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Original article

Carbonic anhydrase inhibitors. Synthesis, and molecular structure of novel series *N*-substituted *N′*-(2-arylmethylthio-4-chloro-5-methylbenzene sulfonyl)guanidines and their inhibition of human cytosolic isozymes I and II and the transmembrane tumor-associated isozymes IX and XII

Beata Żołnowska [a], Jarosław Sławiński [a,*], Aneta Pogorzelska [a], Jarosław Chojnacki [b], Daniela Vullo [c], Claudiu T. Supuran [c,d]

[a]Department of Organic Chemistry, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland
[b]Department of Inorganic Chemistry, Gdańsk University of Technology, Narutowicza 11/12, 80-233 Gdańsk, Poland
[c]Dipartimento di Chimica, Universita degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, 50019 Sesto Fiorentino (Florence), Italy
[d]Università degli Studi di Firenze, NEUROFARBA Dept., Sezione di Scienze Farmaceutiche, 50019 Sesto Fiorentino (Florence), Italy

ABSTRACT

A series of novel *N*-substituted *N′*-(2-arylmethylthio-4-chloro-5-methylbenzenesulfonyl)guanidines **9-41** have been synthesized and investigated as inhibitors of four isoforms of zinc enzyme carbonic anhydrase (CA.EC 4.2.1.1), that is the cytosolic CA I and II, and cancer-associated isozymes CA IX and XII. Against the human CA I investigated compounds showed K_I in the range of 87-6506 nM, toward hCA II ranging from 7.8 to 4500 nM, against hCA IX in the range of 4.7-416 nM and against hCA XII at range of 0.96-540 nM. Compounds **10**, **12-14, 16**, **18-20**, **24-26, 31** and **32** exhibited a powerful inhibitory potency toward hCA IX (K_I = 4.7-21 nM) in comparison to the reference sulfonamides **AAZ**, **MZA**, **EZA**, **DCP** and **IND** (K_I = 24-50 nM). Compound **14** was the most potent inhibitor of hCA I (K_I = 87 nM), hCA IX (K_I = 4.7 nM) and hCA XII (K_I = 0.96 nM), while **26** was the most effective inhibitor of hCA II (K_I = 7.8 nM). The most promising compound **32** exerted the highest selectivity ratios toward hCA IX versus hCA I (hCA I/hCA IX = 261) and hCA II (hCA II/hCA IX = 26). The *in vitro* antitumor activity of compounds **10**, **13**, **14**, **21**, **22**, **25**, **32**, **38** and **41** was evaluated at the US National Cancer Institute (NCI) against a panel of 60 human tumor cell lines. The most active antitumor agents **21** and **25**, inhibiting 32-35 human tumor cell lines with GI_{50} in the range of 2.1-5.0 μM also showed relatively high inhibitory activity toward hCA IX and XII with K_I from 18-40 nM.

Keywords:
Sulfonylguanidine; Sulfonamide; Synthesis; Carbonic anhydrase isozymes I, II, IX and XII inhibitors; Anticancer activity

*Corresponding author. Tel.: +48 58 349 10 98; fax: +48 58 349 12 77.
E-mail address: jaroslaw@gumed.edu.pl (J. Sławiński).

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are metalloenzymes catalyze a reversible hydratation of carbon dioxide to bicarbonate and protons ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$), and

thus played important role in respiration and transport of CO_2 /bicarbonate between metabolizing tissues and lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues and organs, biosynthetic reactions (such as gluconeogenesis and lipid and urea synthesis), bone resorption, calcification, tumorigenicity and many other physiological or pathological processes [1]. The isoforms of CA vary in location and tissue distribution, thus cytosolic (I, II, III, VII, and XIII), membrane-bound (IV, IX, XII, and XIV), mitochondrial (VA and VB), and secreted (VI) forms have been described [2,3]. The isozymes CA IX and XII have been known as the membrane CAs associated with cancers, which were also found in a very limited number of normal tissues, such as gastrointestinal mucosa and gastrointestinal related structures [3-6]. The expression level of CA IX is elevated in response to hypoxia, which is a consequence of the rapid growth of many tumors and an important regulator by a direct transcriptional activation of the *CA9* gene by the hypoxia inducible factor (HIF-1) [7,8]. The general result of CA IX overexpression in tumors is a pH decrease in the extracellular microenvironment from pH ~ 7.4 (normal tissue) to pH ~ 6.8 (hypoxic tumor) that promotes tumor cell survival and invasion [9,10]. Considering the abnormally high expression of CA IX in many hypoxic tumors and its demonstrated role in the tumor acidification processes and oncogenesis, this isoform constitutes attractive target for anticancer therapy.

It has been known that primary sulfonamides act as carbonic anhydrase inhibitors (CAIs) by binding to the catalytic Zn^{2+} ion in the active site of the enzyme and blocking its function [9, 11]. The first investigated aromatic/heterocyclic sulfonamides were clinically used derivatives acetazolamide **AAZ** [12], methazolamide **MZA** [12], ethoxzolamide **EZA** [12], dichlorophenamide **DCP** [12] and indisulam **IND** [13] (Figure 1). Unfortunately, they do not show selective inhibition of the tumor-associated CA IX and XII and are able to inhibit other CA isozymes that have a physiological relevance [14]. However, remarkable progress has been made in developing small-molecule inhibitors e.g. **CAI17**, **U-104** or **I** with reasonable selectivity for extracellular CA IX that show efficacy *in vivo* in preclinical models of human cancer (Figure 1) [15-17].

During recent years we have reported on the strong inhibition of human cytosolic CA I and II and tumor-associated CA IX and XII with some 4-chloro-5-methyl-2-(R-thio)benzenesufonamides of type **A** [18] and **B** [19,20]. Some of these compounds showed a certain degree of selectivity for inhibition of the tumor-associated over the cytosolic isoforms of CAs [18-20]. Considering our previous reports and the existing state of knowledge about connection between CA IX and cancer, we decided to investigate the inhibitory activity

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against CAs for the series of *N*-[2-(R-methylthio)-4-chloro-5-methylbenzenesulfonyl]-*N′*-(sulfamoylaryl/alkyl/heteroaryl)guanidines of type **C** (Figure 2).

2. Results and discussion

2.1. Chemistry

As was presented in Scheme 1, the newly synthesized compounds were obtained starting from the appropriate *N*-(benzenesulfonyl)cyanamide potassium salts **3** [21], **4-7** [22], which were prepared according to the previously reported procedure, by nucleophilic substitution of arylmethyl chloride with dipotassium salt **2**. Novel substrate **8** was synthesized analogously from **2** and 5-chloro-6-(chloromethyl)benzo[*d*][1,3]dioxole. The starting compounds: 3-aminobenzodithiazine **1** and **2** were obtained as was described in [21]. In turn, an 5-amino-1,3,4-thiadiazole-2-sulfonamide was synthesized *via* acidic hydrolysis of 2-acetamido-1,3,4-thiadiazole-5-sulfonamide [23].

Thus, treatment of salts **3-8** with either amino- or hydrazinyl components resulted in affording the desired *N,N′*-substituted guanidines **9-41**. The syntheses were carried out using two approaches marked as **c** and **d** in Scheme 1. First way includes reaction of potassium salt with aminosulfonamide derivative in the presence of *p*-toluenesulfonic acid monohydrate (PTSA) in dry toluene (or dry *p*-dioxane) whereas the second one involves amino-, or hydrazinylsulfonamide hydrochlorides reacting with potassium salt in toluene (*p*-dioxane or acetonitrile). The reactions proceeded in reflux for 2-28 h (reaction times were monitored using TLC method) with satisfactory yields.

The structure of final compounds was confirmed by elemental analyses (C, H, N) and spectral data. IR spectra showed the absence of the (C≡N) group and the presence of a bands at range 3552-3176 cm^{-1} for NH and NH_2, and bands at range 1656-1619 cm^{-1} for C=N bond. In series of 3-(sulfamoylphenyl)guanidines (**9-11**, **16-17**, **21-23**, **27-29**, **33-35**, **38**, **39**), ^{1}H-NMR spectra in DMSO-d_6 revealed singlets at range 6.92-7.50 ppm for NH and SO_2NH_2, and distinctive singlets at ranges 9.30-9.42 or 8.44-8.52 ppm for SO_2NH group. Similarly, it was observed singlets at ranges 7.08-7.10 ppm (for SO_2NH_2), 7.04-8.45 ppm (for NH), and 9.22-9.33 ppm (for SO_2NH) for compounds of series 3-(4-sulfamoylphenylamino)guanidines (**12**, **18**, **24**, **30**, **36**, **40**). The ^{1}H-NMR spectra of 3-(sulfamoylbenzyl)guanidines of type **E** (**13**, **19**, **25**, **31**, **37**, **41**) showed signals for SO_2NH_2 in the range 7.20-7.45 ppm, and broad singlets in 6.70-6.85 ppm corresponded with guanidine NH_2. In addition, these spectra did not exhibit

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signals characteristic for SO_2NH group. However, 3-(2-sulfamoyl-1,3,4-thiadiazol-5-yl)guanidine derivatives (**14**, **20**, **26**, **32**) displayed in the ^{1}H-NMR spectra singlets in the range of 7.00-7.45 ppm for NH, 8.29-8.31 ppm for SO_2NH_2, and broad singlets in the range of 11.85-11.90 ppm attributable to SO_2NH group. Moreover, X-ray analysis was undertaken to confirm proposed structures on the representative compound **17**.

