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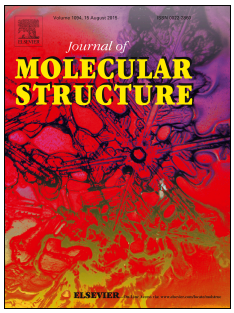

Synthesis, characterization, crystal structures and biological screening of 4-amino quinazoline sulfonamide derivatives

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Synthesis, characterization, crystal structures and biological screening of 4-amino quinazoline sulfonamide derivatives

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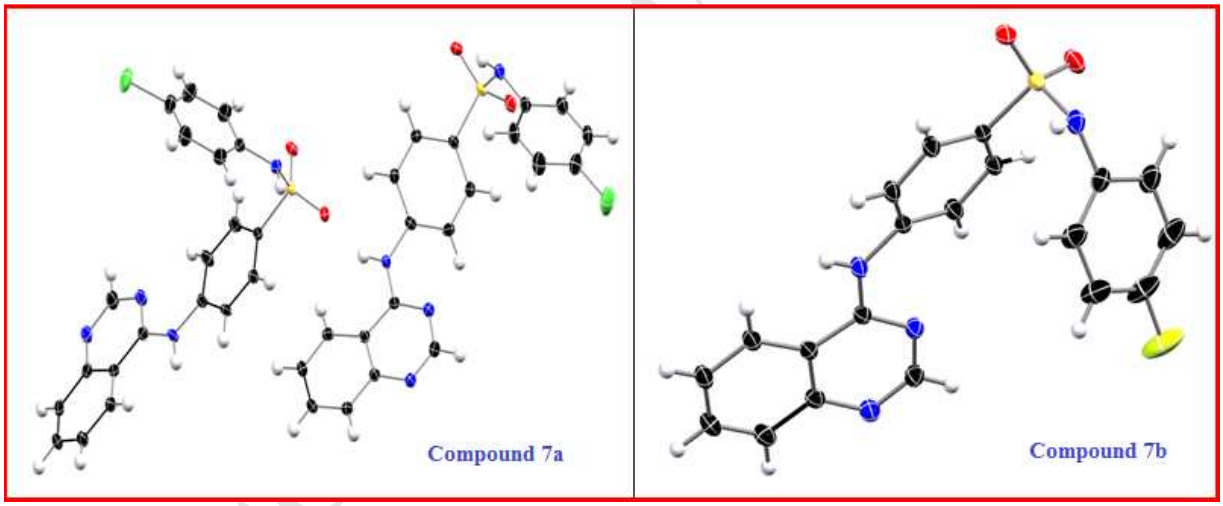
Graphical Abstract



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Abstract

Three quinazolin-4-ylamino derivatives containing phenylbenzenesulfonamides (**7a-7c**) were synthesized by reacting (*E*)-*N'*-(2-cyanophenyl)-*N*,*N*-dimethyl formamidine (**6**) with different 4-amino-*N*-(phenyl)benzenesulfonamides (**4a-4c**) and characterized by different techniques such as HRMS, IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The structural properties were further examined by single crystal X-ray diffraction method. The X-ray data shows that compounds **7a** and **7c** contain two molecules and **7b** contains one molecule in the asymmetric unit. Comparison of conformation of two distinct molecules, "A" and "B", in the asymmetric unit of **7a** and **7c** were studied with the aid of reported literature. The *in vitro* antiproliferative activity of the compounds was tested against two breast cancer cell lines (MDA-MB-231 and MCF7). Compound **7b** observed as a highest potent candidate against MDA-MB-231with IC_{50} of 5.44 µg/mL. Antimicrobial activity was also screened against bacterial and fungal strains. Compound **7a** with chloro substitution was observed as the most potent candidate against the Gram-negative bacterial strains, whereas the compounds showed no significant activity against the fungal strain.

Keywords: X-ray-diffraction, quinazoline-sulfonamide, crystal structure, antimicrobial, antiproliferative activity.

1. Introduction

The biological activity of a variety of quinazoline derivatives depends on the nature and the position of the substituent group in their framework. Out of the broadly identified substitution pattern, 4-aminoquinazolines and their *N*-anilino derivatives were found to be effective especially as anticancer agents [1, 2]. The literature reports also have explained the broad range biological potential of quinazolinone and quinazoline derivatives in antitubercular [3], antimicrobial [4, 5], antimalarial [6], anticonvulsant [7], antiviral [8], anti-inflammatory [9], antidiabetic [10] and many other biological activities. Therefore, the synthesis of 4-aminoquinazolines has attracted broad attention in recent years [11]. On the other hand, sulfonamides and their different derivatives are broadly used in medicine due to their pharmacological properties such as antibacterial activity [12, 13].

