H), 7.21 (s, 2H, 1-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 153.2 (C7), 143.3 (C5), 137.7 (C9), 134.3 (C2), 134.2 (C13), 128.9 (C10), 127.3 (C3), 126.6, (C14), 126.4 (C11), 126.3 (C15), 126.3 (C16), 123.9 (C18), 121.8 (C17), 118.5 (C12), 117.9 (C4) ppm; MS (APCI): *m/z* (%) = 342.2 ($[M+H]^+$, 88); analysis calcd for $C_{17}H_{15}N_3SO_3$ (341.39): C 59.81, H 4.43, N 12.31, S 9.39; found: C 59.68, H 4.61, N 12.17, S 9.17.

4.5.2. 4-(3-(4-Benzylphenyl)ureido)benzensulfonamide (2)

46%; R_F = 0.52 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 231 °C; IR (ATR): ν = 3262*w*, 2362*w*,1651*m*, 1590*m*, 1538*s*, 1388*m*, 1329*s*,1303*m*, 1220*m*, 1151*s*, 1096*m*, 587*s*, 546*s* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 8.98 (s, 1H, 6-H), 8.68 (s, 1H, 8-H), 7.73–7.66 (*m*, 2H, 3-H), 7.60–7.54 (*m*, 2H, 4-H), 7.38–7.33 (*m*, 2H, 10-H), 7.26 (t, J = 7.6 Hz, 2H, 16-H), 7.22–7.18 (*m*, 2H, 15-H), 7.18–7.15 (*m*, 1H, 19-H), 7.16 (s, 2H, 1-H), 7.14–7.11 (*m*, 2H, 11-H), 3.86 (s, 2H, 13-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 152.7 (C7), 143.3 (C5), 142.0 (C14), 137.7 (C9), 137.2 (C2), 135.6 (C12), 129.5 (C3), 129.1 (C16), 128.8 (C15), 127.25 (C12), 126.3 (C19), 119.1 (C10), 117.8 (C4), 40.9 (C13); MS (APCI): *m/z* (%) = 382.2 ($[M+H]^+$, 40); analysis calcd for $C_{20}H_{19}N_3SO_3$ (381.45): C 62.97, H 5.02, N 11.02, S 8.41; found: C 62.82, H 5.23, N 4.76, S 8.22.

4.5.3. 4-(3-(2-Benzylphenyl)ureido)benzensulfonamide (3)

41%; R_F = 0.61 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 220 °C; IR (ATR): ν = 3378*m*, 3347*w*, 3249*w*, 1654*s*, 1592*m*, 1533*s*, 1486*s*, 1339*s*, 1226*m*, 1156*s*, 1096*w*, 838*w*, 742*m*, 656*m*, 576*m*, 538*s* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 9.29 = (s, 1H, 6-H), 8.14 (s, 1H, 8), 7.75–7.72 (*m*, 1H, 12-H), 7.72–7.68 (*m*, 2H, 3-H), 7.61–7.54 (*m*, 2H, 4-H), 7.30–7.24 (*m*, 2H, 18-H), 7.21–7.18 (*m*, 1H, 14-H), 7.21–7.14 (*m*, 2H, 17-H), 7.17 (s, 2H, 1-H), 7.17–7.15 (*m*, 1H, 13-H), 7.06–7.03 (*m*, 1H, 11-H), 7.03–6.99 (*m*, 1H, 19-H), 3.97 (s, 2H, 15-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 153.1 (C7), 143.4 (C5), 140.3 (C9), 137.2 (C2), 136.9 (C10), 132.7 (C16), 129.2 (C17), 128.9 (C18), 127.3 (C3), 127.0 (C14), 126.5 (C13), 124.3 (C11), 123.6 (C12), 117.8 (C4), 36.7 (C15); MS (APCI): *m/z* (%) = 382.3 ($[M+H]^+$, 31); analysis calcd for $C_{20}H_{19}N_3SO_3$ (381.45): C 62.97, H 5.02, N 11.02, S 8.41; found: C 62.70, H 5.25, N 10.81, S 8.21.