Details on data collection, structure solution and refinement are given in Table 1. Compound **17** crystallizes in the monoclinic system, space group $P2_1/c$, with one molecule of organic compound and one molecule of water in the asymmetric part of the unit cell. Atom numbering scheme is presented in Figure 3.

Nitrogen atom N4, being a part of the guanidyl residue, is deprotonated, while the terminal sulfonamide nitrogen N1 bounds two hydrogen atoms. This tautomeric form enables formation of hydrogen bonds of the amide group at N3 with oxygen acceptor atoms: water O5 and O3 in SO_2 moiety. On the other hand bond lengths C7-N3 and C7-N4 are 1.340 and 1.348 Å, indicating partly double character of both bonds. The guanidine residue is not coplanar with the aromatic rings, dihedral angle of mean (C7 N2 N3 N4) plane and most proximate aromatic ring (C1-C6) is 74.99°.

The terminal sulfonamide NH_2 group (with N1 atom) forms intermolecular hydrogen bonds with SO_2 oxygen atoms of neighboring molecules.

The hydrophilic and hydrophobic parts are arranged into layers parallel to the crystallographic (1 0 0) plane. Water molecules are engaged into a network of hydrogen bonding (see Table 1). Most characteristic is a centrosymmetric motif R4,2(8) with four donors coming from two molecules of water and two acceptor oxygen atoms O3 from sulfonamide fragment and its equivalent related by the inversion center. Oxygen atom O5 which belongs to water is also an acceptor of two nitrogen bounded hydrogen atoms H2N and H3B. The oxygen atoms from terminal sulfonamide group S1-O2-O3 are acceptors of two hydrogen atoms from NH_2 group at N3, forming a cycle R2,2(6). Ring stacking interactions are not strong (PLATON program [24]) as the closest distance between centers of gravity is equal to 4.393(4) Å and is found between C1-C6 ring and its symmetry equivalent related by a glide plane (symmetry code: x,-1/2-y,-1/2+z). Trifluoromethyl substituted rings are located at both of inversion center at (½ ½ ½) with the centroid to centroid distance of 4.640(6)Å (Figure 4).

2.2. CA inhibition studies

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The compounds **9-41** as well as standard, clinically used CAIs, such as acetazolamide **AAZ**, methazolamide **MZA**, ethoxzolamide **EZA**, dichlorophenamide **DCP**, and indisulam **IND** (Fig. 1) have been tested for the inhibition of two cytosolic ubiquitous isozymes of human origin hCA I and hCA II, and transmembrane tumor-associated isoforms hCA IX and XII (Table 3). From the inhibition data reported in Table 3, the following points should be noted:

1. All compounds exhibited a weak inhibitory activity against hCA I with K_I values from 87 to 6506 nM. Among this group, R^1 = phenyl derivatives **9-14** stand out as the most active substances, with the K_I = 87 nM for **14**, possessing 2-sulfamoyl-1,3,4-thiadiazol-5-yl substituent at guanidine moiety. On the other hand, all substances are weaker inhibitors than reference compounds **AAZ** - **IND**.
2. Inhibitory activity toward hCA II depended on structural nature of substituent R^1. Thus, compounds containing at R^1 phenyl (**9-14**) or substituted phenyl (**15-26**) group showed moderate inhibitory efficacy (K_I in the range of 7.8-156 nM) comparable to clinically used CAIs. This efficacy decreases for compounds with expanded aromatic fragments at R^1 (**27-41**; K_I in the range of 249-4500 nM) and demonstrates the highest K_I values for *N*-[4-chloro-2-(6-chlorobenzo[*d*][1,3]dioxol-5-ylmethylthio)-5-methylbenzenesulfonyl]guanidines (**38-41**; K_I in the range of 2768-4500 nM).
3. Against the hCA IX isozyme, the newly synthesized compounds showed inhibitory activities with inhibition constants from 4.7 to 416 nM. Moreover, the most potent hCA IX inhibitor i.e., 1-(2-benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(2-sulfamoyl-1,3,4-thiadiazol-5-yl)guanidine (**14**) was 5-fold stronger than reference **IND** (K_I = 24 nM), the most effective hCA IX inhibitor. Furthermore, thirteen molecules possessing at R^1 phenyl (**10**, **12-14**), 3- or 4-substituted phenyl (**16**, **18-20** and **24-26**), and 1-naphthyl (**31** and **32**) groups exhibited a powerful inhibitory potency (K_I from 4.7 to 21 nM) in comparison to the reference sulfonamides **AAZ** – **IND** (K_I in the range of 24-50 nM). The presence in structure of 2-oxo-1,2-dihydroquinolin-4-yl or 4-chloro-2-(6-chlorobenzo[*d*][1,3]dioxol-5-yl groups at R^1 significantly reduced inhibitory activity, in particular for compounds **35-41** (K_I in the range of 197-416 nM).
4. A satisfying inhibition profile of the second tumor-associated isoform hCA XII, was also observed for R^1 = phenyl, 3-, 4-substituted phenyl, and naphthyl series of derivatives, that is: **9**, **10**, **12-14** (K_I: 0.96-19 nM), **15**, **16**, **18-20** (K_I: 6.4-68 nM), **21**, **22**, **24-26** (K_I: 8.1-40 nM), and **27**, **28**, **30-32** (K_I: 11-50 nM), respectively. For these compounds, changing of R^1 = Ph, 3-CF_3Ph, 4-CF_3Ph and 1-naphthyl into either 2-oxo-

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1,2-dihydroquinolin-4-yl (**33**, **34**, **36** and **37**) or 4-chloro-2-(6-chlorobenzo[*d*][1,3]dioxol-5-yl (**38**-**41**), caused considerable influence on decrease of inhibitory activity for each cases (see Table 3).

5. It should be noted that in each phenyl (R^1 = Ph), 3-trifluoromethylphenyl (R^1 = 3-CF_3Ph), 4-trifluoromethylphenyl (R^1 = 4-CF_3Ph), 1-naphthyl (R^1 = 1-naphthyl) series, compounds with 2-sulfamoyl-1,3,4-thiadiazol-5-yl group as substituent of guanidine moiety (**14**, **20**, **26** and **32**) demonstrated the highest inhibitory activity against hCA I, II, IX, and XII (see Table 3). However, in above-mentioned series as well as R^1 = 2-oxo-1,2-dihydroquinolin-4-yl derivatives, the presence of 2-sulfamoylphenyl substituent at guanidine fragment effected on decrease of inhibitory potency against all tested hCA isoforms (see Table 3).
6. All R^1 = 1-naphthyl derivatives were strongly selective towards transmembrane hCA IX, and XII versus cytosolic hCA I, and II. Compound **32** exerted the highest selectivity toward hCA IX versus hCA I (hCA I/hCA IX = 261) and hCA II (hCA II/hCA IX = 26) and represents the most promising inhibitor. This molecule was also much more potent on hCA XII than hCA I, and II (hCA I/hCA XII = 223, hCA II/hCA XII = 23). Significant selectivity ratios towards transmembrane isozymes presented also compd **31** (hCA I/hCA IX = 210, hCA II/hCA XII = 20) that was about 2-fold weaker hCA IX inhibitor than **32**.

2.3. Anticancer activity

Several representative compounds from each structural groups **10**, **13**, **14**, **21**, **22**, **25**, **32**, **38** and **41** were screened at the National Cancer Institute (NCI) *in vitro* tests in the full NCI-60 cell panel at a single dose 10 μM. The data was reported as mean-graph of the percent growth of the treated cells, and presented as inhibition growth percent (IGP) in Table 4.