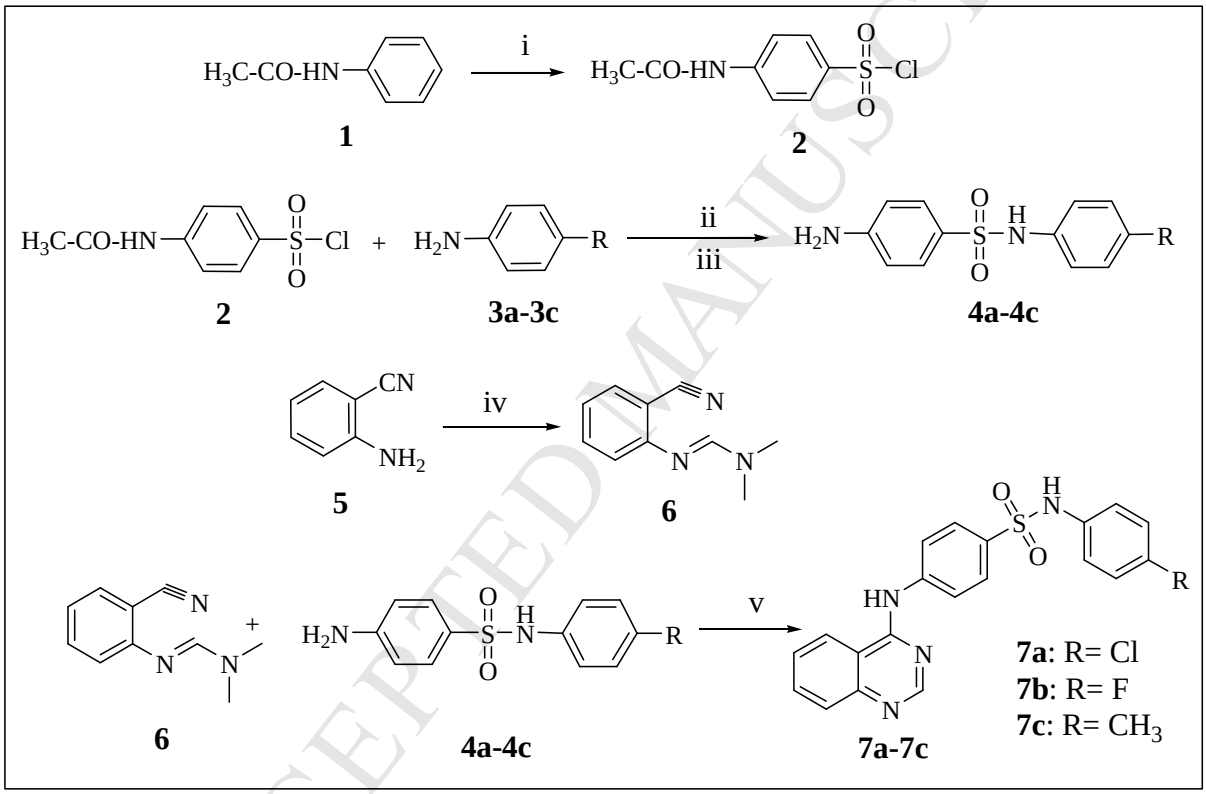
Sulfonamides act as inhibitors of folic acid synthesis in the living system which assists in the flourishing condition of bacteria [14]. The existence of donor and acceptor atoms in sulfonamide derivatives helps them to participate in the arrangement of different hydrogen bond networks. Earlier studies show that this property enables them to form different polymorphic [15, 16] as well as co-crystal structures [17, 18]. Therefore, the sulfonamide derivatives have more significance not only in the field of studying their biological application but also their crystal features as well. Our previous work explained the crystal structure, hydrogen bonding and molecular contacts of quinazoline scaffold containing oxazole and fluorophenyl sulfonamide derivatives [19, 20]. This work focused on the quinazoline scaffold containing phenyl sulfonamide derivatives. The synthesized derivatives were characterized by the HRMS, IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. The lattice parameters, bond angles, bond lengths, dihedral angles, torsion angles, hydrogen bonding (HB) and intermolecular interactions of the grown crystals were obtained by single crystal X-ray diffraction (XRD). Antiproliferative, antibacterial and antifungal activities were also screened to verify the potency of the compounds.

2. Experimental

2.1 Materials and Methods

The melting points (uncorrected) of targeted quinazoline sulfonamide derivatives were determined with DIGITAL MELTING POINT APPARATUS EQ 730 (EQUIPTRONICS) using an open capillary tube with the heating rate of 10 °C/min. HRMS (High-resolution mass spectra)

were recorded on an Agilent 6520 (QTOF) ESI-HRMS instrument. Infrared spectra were recorded in a Shimadzu FT-IR spectrometer using KBr pellets. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker Avance (AC 80) instrument in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. The chemical shift values (δ) were recorded in parts per million (ppm). All synthesis reactions were monitored by thin layer chromatography (TLC) using aluminium TLC plate, silica gel coated with fluorescent indicator F_{254}(Merck). The C, H, N and S elemental analysis was performed using Thermo Finnigan Elemental Micro Analyser.



Scheme 1. Synthesis of compounds **7a-7c**.

Reagents and conditions: i) $ClSO_3H/CHCl_3$, RT, 12 h; ii) Pyridine/$CHCl_3$, RT, 8 h; iii) HCl/Ethanol, 75-80 °C, 4 h; iv) DMF-DMA/DMF, 90 °C, 3-4 h; v) Acetic acid, 100 °C, 3-4 h.