4.5.4. 4-(3-(4-Methoxy-[1,1′-biphenyl]-3-yl)ureido)benzensulfonamide (4)

37%; R_F = 0.53 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 270 °C; IR (ATR): ν = 3370*m*, 3227*w*, 1709*m*, 1597*m*, 1540*s*, 1317*s*, 1247*m*, 1222*m*, 1151*s*, 820*s*, 755*s*, 697*s*, 617*s*, 531*s* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 9.71 (s, 1H, 6-H), 8.49 (d, J = 2.3 Hz, 1H, 14-H), 8.41 (s, 1H, 8-H), 7.76–7.69 (*m*, 2H, 3-H), 7.65–7.59 (*m*, 2H, 4-H), 7.59–7.54 (*m*, 2H, 17-H), 7.47–7.39 (*m*, 2H, 18-H), 7.34–7.28 (*m*, 1H, 19-H), 7.26 (dd, J = 8.4, 2.3 Hz, 1H, 12-H), 7.18 (s, 2H, 13-H), 7.11 (d, J = 8.5 Hz, 1H, 11-H), 3.92 (s, 3H, 15-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 152.7 (C7), 147.9 (C10), 143.3 (C5), 140.7 (C16), 137.3 (C2), 133.2 (C13), 129.3 (C18), 129.1 (C9), 127.4 (C3), 127.3 (C19), 126.7 (C12), 120.8 (C9), 117.7 (C4), 117.3 (C14), 111.7 (C11), 56.5 (C15); MS (APCI): *m/z* (%) = 398.2 ($[M+H]^+$, 20); analysis calcd for $C_{20}H_{19}N_3SO_4$ (397.45): C 60.44, H 4.82, N 10.57, S 8.07; found: C 60.20, H 4.99, N 10.32, S 7.83.

4.5.5. 4-(3-(4-Methoxyphenyl)ureido)benzensulfonamide (5) [43]

68%; R_F = 0.43 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 229 °C; IR (ATR): ν = 3258*m*, 1649*s*, 1592*m*, 1560*s*, 1305*s*, 1251*s*, 1161*s*, 828*m*, 594*m*, 544*s* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 8.94 (s, 1H, 6-H), 8.56 (s, 1H, 8-H), 7.74–7.66 (*m*, 2H, 3-H), 7.61–7.54 (*m*, 2H, 4-H), 7.39–7.28 (*m*, 2H, 10-H), 7.16 (s, 2H, 1-H), 6.91–6.80 (*m*, 2H, 11-H), 3.70 (s, 3H, 13-H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 155.2 (C12), 152.9 (C7), 143.5 (C5), 137.1 (C2), 132.7 (C9), 127.2 (C3), 120.8 (C10), 117.7 (C4), 114.5 (C11), 55.6 (C13); MS (APCI): *m/z* (%) = 322.2 ($[M+H]^+$, 9); analysis calcd for $C_{14}H_{15}N_3SO_4$ (321.35): C 52.33, H 4.70, N 13.08, S 9.98; found: C 52.11, H 4.93, N 12.94, S 9.72.

4.5.6. 4-(3-(3-Methoxyphenyl)ureido)benzensulfonamide (6) [44]

48%; R_F = 0.45 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 238 °C; IR (ATR): ν = 3392*w*, 3280 *w*, 1694*m*, 15901*m*, 1528*m*, 1286*m*, 1152*s*, 771*m*, 549scm $^{-1}$; ^1H NMR (500 MHz, DMSO-d_6): δ = 9.01 (s, 1H, 6-H), 8.76 (s, 1H, 8-H), 7.74–7.69 (*m*, 2H, 3-H), 7.62–7.55 (*m*, 2H, 4-H), 7.20–7.17 (*m*, 1H, 10-H), 7.18 (s, 2H, 1-H), 7.18–7.15 (*m*, 1H, 13-H), 6.93 (ddd, J = 8.1, 2.0, 0.9 Hz, 1H, 14-H), 6.56 (ddd, J = 8.2, 2.5, 0.9 Hz, 1H, 12-H), 3.72 (s, 3H, 15-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 160.2 (C11), 152.6 (C7), 143.2 (C5), 141.0 (C9), 137.3 (C2), 130.1 (C13), 127.3 (C3), 117.9 (C4), 111.2 (C14), 108.1 (C12), 104.6 (C10), 55.4 (C15); MS (APCI): *m/z* (%) = 322.2 ($[M+H]^+$, 3); analysis calcd for $C_{14}H_{15}N_3SO_4$ (321.35): C 52.33, H 4.70, N 13.08, S 9.98; found: C 52.00, H 4.97, N 12.86, S 9.72.