As was shown in Table 4, twenty five cell lines from nine types of cancer exhibited significant sensitivity (IGP ≥ 50%) against some tested compounds. The most distinctive tumor cells belonged to: leukemia HL-60(TB) (toward **21**, **25**, **41**; 58% ≤ IGP ≤ 83%), non-small cell lung HOP-92 (**13**, **21**, **25**, **41**; 57% ≤ IGP ≤ 89%), colon KM12 (**21**, **25**; 50% ≤ IGP ≤ 89%), CNS cancer SNB-75 (**21**, **22**, **25**; 53% ≤ IGP ≤ 71%) and prostate PC-3 (**21**, **22**, **25**, **41**; 54% ≤ IGP ≤ 83%) cell lines. Among benzenesulfonylguanidines (R^1 = Ph), the most active compd **13** with 4-sulfamoylbenzyl substituent on guanidine moiety inhibited fourteen cell lines with IGP from 30% to 63%, whereas compd **10** containing 3-sulfamoylbenzyl

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substituent showed interesting inhibitory activity against cell lines of non-small cell lung HOP-92 (IGP = 46%), prostate PC-3 (IGP = 45%), CNS cancer SNB-75 (IGP = 35%), and leukemia MOLT-4 (IGP = 34%). In turn, **14** with 2-sulfamoyl-1,3,4-thiadiazol-5-yl group reduced of growth of CNS cancer SNB-75 cell lines to 18% as well as leukemia HL-60(TB) and MOLT-4 cell lines to 15% and 14%, respectively. However, benzenesulfonylguanidines (R^1 = 4-CF_3Ph) **21**, **22**, **25** were the most active antiproliferative agents showing high activity against almost all cancer cell lines presented in Table 4. As was given in Table 4, 1-[4-chloro-5-methyl-2-(naphthalen-1-ylmethylthio)benzenesulfonyl]-3-(2-sulfamoyl-1,3,4-thiadiazol-5-yl)guanidine (**32**) exhibited selectivity for the renal cancer A498 cells with IGP = 48%. It is worth to note, that *N*-[2-(6-chlorobenzo[*d*][1,3]dioxol-5-ylmethylthio)benzenesulfonyl]guanidine **41** (X = 4-sulfamoybenzyl) effectively inhibited growth of twenty one cell lines with IGP in the range of 30 - 62%, but its close analogue – **38** (X = 4-sulfamoyphenyl) exhibited lower inhibitory activity with IGP: 20 - 41% against ten cell lines.

Further anticancer evaluations was performed at 5-dose assay on distinctive compounds **21** and **25**. The anticancer activity was reported for each cell lines by GI_{50}, TGI, LC_{50} values and given in Table 5. Thus, compound **21** showed remarkable activity against 32 human tumor cell lines with GI_{50} values in the low micromolar range of 2.1 - 5.0 μM with selectivity toward leukemia HL-60(TB) (GI_{50} = 2.1 μM, TGI = 5.5 μM, LC_{50} = 34.5 μM) and SR (GI_{50} = 2.1 μM, TGI = 5.9 μM, LC_{50} > 100 μM). However, the **25** effectively acted against 35 cell lines in the range of 2.4 - 5.0 μM with selectivity toward leukemia HL-60(TB) (GI_{50} = 2.4 μM, TGI = 6.4 μM, LC_{50} = 54.4 μM) and SR (GI_{50} = 2.4 μM, TGI = 6.8 μM, LC_{50} > 100 μM).

3. Conclusions

We have developed methods for the synthesis of novel *N*-substituted *N*′-(2-arylmethylthio-4-chloro-5-methylbenzenesulfonyl)guanidine derivatives using *N*-(benzenesulfonyl)cyanamide potassium salt and amino-, or hydrazinylsulfonamide derivatives. All new guanidines containing primary sulfonamide group were tested for the inhibition of the physiological CA isoforms (CA I, and II), as well as membrane-bound and tumor-associated isoforms CA IX, and XII. Against the human CA I investigated compounds showed inhibition constant in the range of 87-6506 nM, while toward hCA II in the range of 7.8-4500 nM. Moreover, hCA IX was inhibited with K_I values from 4.7 to 416 nM, while

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second membrane-bound isoform hCA XII was inhibited in the range of 0.96-540 nM. Compounds **10**, **12-14**, **16**, **18-20**, **24-26**, **31** and **32** exhibited a powerful inhibitory potency toward hCA IX (K_I = 4.7-21 nM) in comparison to the reference sulfonamides **AAZ**, **MZA**, **EZA**, **DCP** and **IND** (K_I = 24-50 nM). Compound **32** exerted the highest selectivity ratios hCA IX versus hCA I equal on 261 and versus hCA II (i.e. hCA II/hCA IX = 26) and represents the most promising inhibitor in this series.

Interestingly, compounds **10**, **13**, **14**, **21**, **22**, **25**, **32**, **38** and **41** exhibited diversified antiproliferative activity against many types of tumor cells that was evaluated at the NCI *in vitro* tests. The most active antitumor agents **21** and **25**, inhibiting 32-35 human tumor cell lines with GI_{50} in the range of 2.1-5.0 μM also showed relatively high inhibitory activity toward transmembrane tumor-associated isoforms hCA IX and XII with K_I from 18-40 nM. In addition, compound **32** which presented high selectivity toward isozymes hCA IX and XII displayed significant inhibitory activity against A498 cell line of renal cancer (IGP = 48% at a dose 10 μM). Compounds **10**, **13**, **14** and **22** revealed low or moderate antiproliferative activities while their abilities to inhibition of hCA IX and XII remained at a high level (K_I: 0.96-39 nM). Summing up, we found that some of described compounds exert promising biological activity against both cancer cells and tumor-associated hCAs as compared to, for example, clinically tested U-104 [10].

4. Experimental protocols

4.1. Synthesis

The following instruments and parameters were used: melting points Boethius PHMK apparatus; IR spectra: KBr pellets, 400-4000 cm^{-1} Thermo Mattson Satellite FTIR spectrometer; ^1H NMR and ^{13}C NMR: Varian Gemini 200 apparatus or Varian Unity Plus 500 MHz; chemical shifts are expressed at δ values relative to Me_4Si as standard. The results of elemental analyses for C, H, and N were in agreement with the calculated values within ±0.4% range. Thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 F254 plates and visualized with UV. The commercially unavailable substrates were obtained according to the following methods described previously: *N*-(2-benzylthio-4-chloro-5-methylbenzenesulfonyl)cyanamide potassium salt (**3**) [21], *N*-[4-chloro-5-methyl-2-(3-trifluorobenzylthio)benzenesulfonyl]cyanamide potassium salt (**4**) [22], *N*-[4-chloro-5-methyl-2-(4-trifluorobenzylthio)benzenesulfonyl]cyanamide potassium salt (**5**) [22], *N*-[4-

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chloro-5-methyl-2-(naphthalen-1-ylmethylthio)benzenesulfonyl]cyanamide potassium salt (**6**) [22], *N*-[4-chloro-5-methyl-2-(2-oxo-1,2-dihydroquinolin-4-ylmethylthio)benzenesulfonyl]cyanamide potassium salt (**7**) [22], 2-amino-1,3,4-thiadiazole-5-sulfonamide [23].

*4.1.1. N-[4-Chloro-2-(6-chlorobenzo[d][1,3]dioxol-5-ylmethylthio)-5-methylbenzenesulfonyl] cyanamide potassium salt (**8**)*

The mixture of **2** (1.525 g, 4.5 mmol) and 5-chloro-6-(chloromethyl)benzo[*d*][1,3]dioxole (1.015 g, 4.95 mmol) in water (15 ml) was stirred at 0 °C for 4 h. The solid was filtered off and crystallized from ethanol, giving the title compound **8** (1.838 g, 87%): m.p. 220-222 °C; IR (KBr) 2921 (CH_3), 2177 (C≡N), 1343, 1140 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.25 (s, 2H, SCH_2), 6.05 (s, 2H, O-CH_2O), 7.11 (s, 1H, Ar), 7.12 (s, 1H, Ar), 7.34 (s, 1H, H-3), 7.76 (s, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6) δ 19.26, 34.58, 102.35, 109.78, 110.82, 117.48, 125.43, 126.99, 127.38, 130.87, 131.77, 135.41, 136.03, 140.94, 146.94, 147.86 ppm. Anal. ($C_{16}H_{11}Cl_2KN_2O_4S_2$) C, H, N.

*4.1.2. Procedure for the preparation of N,N'disubstituted guanidines (**9-41**)*

To a suspension of the appropriate *N*-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)cyanamide potassium salts (**3**-**8**) (0.7 mmol) in dry solvent (toluene, *p*-dioxane, acetonitrile) (8 ml) was added the corresponding amino-, or hydrazinylsulfonamide hydrochloride derivative (0.7 mmol) or aminosulfonamide derivative (0.7 mmol) in the presence of *p*-toluenesulfonic acid monohydrate (PTSA) (0.7 mmol). A reaction mixture was stirred at reflux for 2-28 h, and left overnight at 0 °C. The precipitate was filtered off, and dried, then treated with water (10 ml). After vigorously stirring for 30 minutes the precipitate was collected by filtration, dried and crystallized from ethanol (**9-28**, **30**, **33-41**), ethyl acetate (**29, 31**) or methanol (**32**). In this manner the following sulfonamides were obtained.