2.1.1 Structural determination by single crystal X-ray diffraction studies

Single crystal structure data of **7a**, **7b** and **7c** were collected by Agilent/Rigaku SuperNova diffractometer, equipped with Eos detector, using multilayer optics monochromated Mo Kα (λ = 0.71073 Å) radiation and processed with CrysAlisPro(v. 1.171.38.43c) [21]. The structures were solved (direct methods) and refined within Olex^2(v. 1.2.10) [22] program package using

SHELXS [23] and SHELXL [24] programs. All non-hydrogen atoms were anisotropically refined. C–H hydrogen atoms were calculated to their ideal positions and refined using a riding model with U_{iso} parameters 1.2-1.5 times larger to their respective host atoms. O–H and N–H hydrogen atoms were located from the difference density map and refined without any restraints. The crystal lattices of structures of compounds **7a** and **7c** contain spherical voids that contribute to ca. 1 % of the unit cell volume (calculated using Mercury [25] contact surface with 1.2 Å probe radius and 0.7 Å grid spacing). These voids did not, however, show any residual electron density and thus were not considered to include any solvent molecules.

2.1.2 Antiproliferative studies

The compounds (**7a-7c**) were tested for their *in vitro* antiproliferative activity against two breast cancer cell lines MDA-MB-231 and MCF7 by MTT assay [26]. Compounds screened at seven different concentrations (1.0, 6.25, 12.5, 25, 50, 100 and 500 µg/mL) along with Cisplatin, used as a reference standard. The concentration required for 50 % inhibition of cell viability (IC_{50}) was calculated.

2.1.3 Antimicrobial studies

The compounds **7a-7c** were also screened for their *in vitro* antibacterial activity against two representative Gram-positive bacterial strains *Bacillus subtilis and Staphylococcus aureus*; two Gram-negative bacterial species *Escherichia coli* and *Pseudomonas aeruginosa* and a fungal strain *Aspergillus niger* by broth dilution method [27]. Ciprofloxacin and Fluconazole were used as reference drugs in terms of minimum inhibitory concentration (MIC).

2.2 Synthesis

The synthetic routes are shown in Scheme 1 and the molecules were synthesized based on available procedures.

2.2.1 Synthesis of 4-(acetylamino)benzene-1-sulfonyl chloride(2)from acetanilide (1)

Acetanilide (**1)** (10 g, 0.074 moles) was stirred in a minimum quantity of chloroform and cooled to 0 °C. Excess of chlorosulfonic acid (50 mL, 0.74 moles) was added to the cooled solution under stirring. The reaction temperature was maintained between 0-5 °C during the addition. After the completion of the reaction, mixture was slowly added to crushed ice to neutralize the excess of acid and the solid was collected by filtration and dried [28].

2.2.2 Synthesis of sulfonamide intermediates (4a-4c)

Synthesis of the precursor sulfonamides **4a**- **4c** was achieved by treating 0.01 mol of substituted aromatic amines (**3a-3c**) with 0.015 mol of 4-(acetylamino)benzene sulfonylchloride (**2**) in 0.03 mol of pyridine at room temperature (RT) for 8 h. Reaction progress was monitored by TLC (with hexane: EtOAc (1:1) mixture as eluent. Excess pyridine was neutralized with diluted HCl solution and the product was isolated by filtration. The hydrolysis of the above product was carried out by refluxing it with concentrated HCl in ethanol for 4 h. The clear solution was cooled and neutralized by ammonium hydroxide solution. The solid precipitated was filtered and dried at 50-55 °C (Scheme 1).

2.2.3 Synthesis of *N,N*-dimethylformamidine intermediate (6)

The *N,N*-dimethylformamidine intermediate (**6**) was obtained by heating 0.01 mol of 2-aminobenzonitrile (**5**) with 0.015 mol of dimethylformamide-dimethylacetal (DMF-DMA) at 90 °C in DMF solvent [29]. The product was precipitated by diluting with water and isolated by filtration.

2.2.4 Synthesis of 4-amino quinazoline derivatives (7a-7c)

The target quinazoline derivatives (**7a-7c**) were obtained by refluxing 0.01 mol of intermediate **6** with 0.011 mol of synthesized sulfonamides (**4a-4c**) in acetic acid. The products were isolated by filtration [19].

N-(4-Chlorophenyl)-4-[(quinazolin-4-yl)amino]benzene-1-sulfonamide **(7a)**

Yield 74 %; mp: 243-245 ^{o}C; IR (KBr, cm^{-1}): 3378 (N-H), 1621 (C=N), 1322 (SO_2 asym), 1150 (SO_2 sym); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 10.32 (s, 1H, SO_2N-H), 9.99 (s, 1H, N-H), 8.63 (s, 1H, Ar), 8.48-8.50 (d, J=8.4Hz, 1H, Ar), 8.05-8.07 (d, 2H, Ar), 7.81-7.84 (t, 1H, Ar), 7.76-7.78 (d, J=8.4Hz, 1H, Ar), 7.71-7.73 (d, 2H, Ar), 7.59-7.62 (t, 1H, Ar), 7.23-7.25 (d, 2H, Ar), 7.07-7.09 ppm (d, 2H, Ar); ^{13}C NMR (100 MHz, DMSO): δ = 157.3, 154.0, 149.8, 143.5, 136.8, 133.3, 132.7, 129.0, 127.9, 127.9, 127.5, 126.5, 123.0, 121.4, 121.2, 115.2 ppm; HRMS (ESI) m/z calcd: 411.0683 [M+H]$^{+}$, found 411.0681; Anal. calcd for $C_{20}H_{15}N_4SO_2Cl$ (%): C 58.46; H 3.68; N 13.64; S 7.80, found: C 58.38; H 3.58; N 13.69; S 7.74.