4.5.7. 4-(3-(2-Methoxyphenyl)ureido)benzensulfonamide (7) [9,34,45]

52%; R_F = 0.53 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 225 °C; IR (ATR): ν = 3321*w*, 1684*m*, 1591*m*, 1540*s*, 1309*m*, 1149*s*, 844*m*, 744*s*, 665*m*, 610*m*, 546*s* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 9.65 (s, 1H, 6-H), 8.32 (s, 1H, 8-H), 8.10 (dd, J = 8.0, 1.7 Hz, 1H, 14-H), 7.75–7.70 (*m*, 2H, 3-H), 7.63–7.57 (*m*, 2H, 4-H), 7.18 (s, 2H, 1-H), 7.01 (dd, J = 8.2, 1.5 Hz, 1H, 11-H), 6.95 (td, J = 7.7, 1.7 Hz, 1H, 12-H), 6.89 (td, J = 7.6, 1.5 Hz, 1H, 13-H), 3.87 (s, 3H, 15-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 152.62 (C7), 148.3 (C10), 143.4 (C5), 137.2 (C2), 128.7 (C9), 127.3 (C3), 122.7 (C12), 121.0 (C14), 118.9 (C13), 117.7 (C4), 111.3 (C11), 56.3 (C15); MS (APCI): *m/z* (%) = 322.2 ($[M+H]^+$, 6); analysis calcd for $C_{14}H_{15}N_3SO_4$ (321.35): C 52.33, H 4.70, N 13.08, S 9.98; found: C 52.12, H 4.97, N 12.85, S 9.77.

4.5.8. 4-(3-(4-Fluorophenyl)ureido)benzenesulfonamide (8)

81%; R_F = 0.38 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 236 °C (lit.: 242–243 °C [34]); IR (ATR): ν = 3412*w*, 3337*w*, 1673*m*, 1696*m*, 1501*s*, 1327*m*, 1210*m*, 1147*s*, 914*m*, 836*m*, 627*m*, 537*s* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 9.02 (s, 1H, 6-H), 8.79 (s, 1H, 8-H), 7.74–7.68 (*m*, 2H, 3-H), 7.62–7.55 (*m*, 2H, 4-H), 7.45 (dd, J = 9.2, 4.9 Hz, 2H, 10-H), 7.17 (s, 2H, 1-H), 7.12 (td, J = 8.9, 1.3 Hz, 2H, 11-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 158.0 (d, J = 238.9 Hz, C12), 152.8 (C7), 143.3 (C5), 137.3 (C2), 136.1 (d, J = 2.4 Hz, C9), 127.3 (C3), 120.7 (d, J = 8.1 Hz, C10), 117.9 (C4), 115.8 (d, J = 22.4 Hz, C11); MS (APCI): *m/z* (%) = 310.2 ($[M+H]^+$, 99); analysis calcd for $C_{13}H_{12}FN_3SO_3$ (309.32): C 50.48, H 3.91, N 13.58, S 10.37; found: C 50.21, H 4.11, N 13.39, S 10.20.