*4.1.2.1. 1-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(4-sulfamoylphenyl)guanidine (**9**).* Starting from **3** (0.274 g), 4-aminobenzenesulfonamide (0.121 g) and PTSA (0.133 g) in dry toluene for 3 h, the title compound **9** was obtained (0.205 g, 56%): m.p. 260-263 °C; IR (KBr) 3468, 3361, 3399 (NH, SO_2NH_2), 1631, 1512 (C=N, C=C), 1337, 1158 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.32 (s, 3H, CH_3), 4.33 (s, 2H, SCH_2), 7.05 (s, 2H, NH), 7.14-7.40 (m, 9H, arom. and SO_2NH_2), 7.50 (s, 1H, H-3), 7.60 (d, 2H, arom.), 7.92 (s, 1H, H-6), 9.39 (s, 1H,

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SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.22, 36.26, 118.06, 120.86, 124.51, 127.52, 127.63, 128.71, 129.25, 129.70, 132.01, 136.06, 136.43, 137.10, 138.59, 144.81, 154.60 ppm. Anal. ($C_{21}H_{21}ClN_4O_4S_3$) C, H, N.

4.1.2.2. *1-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(3-sulfamoylphenyl)guanidine (**10**).* Starting from **3** (0.274 g), 3-aminobenzenesulfonamide (0.121 g) and PTSA (0.133 g) in dry toluene for 3 h, the title compound **10** was obtained (0.298 g, 81%): 105-107 °C; IR (KBr) 3387, 3297, 3179 (NH, SO_2NH_2), 1633, 1525 (C=N, C=C), 1387, 1164, 1143 (SO_2); ^1H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 4.32 (s, 2H, SCH_2), 6.99 (s, 2H, NH), 7.20-7.28 (m, 3H, arom.), 7.30-7.42 (m, 5H, arom. and SO_2NH_2), 7.48 (s, 2H, H-3 and arom.), 7,64 (t, 1H, arom.), 7.84 (d, 1H, arom.), 7.90 (s, 1H, H-6), 9.39 (s, 1H, SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.22, 36.25, 118.06, 120.85, 124.50, 127.52, 127.63, 128.71, 129.25, 129.70, 130.85, 132.00, 136.06, 136.43, 137.10, 138.59, 138.79, 144.81, 154.60 ppm. Anal. ($C_{21}H_{21}ClN_4O_4S_3$) C, H, N.

*4.1.2.3. 1-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(2-sulfamoylphenyl)guanidine (**11**).* Starting from **3** (0.274 g) and 2-aminobenzenesulfonamide hydrochloride (0.121 g) in dry toluene for 4 h. After standing at refrigerator overnight the precipitate of inorganic salt was filtered out, washed with ethanol. Filtrate conataining product was evaporated under reduced pressure. After crystallization from 50% ethanol the title compound **11** was obtained (0.287 g, 78%): m.p. 100-102 °C; IR (KBr) 3427, 3335 (NH), 1630, 1515 (C=N, C=C), 1389, 1158 (SO_2); ^1H NMR (DMSO-d_6) δ 2.33 (s, 3H, CH_3), 4.39 (s, 2H, CH_2), 7.22-7.38 (m, 5H, NH and arom.), 7.40-7.48 (m, 3H, SO_2NH_2 and arom.), 7.52 (s, 1H, H-3), 7.64-7.76 (m, 3H, arom.), 7.78-7.84 (m, 2H, NH), 7.91 (s, 1H, H-6), 8.52 (s, 1H, $NHSO_2$) ppm; ^{13}C NMR (DMSO-d_6) δ 19.19, 36.36, 124.76, 127.23, 127.37, 127.61, 128.79, 129.40, 129.50, 130.98, 131.95, 132.33, 134.47, 135.21, 136.22, 136.36, 137.03, 138.67, 155.03 ppm. Anal. ($C_{21}H_{21}ClN_4O_4S_3$) C, H, N.

*4.1.2.4. 1-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(4-sulfamoylphenylamino)guanidine (**12**).* Starting from **3** (0.274 g) and 4-hydrazinylbenzenesulfonamide hydrochloride (0.157 g) in dry toluene for 17 h, the title compound **12** was obtained (0.151 g, 40%): m.p. 198-202 °C; IR (KBr) 3371, 3297, 3267 (NH), 1625, 1603, 1551 (C=N, NH_{def}, C=C), 1331, 1155, 1140 (SO_2); ^1H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 4.35 (s, 2H, SCH_2), 6.72 (d, J = 8.83 Hz, 2H, arom.), 7,04 (br s, 1H, NH),

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7.10 (s, 2H, SO_2NH_2), 7.20-7.40 (m, 5H, arom. and NH), 7.40 (s, 1H, arom.), 7.48 (s, 1H, H-3), 7.61 (d, J = 8.83 Hz, 2H, arom.), 7.89 (s, 1H, H-6), 8.44 (s, 1H, NH), 9.25 (s, 1H, SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.22, 36.49, 107.38, 111.87, 127.38, 127.58, 128.80, 129.40, 130.76, 131.97, 134.82, 136.00, 136.41, 136.71, 139.74, 151.06, 158.95 ppm. Anal. ($C_{21}H_{22}ClN_5O_4S_3$) C, H, N.

*4.1.2.5. 2-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(4-sulfamoylbenzyl)guanidine (**13**).* Starting from **3** (0.274 g) and 4-aminomethylbenzenesulfonamide hydrochloride (0.156 g) in dry toluene for 20 h, the title compound **13** was obtained (0.332 g, 88%): m.p. 209-211 °C; IR (KBr) 3442, 3351, 3257 (NH), 2924 (CH_3, CH_2), 1625 (C=N), 1416, 1170, 1137,2 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H, CH_3), 4.27 (s, 2H, SCH_2), 4.41 (d, 2H, $NHCH_2$), 6.80 (br s, 2H, NH_2), 7.20-7.40 (m, 10H, arom and SO_2NH_2), 7.45 (s, 1H, H-3), 7.77 (d, 2H, arom), 7.80 (s, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6) δ 19.21, 36.34, 43.66, 125.94, 127.33, 127.56, 127.68, 128.75, 129.33, 130.56, 131.75, 136.04, 136.47, 136.66, 139.50, 143.07, 143.17, 156.92 ppm. Anal. ($C_{22}H_{23}ClN_4O_4S_3$) C, H, N.

*4.1.2.6. 1-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(2-sulfamoyl-1,3,4-thiadiazol-5-yl)guanidine (**14**).* Starting from **3** (0.274 g), 5-amino-1,3,4-thiadiazole-2-sulfonamide (0.127 g) and PTSA (0.133 g) in dry *p*-dioxane for 3 h. The solvent was evaporated under redused pressure to dryness and residue was treated with water (20 ml). Further isolation according to the general procedure. The title compound **14** was obtained (0.257 g, 69%): 258-261 °C; IR (KBr) 3461, 3366, 3289, 3176 (NH), 1647, 1526 (C=N, C=C), 1340, 1171, 1135 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.33 (s, 3H, CH_3), 4.30 (s, 2H, SCH_2), 7.14-7.50 (m, 7H, arom. and NH), 7.58 (s, 1H, H-3), 7.97 (s, 1H, H-6), 8.30 (s, 2H, SO_2NH_2), 11.90 (s, 1H, SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.24, 36.30, 127.43, 128.45, 128.57, 128.98, 129.23, 131.04, 132.58, 135.89, 136.49, 137.81, 138.16, 162.30 ppm. Anal. ($C_{17}H_{17}ClN_6O_4S_4$) C, H, N.

*4.1.2.7. 1-[4-Chloro-5-methyl-2-(3-trifluoromethylbenzylthio)benzenesulfonyl]-3-(4-sulfamoylphenyl)guanidine (**15**).* Starting from **4** (0.321 g) and 4-aminobenzenesulfonamide (0.121 g), and PTSA (0.133 g) in dry toluene for 3 h, the title compound **15** was obtained (0.286 g, 69%): m.p. 244-245 °C; IR (KBr) 3436, 3334, 3248 (NH), 1623, 1512 (C=N, C=C), 1330, 1162, 1119 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 4.48 (s, 2H, SCH_2), 7.05 (s, 2H, NH), 7.24 (s, 2H, SO_2NH_2), 7.40-7.80 (m, 9H, arom.), 7.92 (s, 1H, H-6), 9.40 (s, 1H, SO_2NH) ppm. Anal. ($C_{22}H_{20}ClF_3N_4O_4S_3$) C, H, N.

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*4.1.2.8. 1-[4-Chloro-5-methyl-2-(3-trifluoromethylbenzylthio)benzenesulfonyl]-3-(3-sulfamoylphenyl)guanidine (**16**).* Starting from **4** (0.321 g) and 3-aminobenzenesulfonamide (0.121 g), and PTSA (0.133 g) in dry toluene for 3 h, the title compound **16** was obtained (0.212 g, 51%): m.p. 130-132 °C; IR (KBr) 3413, 3315 (NH), 2922 (CH_3, CH_2), 1634, 1517 (C=N , C=C) 1331, 1149 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 4.45 (s, 2H, SCH_2), 7.00 (s, 2H, NH), 7.32-7.40 (m, 3H, arom. and SO_2NH_2), 7.42-7.52 (m, 3H, H-3, arom.), 7.60 (t, 2H, arom.), 7.68 (t, 1H, arom.), 7.75 (s, 1H, arom.), 7.82 (d, 1H, arom.), 7.91 (s, 1H, H-6), 9.40 (s, 1H, SO_2NH) ppm. Anal. ($C_{22}H_{20}ClF_3N_4O_4S_3$) C, H, N.