***N*-(4-Fluorophenyl)-4-[(quinazolin-4-yl)amino]benzene-1-sulfonamide (7b)**

Yield 94 %; mp: 276-278 ^{o}C; IR (KBr, cm^{-1}): 3386 (N-H), 1622 (C=N), 1330 (SO_2 asym), 1153 (SO_2 sym); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 10.45 (s, 1H, SO_2N-H), 10.06 (s, 1H, N-H), 8.69 (s, 1H, Ar), 8.55-8.57 (d, 1H, Ar), 8.13-8.15 (d, 2H, Ar), 7.87-7.91 (t, 1H, Ar), 7.82-7.84 (d, 1H, J= 8 Hz, Ar), 7.77-7.79 (d, 2H, Ar), 7.65-7.67 (t, 1H, Ar), 7.29-7.31 (d,1H, Ar), 7.24-7.27 (t, 1H, Ar), 7.09-7.13 ppm (m,1H, Ar); ^{13}C NMR (100 MHz, DMSO): δ = 157.2, 153.9, 152.4, 149.6, 144.2, 137.0, 133.3, 129.2, 128.2, 127.9, 126.5, 121.2, 121.0, 119.2, 116.4, 115.7 ppm; HRMS (ESI) m/z calcd: 395.0972 [M+H]$^{+}$, found 395.0968; Anal. calcd for $C_{20}H_{15}N_4SO_2F$ (%): C 60.90, H 3.83, N 14.20, S 8.13, found: C 60.78, H 3.72, N 14.13, S 8.18.

***N-(4-Methylphenyl)-4-[(quinazolin-4-yl)amino]benzene-1-sulfonamide* (7c)**

Yield 91 %; mp: 243-245 ^{o}C; IR (KBr, cm^{-1}): 3361 (NH), 1624 (C=N), 1313 (SO_2 asym), 1150 (SO_2 sym); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 10.07 (s, 1H, SO_2N-H), 10.02 (s, 1H, N-H), 8.68 (s. 1H, Ar), 8.54-8.56 (d, 1H, Ar), 8.09-8.11 (d, 2H, Ar), 7.81-7.87 (m, 2H, Ar), 7.76-7.78 (d, 2H, Ar), 7.64-7.67 (t, 1H, Ar), 7.03 (s, 4H, Ar), 2.17 ppm (s, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO): δ = 157.3, 154.0, 149.7, 143.2, 135.2, 133.2, 133.2, 133.1, 129.5, 127.8, 126.5, 122.99, 121.1, 120.4, 115.2, 20.25 ppm; HRMS (ESI) m/z calcd: 391.1223 [M+H]$^{+}$, found 391.1228; Anal. calcd for $C_{21}H_{18}N_4SO_2$ (%): C 64.60, H 4.65, N 14.35, S 8.21, found: C 64.64, H 4.58, N 14.42, S 8.10.

3 Results and discussion

3.1 Synthesis

In the present work, we have synthesized three *N*-(quinazolin-4-yl)sulfonamide derivatives by condensing different aryl sulfonamides with (*E*)-*N'*-(2-cyanophenyl)-*N*,*N*-dimethylformamidine. It is assumed that the synthesized aromatic amine first attacks on the carbon of the *N*,*N*-dimethylamine following an ejection of *N*,*N*-dimethylamine [29]. The intermediate aromatic amidine then undergoes cyclization into a quinazoline frame in which the endocyclic and exocyclic nitrogen atoms interchange their place *via* Dimroth rearrangement to obtain the expected 4-anilinoquinazoline (Scheme 1). The attack of an amine into the cyano group is impossible in practice without a probable catalytic action of an acid [30].

3.2 Spectral analysis

The primary characterization of the compounds included measuring of melting point, elemental analysis and mass spectra. The HRMS results have displayed (M+1) peak as a major peak. The molecular composition of the compounds was assigned on the basis of IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The IR spectra of final compounds **7a**, **7b** and **7c** show absorption bands with medium to strong intensities as follows: the range of 3369-3386 cm^{-1} is assigned for N-H stretching frequency and high intense bands in the ranges 1313-1330 and 1150-1153 cm^{-1} for asymmetric and symmetric stretching modes of O=S=O group (Fig. 1 and Fig. S2).