4.5.9. 4-(3-(3-Fluorophenyl)ureido)benzensulfonamide (9) [44]

72%; R_F = 0.39 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 240 °C; IR (ATR): ν = 3297*w*, 1701*m*, 1541*m*, 1312*m*, 1157*s*, 829*m*, 773*m*, 657*m*, 540*m*, 526*s* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 9.09 (s, 1H, 6-H), 8.99 (s, 1H, 8-H), 7.76–7.69 (*m*, 2H, 3-H), 7.63–7.56 (*m*, 2H, 4-H), 7.47 (dt, J = 11.9, 2.3 Hz, 1H, 10-H), 7.30 (td, J = 8.2, 6.9 Hz, 1H, 12-H),

7.19 (s, 2H, 1-H), 7.15–7.10 (*m*, 1H, 14-H), 6.83–6.75 (*m*, 1H, 13-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 162.8 (d, J = 240.8 Hz, C11), 152.6 (C7), 143.01 (C5), 141.7 (d, J = 11.1 Hz, C9), 137.6, 130.8 (d, J = 9.6 Hz, C13), 127.3 (C3), 118.1 (C4), 114.6 (d, J = 2.7 Hz, C14), 109.0 (d, J = 21.1 Hz, C10), 105.6 (d, J = 26.4 Hz, C12); MS (APCI): *m/z* (%) = 310.2 ([M + H]$^+$, 99); analysis calcd for $C_{13}H_{12}FN_3SO_3$ (309.32): C 50.48, H 3.91, N 13.58, S 10.37; found: C 50.23, H 3.68, N 13.42, S 10.13.

4.5.10. 4-(3-(2-Fluorophenyl)ureido)benzensulfonamide (**10**) [6]

67%; R_F = 0.55 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 233 °C; IR (ATR): ν = 3396*w*, 3307*w*, 3204*w*, 1703*m*, 1541*m*, 1305*s*, 1253*m*, 1149*s*, 1100*m*, 906*m*, 836*m*, 761*s*, 676*m*, 610*m*, 548*s*, cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.23 (s, 1H, 6-H), 8.70 (s, 1H, 8-H), 8.11 (td, J = 8.2, 1.7 Hz, 1H, 14-H), 7.76–7.70 (*m*, 2H, 3-H), 7.63–7.56 (*m*, 2H, 4-H), 7.27–7.20 (*m*, 1H, 11-H), 7.20–7.10 (*m*, 1H, 13-H), 7.19 (s, 2H, 1-H), 7.06–6.98 (*m*, 1H, 12-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 152.7 (d, J = 241.8 Hz, C10), 152.4 (C7), 143.0 (C5), 137.6 (C2), 127.6 (d, J = 10.2 Hz, C9), 127.4 (C3), 125.0 (d, J = 3.4 Hz, C14), 123.4 (d, J = 7.5 Hz, C12), 121.3 (d, J = 1.6 Hz, C13), 117.9 (C4), 115.5 (d, J = 19.0 Hz, C11); MS (APCI): *m/z* (%) = 310.2 ([M + H]$^+$, 46); analysis calcd for $C_{13}H_{12}FN_3SO_3$ (309.32): C 50.48, H 3.91, N 13.58, S 10.37; found: C 50.15, H 4.19, N 13.51, S 10.04.

4.5.11. 4-(3-Ethylureido)benzensulfonamide (**11**) [44]

63%; R_F = 0.35 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1) ; m.p.: 232 °C; IR (ATR): ν = 3295*w*, 1661*m*, 1593*m*, 1535*m*, 1317*m*, 1233*m*, 1159*s*, 1097*m*, 832*m*, 741*m*, 589*m*, 537*s* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.79 (s, 1H, 6-H), 7.68–7.60 (*m*, 2H, 3-H), 7.55–7.47 (*m*, 2H, 4-H), 7.11 (s, 2H, 1-H), 6.22 (t, J = 5.6 Hz, 1H, 8-H), 3.10 (qd, J = 7.1, 5.5 Hz, 2H, 9-H), 1.04 (t, J = 7.2 Hz, 3H, 10-H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 155.16 (C7), 144.18 (C5), 136.36 (C2), 127.16 (C3), 117.20 (C4), 34.44 (C9), 15.77 (C10); MS (APCI): *m/z* (%) = 244.1 ([M + H]$^+$, 29); analysis calcd for $C_9H_{13}N_3SO_3$ (243.28): C 44.43, H 5.39, N 17.27, S 13.18; found: C 44.30, H 17.47, N 17.00, S 12.86.