*4.1.2.9. 1-[4-Chloro-5-methyl-2-(3-trifluoromethylbenzylthio)benzenesulfonyl]-3-(2-sulfamoylphenyl)guanidine (**17**).*Starting from **4** (0.321 g) and 2-aminobenzenesulfonamide hydrochloride (0.146 g) in dry toluene for 4 h, the title compound **17** was obtained (0.261 g, 63%): m.p. 100-101 °C; IR (KBr) 3526, 3439, 3345, 3264 (NH), 2958, 2922 (CH_3), 1629, 1516 (C=N, C=C), 1331, 1156.7, 1126 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.48 (s, 2H, CH_2), 7.19-7.26 (m, 4H, arom.), 7.50 (s, 1H, H-3), 7.53-7.66 (m, 6H, arom. and NH), 7.79 (s, 2H, arom. and NH), 7.89 (s, 1H, H-6), 8.56 (s, 1H, $NHSO_2$) ppm; ^{13}C NMR (DMSO-d_6) δ 19.18, 35.68, 124.27, 124.37, 124.76, 125.96, 126.03, 127.28, 127.57, 127.94, 129.88, 131.02, 132.22, 132.43, 133.46, 134.42, 135.22, 135.32, 136.99, 138.29, 139.12, 155.05 ppm. Anal. ($C_{22}H_{20}ClF_3N_4O_4S_3$) C, H, N.

*4.1.2.10. 1-[4-Chloro-5-methyl-2-(3-trifluoromethylbenzylthio)benzenesulfonyl]-3-(4-sulfamoylphenylamino)guanidine (**18**).*Starting from **4** (0.321 g) and 4-hydrazinylbenzenesulfonamide hydrochloride (0.121 g) in dry toluene for 16 h, the title compound **18** was obtained (0,328 g, 77%): m.p. 214-216 °C; IR (KBr) 3434, 3309, 3258 (NH), 1620, 1600, 1578 (C=N, NH_{def}, C=C), 1331, 1148, 1118 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 4.47 (s, 2H, SCH_2), 6.70 (d, J = 8.67 Hz, 2H, arom.), 7.10 (s, 3H, NH, SO_2NH_2), 7.46 (s, 1H, H-3), 7.50-7.64 (m, 5H, arom. and NH), 7.74 (s, 1H, arom.), 7.78 (s, 1H, arom.), 7.90 (s, 1H, H-6), 8.45 (s, 1H, NH), 9.25 (s, 1H, SO_2NH) ppm. Anal. ($C_{22}H_{21}ClF_3N_5O_4S_3$) C, H, N.

*4.1.2.11. 2-(4-Chloro-5-methyl-2-(3-trifluoromethylbenzylthio)benzenesulfonyl)-3-(4-sulfamoylbenzyl)guanidine (**19**).* Starting from **4** (0.321 g) and 4-aminomethylbenzenesulfonamide hydrochloride (0.156 g) in dry toluene for 16 h, the title

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compound **19** was obtained (0,323 g, 76%): m.p. 145-148 °C; IR (KBr) 3448 (NH), 1630, 1546 (C=N, C=C), 1398, 1160, 1129 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H, CH_3), 4.39 (s, 4H, SCH_2, $NHCH_2$), 6.08 (br s, 2H, NH_2), 7.20-7.40 (m, 5H, arom. and SO_2NH_2), 7.45 (s, 1H, H-3), 7.50-7.78 (m, 6H, arom.), 7.80 (s, 1H, H-6) ppm. Anal. ($C_{23}H_{22}ClF_3N_4O_4S_3$) C, H, N.

*4.1.2.12. 1-[4-Chloro-2-(3-trifluoromethylbenzylthio)-5-methylbenzenesulfonyl]-3-(2-sulfamoyl-1,3,4-thiadiazol-5-yl)guanidine (**20**).* Starting from **4** (0.321 g), 5-amino-1,3,4-thiadiazole-2-sulfonamide (0.127 g) and PTSA (0.133 g) in dry *p*-dioxane for 4 h. The solvent was evaporated under redused pressure to dryness and residue was treated with water (20 ml). Further isolation according to the general procedure gave the title compound **20** (0.269 g, 64%): 230-233 °C; IR (KBr) 3434, 3336 (NH), 2923, 2854 (CH_3, CH_2), 1650, 1519 (C=N, C=C), 1363, 1170, 1148 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H, CH_3), 4.43 (s, 2H, SCH_2), 7.28-7.62 (m, 6H, arom. and NH), 7.69 (s, 1H, H-3), 7.98 (s, 1H, H-6), 8.29 (s, 2H, SO_2NH_2), 11.90 (s, 1H, SO_2NH) ppm. Anal. ($C_{18}H_{16}ClF_3N_6O_4S_4$) C, H, N.

*4.1.2.13. 1-[4-Chloro-5-methyl-2-(4-trifluoromethylbenzylthio)benzenesulfonyl]-3-(4-sulfamoylphenyl)guanidine (**21**).* Starting from **5** (0.321 g) and 4-aminobenzenesulfonamide (0.121 g), and PTSA (0.133 g) in dry toluene for 16 h, the title compound **21** was obtained (0.307 g, 74%): m.p. 241-243 °C; IR (KBr) 3429, 3366, 3266 (NH), 2923 (CH_3, CH_2), 1619 (C=N), 1346, 1165, 1148 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 4.44 (s, 2H, SCH_2), 7.05 (s, 2H, NH), 7.24 (s, 2H, SO_2NH_2), 7.49 (s, 1H, H-3), 7.54 (d, 2H, J = 8.8 Hz, arom.), 7.58 (d, 2H, arom.), 7.60 (d, 2H, arom.), 7.68 (d, 2H, J = 8.8 Hz, arom.), 7.92 (s, 1H, H-6), 9.39 (s, 1H, SO_2NH) ppm. Anal. ($C_{22}H_{20}ClF_3N_4O_4S_3$) C, H, N.

*4.1.2.14. 1-[4-Chloro-5-methyl-2-(4-trifluoromethylbenzylthio)benzenesulfonyl]-3-(3-sulfamoylphenyl)guanidine (**22**).* Starting from **5** (0.321 g) and 3-aminobenzenesulfonamide (0.121 g), and PTSA (0.133 g) in dry toluene for 2 h, the title compound **22** was obtained (0.249 g, 60%): m.p. 188-191 °C; IR (KBr) 3420, 3316 (NH), 2924 (CH_3, CH_2), 1633, 1518 (C=N, C=C), 1384, 1359, 1161 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.33 (s, 3H, CH_3), 4.46 (s, 2H, SCH_3), 7.03 (s, 2H, NH), 7.34-7.42 (m, 3H, arom. and SO_2NH_2), 7.50 (s, 2H, H-3, arom.), 7.58 (d, 2H, arom.), 7.62 (d, 2H, arom.), 7.70 (t, 1H, arom.), 7.80 (d, 1H, arom.), 7.93 (s, 1H, H-6), 9.42 (s, 1H, SO_2NH) ppm. Anal. ($C_{22}H_{20}ClF_3N_4O_4S_3$) C, H, N.

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*4.1.2.15. 1-[4-Chloro-5-methyl-2-(4-trifluoromethylbenzylthio)benzenesulfonyl]-3-(2-sulfamoylphenyl)guanidine (**23**).* Starting from **5** (0.321 g) and 2- aminobenzenosulfonamide hydrochloride (0.146 g) in dry toluene for 4 h, the title compound **23** was obtained (0.208 g, 50%): m.p. 103-105 °C; IR (KBr) 3552, 3445, 3334 (NH), 1629, 1519 (C=N, C=C), 1447, 1161, 1125 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.46 (s, 2H, CH_2), 7.10-7.30 (m, 2H, arom.), 7.40-7.70 (m, 9H, arom., NH and SO_2NH_2), 7.75-7.85 (m, 2H, arom.), 7.87 (s, 1H, H-6), 8.54 (s, 1H, $NHSO_2$) ppm. Anal. ($C_{22}H_{20}ClF_3N_4O_4S_3$) C, H, N.