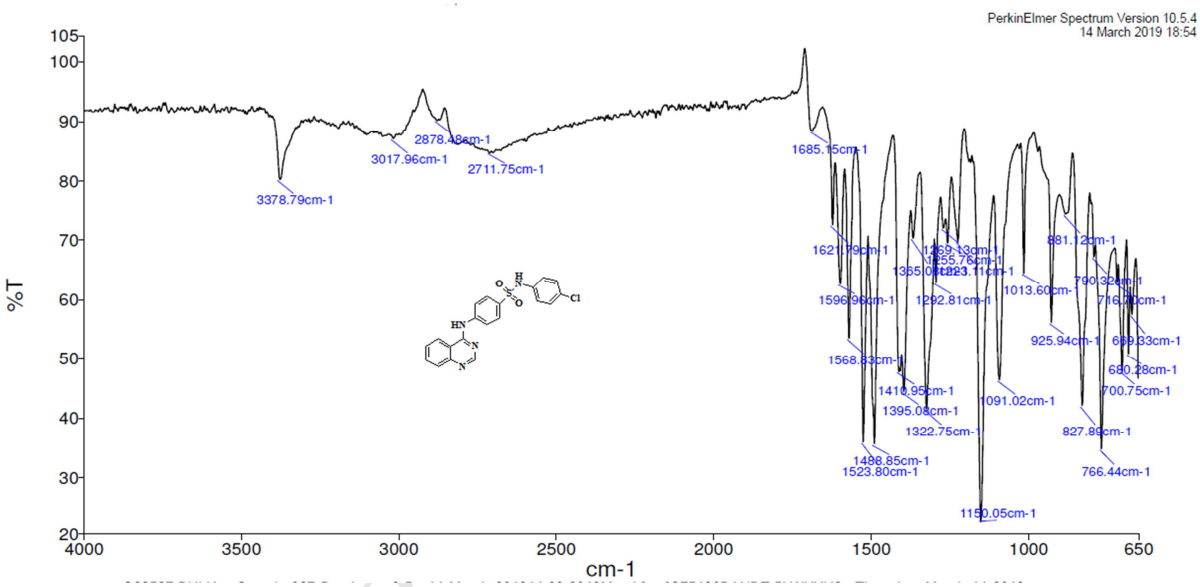


Fig. 1. IR spectrum of *N-(4-Chlorophenyl)-4-[(quinazolin-4-yl)amino]benzene-1-sulfonamide* (**7a**)

The quinazoline ring protons are observed at the downfield region and among that, pyrimidine proton (H1) manifest the highest chemical shift value among the other aromatic protons (δ 8.63-8.69 ppm) (Fig. 2). The aromatic protons *ortho* to the NH group are observed in the upfield region. This shielding effect is due to the electron cloud around the *ortho* protons produced by the electron-rich amino group. The aromatic protons *ortho* to the SO_2 group were observed at more deshielding region due to the electron withdrawing nature of sulfonyl group. The two N-H protons (H_a & H_b) can be seen as two broad peaks in the downfield region. Since the sulfonamide NH (H_b) protons are more acidic compared to phenyl NH (H_a) protons and thereby

can be observed in the most deshielded region [31]. The characteristic peaks in the ^{13}C NMR spectra also support the conformation of the synthesized compounds. The carbon atoms bonded to the functional groups and heteroatoms have shown a slight variation in the chemical shift values. The ^{13}C NMR spectra of carbon atoms, which are directly bonded to the nitrogen atom, show up in the more deshielded region compared to other phenyl carbon atoms. C1 and C8 carbons in the diazine ring (ring b) bonded to two nitrogen atoms emerge in the most deshielded region154 and 157 ppm, respectively, due to the less electron charge density around these atoms. The chemical shift value for the carbon bonded to the electronegative fluorine atom, in compound **7b**, is observed at 152.44 ppm.

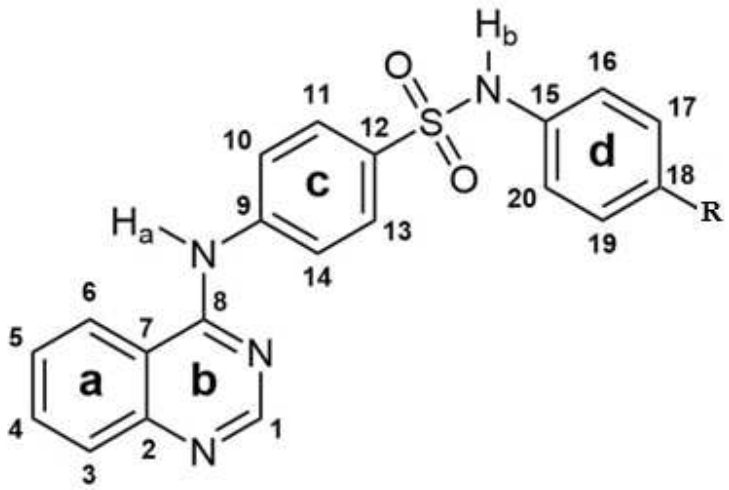


Fig. 2. Atom numbering of core unit to assign the ^{1}H and ^{13}C NMR values of compounds **7a**, **7b** and **7c**.

3.3 Single crystal XRD studies

The solid-state structures of compounds **7a-7c** were determined by single crystal X-ray diffraction (see Table 1 for crystallographic data). Single crystals of all three compounds were obtained by dissolving each of the highly pure compound (100 mg) in 15-20 mL of acetone at 35-40 ºC. The clear solution was allowed for crystal growth by slow evaporation of the solvent without any disruption.

Table 1. Crystallographic structure and refinement data for compounds **7a-7c**.