4.5.12. 4-(3-Isopropylureido)benzensulfonamide (**12**) [44]

49%; R_F = 0.7 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 223 °C; IR (ATR): ν = 3325*w*, 1634*s*, 1133*m*, 1247*m*, 1149*s*, 1099*m*, 739*m*, 587*m*, 539*m* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.65 (*s*, 1H, 6-H), 7.68–7.60 (*m*, 2H, 3-H), 7.53–7.46 (*m*, 2H, 4-H), 7.11 (s, 2H, 1-H), 6.12 (d, J = 7.5 Hz, 1H, 8-H), 3.82–3.66 (*m*, 1H, 9-H), 1.08 (d, J = 6.6 Hz, 5H, 10-H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 154.5 (C7), 144.1 (C5), 136.4 (C2), 127.2 (C3), 117.1 (C4), 41.5 (C9), 23.3 (C10); MS (APCI): *m/z* (%) = 256.2 ([M + H]$^+$, 99); analysis calcd for $C_{10}H_{15}N_3SO_3$ (257.31): C 46.68, H 5.88, N 16.33, S 12.46; found: C 46.42, H 6.03, N 16.02, S 12.18.

4.5.13. 4-(3-Butylureido)benzensulfonamide (**13**) [46–49]

64%; R_F = 0.41 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 195 °C (lit.: 196–199 °C [48]); IR (ATR): ν = 3403*w*, 3296*m*, 3088*w*, 2963*w*, 1687*s*, 1593*m*, 1533*m*, 1324*m*, 1228*m*, 1154*s*, 830*s*, 598*m*, 542*s* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.76 (s, 1H, 6-H), 7.67–7.61 (*m*, 2H, 3-H), 7.54–7.47 (*m*, 2H, 4-H), 7.11 (s, 2H, 1-H), 6.23 (t, J = 5.6 Hz, 1H, 8-H), 3.07 (q, J = 6.9 Hz, 2H, 9-H), 1.45–1.35 (*m*, 2H, 10-H), 1.35–1.24 (*m*, 2H, 11-H), 0.88 (t, J = 7.3 Hz, 3H, 12-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 155.2 (C7), 144.2 (C5), 136.4 (C2), 127.2 (C3), 117.2 (C4), 39.2 (C9), 32.2 (C10), 20.0 (C11), 14.1 (C12); MS (APCI): *m/z* (%) = 272.2 ([M + H]$^+$, 23); analysis calcd for $C_{11}H_{17}N_3SO_3$ (271.34): C 48.69, H 6.32, N 15.49, S 11.82; found: C 48.70, H 6.51, N 15.24, S 11.69.

4.5.14. 4-(3-Hexylureido)benzensulfonamide (**14**)

72%; R_F = 0.49 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 201 °C; IR (ATR): ν = 3410*m*, 3352*m*, 3201*w*, 2938*m*, 2850*m*, 1682*s*, 1596*m*, 1307*s*, 1237*m*, 1151*s*, 825*m*, 590*m*, 541*m*, 513*m* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.77 (s, 1H, 6-H), 7.68–7.60 (*m*, 2H, 3-H), 7.54–7.46 (*m*, 2H, 4-H), 7.12 (s, 2H, 1-H), 6.24 (t, J = 5.7 Hz, 1H, 8-H), 3.06 (td, J = 6.9, 5.7 Hz, 2H, 9-H), 1.40 (tt, J = 7.2, 3.8 Hz, 2H, 10-H), 1.30–1.21 (*m*, 4H, 12-H + 13-H), 1.26–1.25 (*m*, 2H, 13-H), 0.89–0.81 (*m*, 3H, 14-H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 155.2 (C7), 144.2 (C5), 136.3 (C2), 127.2 (C3), 117.2 (C4), 39.5 (C9), 31.4 (C12), 30.0 (C110), 26.5 (C11), 22.5 (C13), 14.4 (C14); MS (APCI): *m/z* (%) = 300.2 ([M + H]$^+$, 32); analysis calcd for $C_{13}H_{21}N_3SO_3$ (299.39): C 52.15, H 7.07, N 14.04, S 10.71; found: C 52.00, H 7.23, N 13.77, S 10.51.