*4.1.2.16. 1-[4-Chloro-5-methyl-2-(4-trifluoromethylbenzylthio)benzenesulfonyl]-3-(4-sulfamoylphenylamino)guanidine (**24**).* Starting from **5** (0.321 g) and 4-hydrazinylbenzenosulfonamide hydrochloride (0.157 g) in dry toluene for 14 h, the title compound **24** was obtained (0.311 g, 73%): m.p. 210-212 °C; IR (KBr) 3369, 3301, 3268 (NH), 2923, 2852 (CH_3, CH_2), 1625, 1552 (C=N, C=N), 1330, 1156, 1140 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 4.47 (s, 2H, SCH_2), 6.73 (d, 2H, J = 8.83 Hz, arom.), 7.08 (s, 3H, NH, SO_2NH_2), 7.47 (s, 1H, H-3), 7.50 (br s, 1H, NH), 7.62 (d, 2H, J = 8.83 Hz, arom.), 7.68 (s, 4H, arom.), 7.89 (s, 1H, H-6), 8.45 (s, 1H, NH), 9.25 (s, 1H, SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.22, 35.82, 111.88, 125.50, 125.57, 125.65, 127.38, 128.09, 130.10, 130.80, 132.45, 134.87, 134.99, 136.70, 140.19, 141.77, 151.04, 158.97 ppm. Anal. ($C_{22}H_{21}ClF_3N_5O_4S_3$) C, H, N.

*4.1.2.17. 1-[4-Chloro-5-methyl-2-(4-trifluoromethylbenzylthio)benzenesulfonyl]-3-(4-sulfamoylbenzyl)guanidine (**25**).* Starting from **5** (0.321 g) and 4-aminomethylbenzenosulfonamide hydrochloride (0.156 g) in dry *p*-dioxane for 2 h, the title compound **25** was obtained (0.270 g, 65%): m.p. 214-217 °C; IR (KBr) 3448, 3393, 3351 (NH), 2924 (CH_3, CH_2), 1634, 1582 (C=N, C=C), 1325, 1159 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.41 (s, 2H, SCH_2), 4.44 (d, 2H, $NHCH_2$), 6.85 (br s, 2H, NH_2), 7.33-7.39 (m, 5H, SO_2NH_2 and arom.), 7.48 (s, 1H, H-3), 7.55-7.79 (m, 6H, NH and arom.), 7.83 (s, 1H, H-6) ppm. Anal. ($C_{23}H_{22}ClF_3N_4O_4S_3$) C, H, N.

*4.1.2.18. 1-[4-Chloro-5-methyl-2-(4-trifluoromethylbenzylthio)benzenesulfonyl]-3-(2-sulfamoyl-1,3,4-thiadiazol-5-yl)guanidine (**26**).* Starting from **5** (0.321 g), 5-amino-1,3,4-thiadiazole-2-sulfonamide (0.127 g), and PTSA (0.133 g) in dry *p*-dioxane for 11 h. The solvent was evaporated under redused pressure to dryness and residue was treated with water (20 ml). Further isolation according to the general procedure gave the title compound **26**

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(0.206 g, 49%): m.p. 242-245 °C; IR (KBr) 3429, 3316 (NH); 2998, 2854 (CH_3, CH_2) 1648, 1515 (C=N, C=C), 1325, 1163, 1132 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.40 (s, 2H, SCH_2), 7.40 (s, 1H, NH), 7.46 (s, 1H, NH), 7.50 (s, 1H, H-3), 7.54 (s, 2H, arom.), 7.58 (s, 2H, arom.), 8.00 (s, 1H, H-6), 8.31 (s, 2H, SO_2NH_2), 11.90 (s, 1H, SO_2NH) ppm. Anal. ($C_{18}H_{16}ClF_3N_6O_4S_4$) C, H, N.

*4.1.2.19. 1-[4-Chloro-5-methyl-2-(naphthalen-1-ylmethylthio)benzenesulfonyl]-3-(4-sulfamoylphenyl)guanidine (**27**).* Starting from **6** (0.309 g) and 4-aminobenzenesulfonamide (0.121 g), and PTSA (0.133 g) in dry toluene for 5 h, the title compound **27** was obtained (0.254 g, 63%): m.p. 235-237 °C; IR (KBr) 3429, 3366, 3266 (NH), 2923 (CH_3, CH_2), 1619 (C=N), 1346, 1165, 1148 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 4.81 (s, 2H, SCH_2), 7.00 (s, 2H, NH, NH=), 7.24 (s, 2H, SO_2NH_2), 7.41-7.58 (m, 7H, arom. and H-3), 7.62 (d, 2H, arom.), 7.86 (s, 1H, arom.), 7.92 (d, 1H, arom.), 7.96 (s, 1H, H-6), 8.42 (d, 1H, arom.), 9.31 (s, 1H, SO_2NH) ppm; ^{13}C NMR (DMSO-d_6) δ 19.28, 34.63, 120.47, 124.36, 125.82, 126.28, 126.58, 126.76, 128.19, 128.57, 128.81, 130.86, 131.62, 131.80, 132.27, 133.68, 136.44, 137.29, 138.68, 141.21, 154.31 ppm. Anal. ($C_{25}H_{23}ClN_4O_4S_3$) C, H, N.

*4.1.2.20. 1-[4-Chloro-5-methyl-2-(naphthalen-1-ylmethylthio)benzenesulfonyl]-3-(3-sulfamoylphenyl)guanidine (**28**).* Starting from **6** (0.309 g) and 4-aminobenzenesulfonamide (0.121 g), and PTSA (0.133 g) in dry toluene for 3 h, the title compound **28** was obtained (0.125 g, 31%): m.p. 140-143 °C; IR (KBr) 3445, 3350, 3273 (NH), 2922 (CH_3, CH_2), 1628, 1518 (C=N, C=C), 1346, 1149 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.80 (s, 2H, SCH_2), 6.92 (s, 2H, NH), 7.1 (t, 1H, arom.), 7.35 (s, 2H, SO_2NH_2), 7.38-7.45 (m, 3H, arom.), 7.52 (t, 2H, arom.), 7.45 (t, 1H, arom.), 7.60 (s, 1H, H-3), 7.72 (d, 1H, arom.), 7.86 (d, 1H, arom.), 7.92 (s, 1H, H-6), 7.94 (d, 1H, arom.), 8.18 (d, 1H, arom.), 9.30 (s, 1H, SO_2NH) ppm. Anal. ($C_{25}H_{23}ClN_4O_4S_3$) C, H, N.

*4.1.2.21. 1-[4-Chloro-5-methyl-2-(naphthalen-1-ylmethylthio)benzenesulfonyl]-3-(2-sulfamoylphenyl)guanidine (**29**).* Starting from **6** (0.309 g) and 2-aminobenzenesulfonamide (0.121 g), and PTSA (0.133 g) in dry toluene for 4 h, the title compound **29** was obtained (0.226 g, 56%): m.p. 122-124 °C; IR (KBr) 3543, 3436, 3339, 3261 (NH), 2926 (CH_3, CH_2), 1630 (C=N), 1340, 1150, 1125 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.82 (s, 2H, SCH_2), 6.96 (t, 1H, arom.), 7.16 (t, 1H, arom.), 7.46 (t, 1H, arom.), 7.50-7.54 (m, 2H, NH), 7.58 (d, 1H, arom.), 7.60 (s, 5H, arom., SO_2NH_2), 7.64 (s, 1H, H-3), 7.75 (d, 1H, arom.), 7.9

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(d, 1H, arom.), 7.92 (s, 1H, H-6), 7.95 (d, 1H, arom.), 8.25 (d, 1H, arom.), 8.44 (s, 1H, SO_2NH) ppm. Anal. ($C_{25}H_{23}ClN_4O_4S_3$) C, H, N.

*4.1.2.22. 1-[4-Chloro-5-methyl-2-(naphthalen-1-ylmethylthio)benzenesulfonyl]-3-(4-sulfamoylphenylamino)guanidine (**30**).* Starting from **6** (0.309 g) and 4-hydrazinylbenzenosulfonamide hydrochloride (0.157 g) in dry toluene for 20 h, the title compound **30** was obtained (0.273 g, 66%): m.p. 211-213 °C; IR (KBr) 3373, 3295 (NH), 2922, 2852 (CH_3, CH_2), 1622, 1548 (C=N, C=C), 1329, 1155, 1142 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 4.81 (s, 2H, SCH_2), 6.70 (d, 2H, J = 8.50 Hz, arom.), 7.00 (br s, 1H, NH), 7.10 (s, 2H, SO_2NH_2), 7.40-7.70 (m, 8H, arom. and NH), 7.80-8.00 (m, 3H, arom. and H-6), 8.25 (d, 1H, arom.), 8.40 (s, 1H, NH), 9.22 (s, 1H, SO_2NH) ppm. Anal. ($C_{25}H_{24}ClN_5O_4S_3$) C, H, N.