Parameters	**7a**	**7b**	**7c**
CCDC No.	1857204	1563909	1857205
Empirical formula	$C_{20}H_{15}ClN_4O_2S$	$C_{20}H_{15}FN_4O_2S$	$C_{21}H_{18}N_4O_2S$
Formula weight	410.87	394.42	390.45
Temperature/K	120.00(10)	293.00(2)	120.00(10)
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/c$	$P2_1/n$
a/Å	16.5427(3)	8.2860(4)	16.6600(4)
b/Å	13.1893(2)	13.6797(4)	13.3798(2)
c/Å	18.1771(3)	16.7470(8)	18.1035(4)
α/°	90	90	90
β/°	108.0859(18)	99.669(5)	107.919(2)
γ/°	90	90	90
Volume/Å^3	3770.05(11)	1871.30(14)	3839.67(15)
Z	8	4	8
ρ_{calc}g/cm^3	1.448	1.4	1.351
μ/mm^{-1}	0.338	0.207	0.193
F(000)	1696	816	1632
Crystal size/mm^3	0.30 × 0.30 × 0.20	0.3 × 0.24 × 0.23	0.35 × 0.30 × 0.30
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection/°	4.006 to 51.998	4.934 to 51.994	3.854 to 52.000
Index ranges	$-18 \le h \le 20$, $-15 \le k \le 16$, $-18 \le l \le 22$	$-10 \le h \le 10$, $-16 \le k \le 16$, $-20 \le l \le 20$	$-20 \le h \le 19$, $-15 \le k \le 16$, $-16 \le l \le 22$
Reflections collected	13608	20021	13976
Independent reflections	7360 [R_{int} = 0.0285, R_{sigma} = 0.0515]	3667 [R_{int} = 0.0321, R_{sigma} = 0.0208]	7534 [R_{int} = 0.0283, R_{sigma} = 0.0555]
Data/restraints/parameters	7360/0/521	3667/0/261	7534/0/523
Goodness-of-fit on F^2	1.032	1.058	1.030
Final R indexes [I>=2σ (I)]	R_1 = 0.0413, wR_2 = 0.0915	R_1 = 0.0425, wR_2 = 0.1081	R_1 = 0.0448, wR_2 = 0.0994
Final R indexes [all data]	R_1 = 0.0544, wR_2 = 0.1002	R_1 = 0.0505, wR_2 = 0.1137	R_1 = 0.0609, wR_2 = 0.1102
Largest diff. peak/hole / e Å^{-3}	0.30/-0.52	0.19/-0.25	0.39/-0.45

Compound **7a** crystallizes in a monoclinic space group $P2_1/n$ with two molecules, “A” and “B”, in the asymmetric unit and eight in the unit cell altogether (Fig. 2). The orientation of the

sulfonamide functionality in both A and B molecules is consistent with the observation that the staggered geometry is preferred in the solid state structures of unhindered sulfonamides [32] although the eclipsed conformer has been addressed as the global energy minimum in the gas phase [33]. Molecules A and B both show the twisting of the 4-chlorophenyl group (ring d in Fig. 2) in a way that the N–H hydrogens of the sulfonamide and the anilinoquinazole face toward the same side of the central aryl ring (ring c in Fig. 2). However, in molecule B the 4-chlorophenyl group shows a higher degree of rotation away from the arylsulfonamide group (See ESI Fig. S8). The quinazoline moieties in each of the unique molecules in the asymmetric unit show major similarities but also small differences regarding their orientation in respect to the aryl sulfonamide backbone. In both distinct molecules the quinazoline unit shows *anti* orientation in respect to the C(6)–H atom and the aniline moiety. This orientation seems to be typical for anilinoquinazoles with secondary amine as the linker as it is most likely sterically favored. On the other hand, the quinazoline moiety in molecule A is nearly coplanar (7.5 ° tilt angle) with the backbone whereas in B it is tilted away from the plane by ~34°. Comparison of the relevant geometrical parameters of the sulfonamide environment indicates that they are consistent with several other sulfonamides and sulfanilamides reported e.g. by Lahtinen *et al.*, Pervolich *et al.* and Parkin *et al.* [34-36]. Similar molecular conformations can be exemplified for instance by the crystal structures of *N*-[4-(phenylsulfamoyl)phenyl]benzamide [34], 3-(((4-acetamidophenyl)sulfonyl)amino)benzoic acid [37] and 4-amino-*N*-(3-chloro-4-methylphenyl) benzenesulfonamide [38]; dihedral angles shown in ESI Table S1.

The solid-state structures of secondary sulfonamides have the tendency of showing an abundance of intermolecular hydrogen bonds (HB) [35, 38-39]. Indeed, the most noticeable intermolecular interactions in **7a** are the hydrogen bonds between the amino group of the 4-anilinoquinazoline and sulfonamide S=O and the sulfonamide N–H and quinazoline N groups (see hydrogen bonding parameters in Table 2). These constitute several different hydrogen bonding networks with two examples illustrated in Fig 2. Additional significant intermolecular contacts include two distinct π-π dimers which are formed between the aromatic quinazoline groups with respective inter-quinazole distances of 3.54 and 3.57 Å (see ESI Fig. S8).