4.5.15. 4-(3-Phenylureido)benzensulfonamide (**15**) [39,45,50–58]

68%; R_F = 0.4 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 229 °C (lit.: 233–235 °C [34]); IR (ATR): ν = 3306*w*, 1699*s*, 1593*s*, 1526*m*, 1316*m*, 1147*s*, 746*m*, 647*m*, 534*s* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.02 (s, 1H, 6-H), 8.75 (s, 1H, 8-H), 7.75–7.69 (*m*, 2H, 3-H), 7.63–7.56 (*m*, 2H, 4-H), 7.48–7.41 (*m*, 2H, 10-H), 7.32–7.24 (*m*, 2H, 11-H), 7.18 (s, 2H, 1H), 6.98 (tt, J = 7.3, 1.1 Hz, 1H, 12-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 152.7 (C7), 143.3 (C5), 139.8 (C9), 137.3 (C2), 129.3 (C11), 127.3 (C3), 122.7 (C12), 118.9 (C10), 117.9 (C4); MS (APCI): *m/z* (%) = 292.2 ([M + H]$^+$, 84); analysis calcd for $C_{13}H_{13}N_3SO_3$ (291.33): C 53.60, H 4.50, N 14.42, S 11.01; found: C 53.42, H 4.77, N 14.05, S 10.78.

4.5.16. N-((4-Sulfamoylphenyl)carbamoyl)benzamide (**16**)

44%; R_F = 0.7 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 263 °C; IR (ATR): ν = 3339*w*, 3245*w*, 1700*s*, 1593*m*, 1160*m*, 1097*m*, 750*m*, 704*s*, 544*s* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.12 (s, 1H, 8-H), 11.03 (s, 1H, 6-H), 8.04–7.98 (*m*, 2H, 11-H), 7.82–7.73 (*m*, 4H, 3-H + 4-H), 7.69–7.62 (*m*, 1H), 7.57–7.50 (*m*, 2H, 4-H), 7.27 (s, 2H, 1-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.2 (C9), 151.6 (C7), 141.1 (C4), 139.3 (C2), 133.6 (C10), 132.6 (C13), 129.1 (C4), 128.8 (C12), 127.3 (C11), 119.9 (C4); MS (APCI): *m/z* (%) = 320.2 ([M + H]$^+$, 6); analysis calcd for $C_{14}H_{13}N_3SO_4$ (319.34): C 52.66, H 4.10, N 13.16, S 10.04; found: C 52.41, H 4.28, N 13.02, S 9.76.

4.5.17. Ethyl 4-(3-(4-sulfamoylphenyl)ureido)benzoate (**17**) [44]

84%; R_F = 0.4 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 263 °C; IR (ATR): ν = 3298*w*, 1698*s*, 1591*s*, 1411*m*, 1370*m*, 1150*m*, 1100*m*, 1016*m*, 902*w*, 768*m*, 642*m*, 572*m*, 538*s* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.18 (s, 1H, 8-H), 9.14 (s, 1H, 6-H), 7.91–7.86 (*m*, 2H, 11-H), 7.77–7.70 (*m*, 2H, 3-H), 7.64–7.60 (*m*, 2H, 4-H), 7.60–7.56 (*m*, 2H, 10-H), 7.19 (s, 2H, 1-H), 4.26 (q, J = 7.0 Hz, 2H, 14-H), 1.29 (t, J = 7.1 Hz, 3H, 15-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 165.8 (C13), 152.4 (C7), 144.4 (C9), 142.9 (C5), 137.7 (C2), 130.8 (C11), 127.3 (C3), 123.56 (C12), 118.2 (C4), 118.0 (C10), 60.8 (C14), 14.7 (C15); MS (APCI): *m/z* (%) = 364.2 ([M + H]$^+$, 12); analysis calcd for $C_{16}H_{17}N_3SO_5$ (363.39): C 52.88, H 4.72, N 11.56, S 8.82; found: C 52.51, H 4.99, N 11.31, S 8.62.