*4.1.2.23. 2-[4-Chloro-5-methyl-2-(naphthalen-1-ylmethylthio)benzenesulfonyl]-3-(4-sulfamoylbenzyl)guanidine (**31**).* Starting from **6** (0.309 g) and 4-aminomethylbenzenesulfonamide hydrochloride (0.156 g) in dry *p*-dioxane for 3 h. The solvent was evaporated under redused pressure to dryness and residue was treated with water (20 ml). Further isolation according to the general procedure gave the title compound **31** (0.338 g, 82%): m.p. 181-184 °C; IR (KBr) 3440, 3351, 3257 (NH), 2922 (CH_3, CH_2), 2853 (CH_3, CH_2), 1624, 1534 (C=N, C=C), 1398, 1160, 1137, (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.29 (s, 2H, SCH_2), 4.74 (s, 2H, $NHCH_2$), 6.72 (br s, 2H, NH_2), 7.20 (d, 2H, arom.), 7.30 (s, 3H, SO_2NH_2 and arom.), 7.44 (t, 1H, arom.), 7.50 (s, 3H, arom.), 7.59 (s, 1H, H-3), 7.7 (d, 2H, arom.), 7.83 (s, 1H, H-6), 7.85 (d, 1H, arom.), 7.95 (d, 1H, arom.), 8.18 (s, 1H, arom.) ppm. Anal. ($C_{26}H_{25}ClN_4O_4S_3$) C, H, N.

*4.1.2.24. 1-[4-Chloro-5-methyl-2-(naphthalen-1-ylmethylthio)benzenesulfonyl]-3-(2-sulfamoyl-1,3,4-thiadiazol-5-yl)guanidine (**32**).* Starting from **6** (0.309 g), 5-amino-1,3,4-thiadiazole-2-sulfonamide (0.146 g), and PTSA (0.133 g) in dry *p*-dioxane for 2 h. The solvent was evaporated under reduced pressure to dryness and residue was treated with water (20 ml). Further isolation according to the general procedure gave the title compound **32** (0.122 g, 30%): m.p. 223-225 °C; IR (KBr) 3455, 3354 (NH), 2924 (CH_3, CH_2), 1656, 1511 (C=N, C=C), 1384, 1359, 1161 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 4.76 (s, 2H, SCH_2), 7.24-7.54 (m, 6H, arom. and NH), 7.68 (s, 1H, H-3), 7.80 (d, 1H, arom.), 7.90 (d, 1H,

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arom.), 7.98 (s, 1H, H-6), 8.06 (d, 1H, arom.), 8.29 (s, 2H, SO_2NH_2), 11.85 (s, 1H, SO_2NH) ppm. Anal. ($C_{21}H_{19}ClN_6O_4S_4$) C, H, N.

*4.1.2.25. 1-[4-Chloro-5-methyl-2-(2-oxo-1,2-dihydroquinolin-4-ylmethylthio)benzenesulfonyl]-3-(4-sulfamoylphenyl)guanidine (**33**).* Starting from **7** (0.321 g) and 4-aminobenzenosulfonamide hydrochloride (0.146 g) in dry toluene for 7 h, the title compound **33** was obtained (0.282 g, 68%): m.p. 239-243 °C; IR (KBr) 3463, 3338 (NH), 1653 (C=O), 1635, 1516 (C=N, C=C), 1420, 1154 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H, CH_3), 4.65 (s, 2H, CH_2), 6.58 (s, 1H, arom.), 6.85-7.36 (m, 6H, arom. and NH), 7.51-7.80 (m, 6H, arom. and SO_2NH_2), 7.91-7.98 (m, 2H, H-6 and arom.), 9.38 (s, 1H, $NHSO_2$), 11.86 (s, 1H, NH quin) ppm; ^{13}C NMR (DMSO-d_6) δ 19.29, 33.22, 112.70, 116.00, 118.37, 120.65, 122.01, 122.11, 125.24, 126.72, 127.69, 128.43, 130.97, 132.82, 134.98, 137.29, 138.74, 139.15, 141.16, 146.23, 154.33, 161.69 ppm. Anal. ($C_{24}H_{22}ClN_5O_5S_3$) C, H, N.

*4.1.2.26. 1-[4-Chloro-5-methyl-2-(2-oxo-1,2-dihydroquinolin-4-ylmethylthio)benzenesulfonyl]-3-(3-sulfamoylphenyl)guanidine (**34**).* Starting from **7** (0.321 g) and 3-aminobenzenesulfonamide (0.121 g), and PTSA (0.133 g) in dry toluene for 11 h, the title compound **34** was obtained (0.278 g, 67%): m.p. 176-178 °C; IR (KBr) 3445, 3350, 3273 (NH), 2922 (CH_3, CH_2), 1628, 1518 (C=N, C=C), 1346, 1149 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.60 (s, 2H, SCH_2), 6.54 (s, 1H, arom.), 6.99 (s, 2H, NH), 7.15 (t, 1H, arom.), 7.24-7.38 (m, 4H, arom. and SO_2NH_2), 7.40-7.54 (m, 3H, H-3 and arom.), 7.62 (s, 1H, arom.), 7.72 (d, 1H, arom.), 7.81-7.98 (m, 2H, H-6, arom.), 9.38 (s, 1H, SO_2NH), 11.78 (s, 1H, NHCO) ppm. Anal. ($C_{24}H_{22}ClN_5O_5S_3$) C, H, N.

*4.1.2.27. 1-[4-Chloro-5-methyl-2-(2-oxo-1,2-dihydroquinolin-4-ylmethylthio)benzenesulfonyl]-3-(2-sulfamoylphenyl)guanidine (**35**).* Starting from **7** (0.321 g) and 2-aminobenzenesulfonamide (0.121 g), and PTSA (0.133 g) in dry toluene for 16 h, the title compound **35** was obtained (0.298 g, 72%): m.p. 207-210 °C; IR (KBr) 3426, 3378, 3285 (NH), 2920 (CH_3, CH_2), 1665 (C=O), 1519 (C=C), 1346, 1166, 1131 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.63 (s, 2H, SCH_2), 6.59 (s, 1H, arom.), 7.12-7.36 (m, 4H, arom. and NH), 7.40-7.66 (m, 7H, arom. and SO_2NH_2), 7.76 (t, 1H, arom.), 7.92 (s, 1H, H-6), 7.96 (s, 1H, arom.), 8.46 (s, 1H, SO_2NH), 11.77 (s, 1H, NHCO) ppm. Anal. ($C_{24}H_{22}ClN_5O_5S_3$) C, H, N.

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*4.1.2.28. 1-[4-Chloro-5-methyl-2-(2-oxo-1,2-dihydroquinolin-4-ylmethylthio)benzenesulfonyl]-3-(4-sulfamoylphenylamino)guanidine (**36**).* Starting from **7** (0.321 g) and 4-hydrazinylbenzenosulfonamide hydrochloride (0.157 g) in dry acetonitrile for 13 h, the title compound **36** was obtained (0.310 g, 73%): m.p. 237-241 °C; IR (KBr) 3437, 3319 (NH), 1655 (C=O), 1622 (C=N), 1418, 1187, 1150 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.65 (s, 2H, SCH_2), 6.70 (d, 3H, arom.), 7.07 (s, 3H, NH, SO_2NH_2), 7.24 (t, 1H, arom.), 7.34 (d, 1H, arom.), 7.48-7.56 (m, 3H, arom., H-3, NH), 7.62 (d, 2H, arom.), 7.90 (s, 1H, H-6), 7.98 (s, 1H, arom.), 8.45 (s, 1H, NH), 9.33 (s, 1H, SO_2NH), 11.83 (s, 1H, NHCO) ppm. Anal. ($C_{24}H_{23}ClN_6O_5S_3$) C, H, N.

*4.1.2.29. 1-[4-Chloro-5-methyl-2-(2-oxo-1,2-dihydroquinolin-4-ylmethylthio)benzenesulfonyl]-3-(4-sulfamoylbenzyl)guanidine (**37**).* Starting from **7** (0.321 g) and 4-aminomethylbenzenosulfonamide hydrochloride (0.156 g) in dry acetonitrile for 28 h, the title compound **37** was obtained (0.273 g, 66%): m.p. 188-190 °C; IR (KBr) 3436, 3321 (NH), 2923 (CH_3, CH_2) 1655 (C=O), 1569 (C=C), 1339, 1154, 1128 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.33 (s, 2H, SCH_2), 4.57 (s, 2H, $NHCH_2$), 6.60 (s, 1H, arom.), 6.80 (br s, 2H, NH_2), 7.20 (t, 1H, arom.), 7.23-7.41 (m, 6H, SO_2NH_2 and arom.), 7.50 (s, 2H, H-3 and arom.), 7.70 (d, 2H, arom.), 7.82 (s, 1H, H-6), 7.90 (d, 1H, arom.), 11.77 (s, 1H, NHCO) ppm. Anal. ($C_{25}H_{24}ClN_5O_5S_3$) C, H, N.