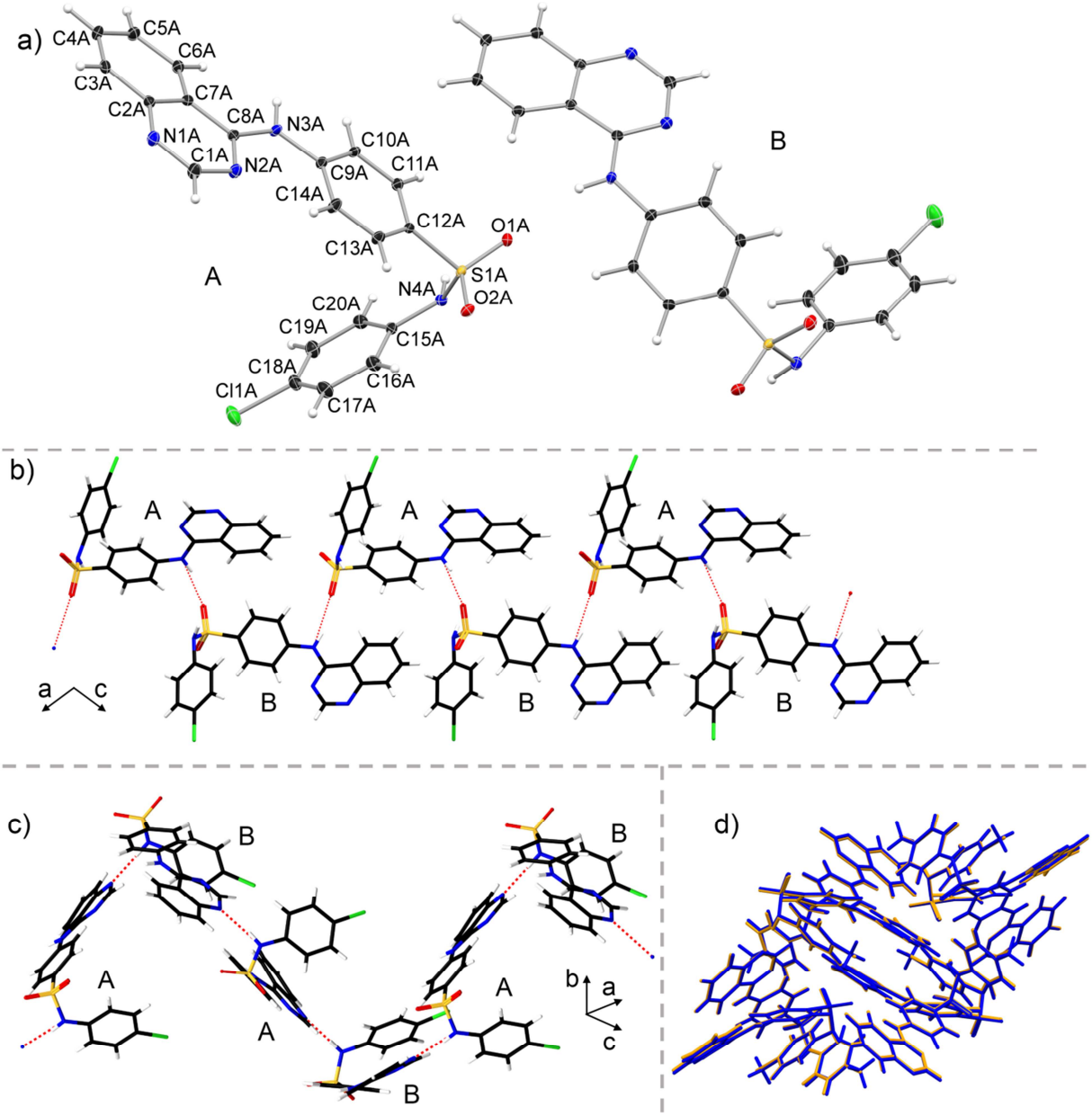


Fig. 3. a) Illustration of the asymmetric unit of structure of **7a** with distinct molecules labelled "A" and "B" (ellipsoids presented at 30 % probability level). Atomic labels are shown for molecule A. b), and c) Examples of the different intermolecular N–H···O=Sand N–H···N hydrogen bond networks formed in the crystal lattice of **7a**. d) Overlay of crystal lattices (view along *a*-axis) of the structure of **7a** (orange) and **7c** (blue).

Interestingly, the solid state structure of the methyl derivative **7c** is isostructural with **7a** and shows nearly identical crystallographic unit cell parameters and molecular packing (Table 1,Table 2, Fig. 3d). The isostructurality is most likely due to the similar sizes of the CH_3- and Cl-

groups and the lack of eagerness of these groups to engage in any significant attractive intermolecular interactions. Since the structural interpretation made for the structure **7a** is similarly valid for **7c**, no further analysis is presented for **7c**. The crystal structure of the fluorine derivative **7b** was solved and refined in space group $P2_1/c$ with one molecule in the asymmetric unit and four in the unit cell. The molecular geometry of **7b** resembles that of the "B" molecules in the structures of **7a** and **7c** and there by shows conventional dihedral angles with analogous sulfonamides, as discussed above. Since only one conformer is observed in **7b**, the molecular packing in the lattice is different to **7a** and **7c**. This is manifested for example by the formation of linear chains of molecules in the direction of the crystallographic *c*-axis by N–H···N hydrogen bonds (Fig. 4b). The N–H···O=S hydrogen bonding patern seems similar to **7a**/**7c** when viewed perpendicular to the plane formed by the HB network (Fig. 4c). View along the 1D HB chain, however, shows that with a single conformer in case of **7b** a network with two alternating molecules is formed, whereas in **7a** and **7c** a four-fold helical HB-network is observed (Fig. 4d). The quinazoline π-π dimer is found also in **7b**, but it shows slipped geometry with fairly short quinazoline inter-planar distance of 3.25 Å.

Table 2. Hydrogen bond parameters derived from the solid state structure of **7a**-**7c**.