4.5.18. 4-(3-(4-Sulfamoylphenyl)ureido)benzoic acid (**18**)

Compound **12** (200 mg, 0.55 mmol) was suspended in methanol (10 ml) and potassium hydroxide (310 mg, 5.5 mmol) was added. The mixture was heated under reflux for 4 h. After cooling to room temperature the white solid was filtered off and washed with methanol to give compound **18** as a colorless crystalline solid (196 mg, 0.54 mmol, 98%); R_F = 0.11 (SiO_2, $CHCl_3$/MeOH/DMF, 16:1:1); m.p.: 299–303 °C; IR (ATR): ν = 1655*s*, 1590*s*, 1535*s*, 1329*m*, 1218*m*, 1151*s*, 764*s*, 640*m*, 541*s* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.60 (s, 1H, 14-H), 9.15 (s, 1H, 8-H), 9.15 (s, 1H, 6-H), 7.92–7.84 (*m*, 2H, 11-H), 7.77–7.69 (*m*, 2H, 3-H), 7.64–7.59 (*m*, 2H, 4-H), 7.58–7.54 (*m*, 2H, 10-H), 7.19 (s, 2H, 1-H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 167.4 (C13), 152.5 (C7), 144.1 (C9), 142.93 (C5), 137.65 (C2), 131.0 (C10), 127.3 (C3), 124.5 (C12), 118.2 (C4), 117.9 (C11); MS (APCI): *m/z* (%) = 336.2 ([M + H]$^+$, 99) analysis calcd for $C_{14}H_{13}N_3SO_5$ (335.34): C 50.14, H 3.91, N 12.53, S 9.56; found: C 49.87, H 4.17, N 12.32, S 9.37.

*4.5.19. 4-(3-(5-chloro-2-phenoxyphenyl)ureido)benzensulfonamide (**19**)*

Sulfanilamide (206 mg, 1.2 mmol) was dissolved in acetonitrile (10 ml) and 5-chloro-2-phenoxyphenylisocyanate (294 mg, 1.2 mmol) was added. The reaction was stirred for 24 h at room temperature. After removing the solvent under reduced pressure, the residue was purified by column chromatography (SiO_2, hexane/ethyl acetate, 2:1) to give compound **19** as an off-white crystalline solid (72%, 360 mg, 0.86 mmol); R_F = 0.35 (SiO_2, hexane/ethyl acetat, 2:1); m.p.: 209 °C; IR (ATR): ν = 3361*m*, 1713*m*, 1590*s*, 1529*s*, 1473*m*, 1310*m*, 1218*s*, 1146*m*, 838*m*, 612*m*, 535*s*, 492*s* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 9.68 (s, 1H, 6-H), 8.74 (s, 1H, 8-H), 8.36 (d, J = 2.6 Hz, 1H, 10-H), 7.75–7.69 (*m*, 2H, 3-H), 7.61–7.54 (*m*, 2H, 4-H), 7.46–7.38 (*m*, 2H, 17-H), 7.19 (s, 2H, 1-H), 7.20–7.16 (*m*, 1H, H18), 7.11–7.04 (*m*, 2H, 16-H), 7.00 (dd, J = 8.7, 2.6 Hz, 1H, 12-H), 6.82 (d, J = 8.7 Hz, 1H, 13-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.7 (C15), 152.4 (C7), 144.5 (C14), 142.8 (C5), 137.7 (C2), 132.6 (C28), 130.7 (C17), 128.0 (C11), 127.4 (C3), 124.6 (C15), 122.4 (C12), 119.8 (C13), 119.2 (C10), 119.1 (C16), 118.0 (C4); MS (APCI): m/z (%) = 418.1 ($[M+H]^+$, 13%) analysis calcd for $C_{19}H_{16}ClN_3SO_4$ (417.87): C 54.61, H 3.86, N 10.06, S 7.67; found: C 54.41, H 4.03, N 9.75, S 7.50.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103123.

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