*4.1.2.30. 1-[4-Chloro-2-(6-chlorobenzo[d][1,3]dioxol-5-ylmethylthio)-5-methylbenzenesulfonyl]-3-(4-sulfamoylphenyl)guanidine (**38**).* Starting from **8** (0.328 g) and 4-aminobenzenesulfonamide (0.121 g), and PTSA (0.133 g) in dry toluene for 22 h, the title compound **38** was obtained (0.232 g, 55%): m.p. 229-232 °C; IR (KBr) 3411, 3312 (NH), 2919 (CH_3, CH_2), 1622, 1506 (C=N, C=C), 1347, 1163, 1141 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 4.29 (s, 2H, SCH_2), 6.03 (s, 2H, OCH_2), 7.00 (s, 1H, arom.), 7.02 (s, 2H, NH), 7.04 (s, 1H, arom.), 7.21 (s, 2H, SO_2NH_2), 7.48 (s, 1H, H-3), 7.51 (d, 2H, J = 8.93 Hz, arom.), 7.62 (d, 2H, J = 8.93 Hz, arom.), 7.94 (s, 1H, H-6), 9.36 (s, 1H, SO_2NH) ppm. Anal. ($C_{22}H_{20}Cl_2N_4O_6S_3$) C, H, N.

*4.1.2.31. 1-[4-Chloro-2-(6-chlorobenzo[d][1,3]dioxol-5-ylmethylthio)-5-methylbenzenesulfonyl]-3-(3-sulfamoylphenyl)guanidine (**39**).* The mixture of **8** (0.328 g) and 3-aminobenzenosulfonamide hydrochloride (0.146 g) in dry toluene was refluxed for 4 h. After reaction mixture was left at refrigerator overnight. Precipitate (A) was collected by

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filtration (filtrate was left to further work-up), dried, treated with water (5 ml) and stirred for 30 min at rt. In this manner was obtained 0.104 g of pure product. Filtrate A was evaporated under reduced pressure and residue was purified by crystallization from 80% ethanol, giving 0.148 g as a second fraction of product. The fractions was connected giving title compound **39** (0.329 g, 78%): m.p. 152-155 °C; IR (KBr) 3430, 3313 (NH), 2921 (CH_3), 1632, 1519 (C=N, C=C), 1341, 1150 (SO_2); ^{1}H NMR (DMSO-*d*$_6$) δ 2.35 (s, 3H, CH_3), 4.30 (s, 2H, CH_2), 6.05 (s, 2H, CH_2O), 7.00 (s, 3H, NH, arom.), 7.08 (s, 1H, arom.), 7.30-7.40 (m, 3H, SO_2NH_2, arom.), 7.47-7.51 (d, 2H, arom. and H-3), 7.60 (s, 1H, arom.), 7.80 (d, 1H, arom.), 7.94 (s, 1H, H-6), 9.40 (s, 1H, $NHSO_2$) ppm. Anal. ($C_{22}H_{20}Cl_2N_4O_6S_3$) C, H, N.

*4.1.2.32. 1-[4-Chloro-2-(6-chlorobenzo[d][1,3]dioxol-5-ylmethylthio)-5-methylbenzenesulfonyl]-3-(4-sulfamoylphenylamino)guanidine (**40**).* Starting from **8** (0.328 g) and 4-hydrazinylbenzenosulfonamide hydrochloride (0.157 g) in dry toluene for 5 h, the title compound **40** was obtained (0.312 g, 72%): m.p. 157-160 °C; IR (KBr) 3449, 3306 (NH), 2920 (CH_3, CH_2), 1629, 1600, 1563 (C=N, NH_{def}, C=C), 1335, 1156, 1111 (SO_2); ^{1}H NMR (DMSO-*d*$_6$) δ 2.34 (s, 3H, CH_3), 4.29 (s, 2H, SCH_2), 6.06 (s, 2H, OCH_2), 6.70 (d, 2H, *J* = 8.88 Hz, arom.), 7.05 (br s, 1H, NH), 7.08 (s, 3H, arom. and SO_2NH_2), 7.11 (s, 1H, arom.), 7.43 (s, 1H, H-3), 7.50 (s, 1H, NH), 7.60 (d, 2H, *J* = 8.88 Hz, arom.), 7.90 (s, 1H, H-6), 8.42 (s, 1H, NH), 9.25 (s, 1H, SO_2NH) ppm. Anal. ($C_{22}H_{21}Cl_2N_5O_6S_3$) C, H, N.

*4.1.2.33. 2-[4-Chloro-2-(6-chlorobenzo[d][1,3]dioxol-5-ylmethylthio)-5-methylbenzenesulfonyl]-3-(4-sulfamoylbenzyl)guanidine (**41**).* Starting from **8** (0.328 g) and 4-aminomethylbenzenesulfonamide hydrochloride (0.156 g) in dry toluene for 26 h, the title compound **41** was obtained (0.303 g, 70%): m.p. 146-150 °C; IR (KBr) 3446, 3351 (NH), 2924 (CH_3, CH_2), 1625, 1540 (C=N, C=C), 1341, 1163, 1130 (SO_2); ^{1}H NMR (DMSO-*d*$_6$) δ 2.30 (s, 3H, CH_3), 4.25 (s, 2H, SCH_2), 4.40 (s, 2H, $NHCH_2$), 6.06 (s, 2H, OCH_2), 6.80 (br s, 2H, NH_2). 7.04 (s, 1H, arom.), 7.10 (s, 1H, arom.), 7.32 (s, 3H, NH, SO_2NH_2), 7.35 (d, 2H, arom.), 7.42 (s, 1H, H-3), 7.75 (d, 2H, arom.), 7.80 (s, 1H, H-6) ppm. Anal. ($C_{23}H_{22}Cl_2N_4O_6S_3$) C, H, N.

4.2. X-ray structure determination

Experimental diffraction data were collected on a KM4 CCD kappa-geometry diffractometer (Oxford diffraction), equipped with a Sapphire2 CCD detector. An enhanced

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X-ray Mo Ka radiation source with a graphite monochromator was used. Determination of the unit cell and diffraction data collection were carried out at room temperature (298K). All calculations (data reduction, structure solution, and refinement) were carried out using CrysAlisPro package [26]. The structure was solved by direct methods, and all nonhydrogen atoms were refined with anisotropic thermal parameters by full-matrix least squares procedure based on F^2. Final refinements were carried out using the SHELX-97 package [27], run under control of WinGX program [28]. Scattering power of all the crystals tested was low, so in spite of long frame exposure time (240 s), the ratio of observed to unique reflections is only 40%.

Trifluoromethyl (CF_3) group was found disordered over two positions with occupancies of fluorine atoms of 0.65(4)/0.35(4). All hydrocarbon H atoms were refined using isotropic model with U_{iso} (H) values fixed to be 1.2 times U_{eq} of C atoms for CH and CH_2 and 1.5 times U_{eq} for CH_3. Bond lengths C–H were fixed at 0.98 Å for methyl groups, and 0.95 Å for methylene and methine groups. Hydrogen atoms attached to N1 and O5 were found in the Fourier map and refined as constrained to bond lengths 0.85 and 0.82 Å, respectively. Hydrogen atoms on N2 and N3 were refined as riding, fitted to aromatic NH or amide NH_2 group (Shelx AFIX 43 and 93). No residual electron density was found in vicinity of N4, which points to its deprotonation.

Crystallographic data for structure of **17** reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC940767. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: (+44) 1223-336-033; Email: deposit@ccdc.cam.ac.uk).

4.3.1. CA inhibition assay

An Applied Photophysics (Oxford, UK) stopped-flow instrument has been used for assaying the CA-catalyzed CO_2 hydration activity [25]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining constant the ionic strength), following 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

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the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (1 mM) were prepared in distilled-deionized water with 10-20% (v/v) DMSO (which is not inhibitory at these concentrations) and dilutions up to 0.1 nM were done thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of E-I complex. The inhibition constants were obtained by non-linear last-squares methods using PRISM 3, as reported earlier [29-31], and represent the mean from at least three different determinations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at

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Figure and scheme captions

Fig 1. Carbonic anhydrase IX inhibitors with anticancer *in vivo* activity.

Fig 2. Structures of benzenesulfonamides **A**, **B**, and **C**.

Fig 3. Molecular structure of **17**. Displacement ellipsoids drawn at 50% level, atoms of disordered CF_3 group shown as balls. Only selected hydrogen bonds drawn: intramolecular (N3–H3A...O3) and towards water molecule (O5–H5A...O3).

Fig 4. Packing and intermolecular interactions in **17**. Hydrogen bonds drawn as blue lines.

Scheme 1. Reagents and conditions: a) K_2CO_3 excess, THF, reflux, 24 h; b) R^1CH_2Cl (or Br), ethanol or water, rt or 0 °C, 1-4 h; c) X-NH_2, 4-$MePhSO_2OH$, toluene (or *p*-dioxane), reflux, 2-22 h; d) X-NH_3Cl, toluene (*p*-dioxane or acetonitrile), reflux, 2-28 h.

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