			7a			
D	H	A	*d*(D–H)/Å	*d*(H–A)/Å	*d*(D–A)/Å	D–H–A/°
N3A	H3A	O1B[1]	0.88(2)	2.28(2)	3.143(2)	164(2)
N4A	H4A	N1B[2]	0.85(2)	2.07(2)	2.912(2)	172(2)
N3B	H3B	O1A	0.85(2)	2.21(2)	3.027(2)	162(2)
N4B	H4B	N1A[3]	0.91(3)	2.00(3)	2.907(3)	178(3)
			7b			
D	H	A	*d*(D–H)/Å	*d*(H–A)/Å	*d*(D–A)/Å	D–H–A/°
N3	H3	O1[4]	0.81(2)	2.23(2)	2.993(2)	156(2)
N4	H4	N1[5]	0.81(2)	2.13(2)	2.927(2)	168.3(19)
			7c			
D	H	A	*d*(D–H)/Å	*d*(H–A)/Å	*d*(D–A)/Å	D–H–A/°
N3A	H3A	O1B[1]	0.86(2)	2.32(2)	3.151(2)	162(2)
N4A	H4A	N1B[2]	0.89(2)	2.07(2)	2.944(3)	169(2)
N3B	H3B	O1A	0.82(2)	2.24(2)	3.032(2)	163(2)
N4B	H4B	N1A[3]	0.87(3)	2.06(3)	2.926(3)	173(2)

[1]-1/2+X,1/2-Y,1/2+Z; [2]1-X,1-Y,1-Z; [3]1/2-X,1/2+Y,1/2-Z, [4]2-X,-1/2+Y,1/2-Z; [5]+X,1/2-Y,-1/2+Z

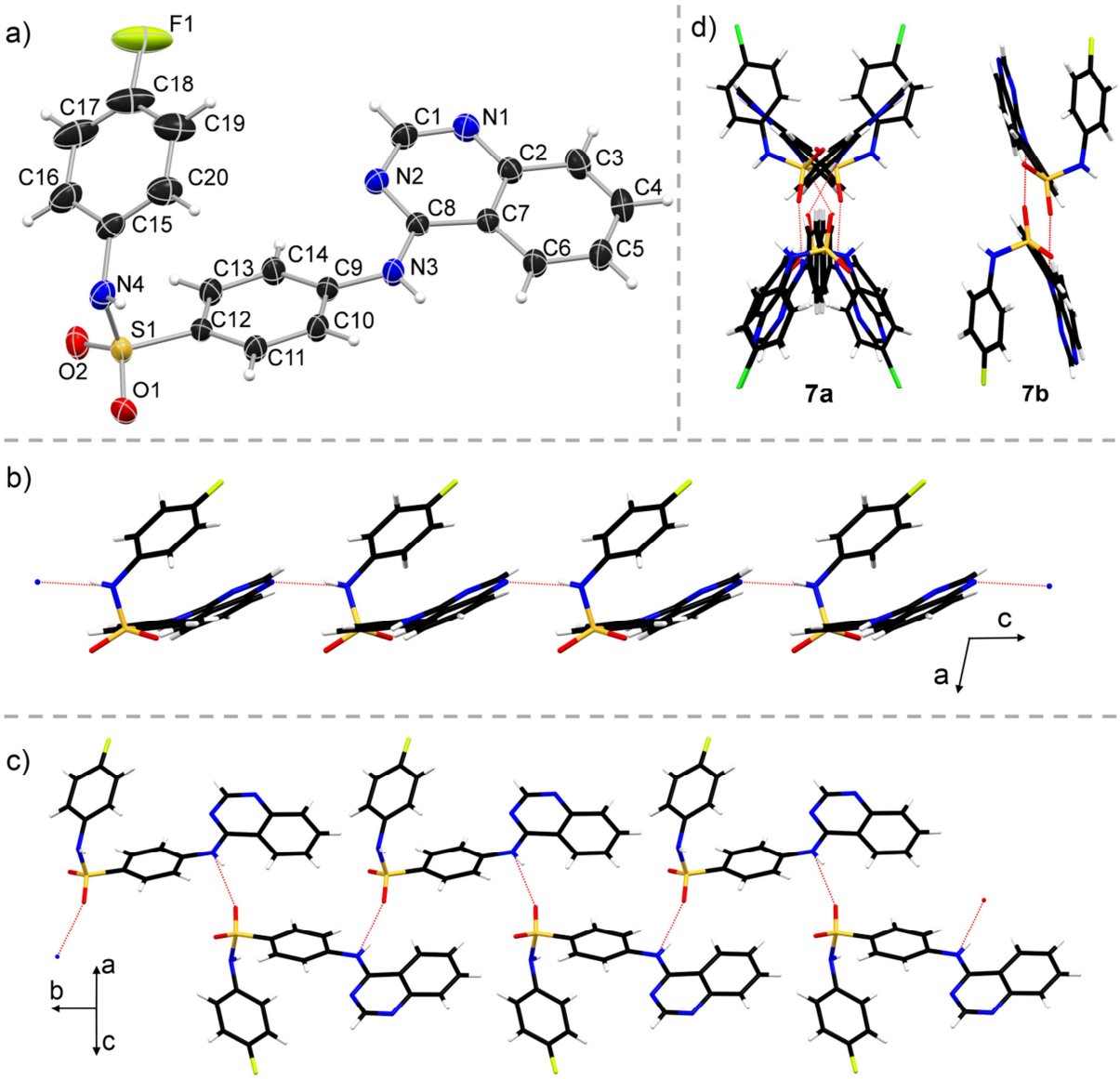


Fig. 4. a) Illustration of the asymmetric unit of structure of **7b**with atomic labels shown (ellipsoids presented at 30 % probability level). b) and c) Examples of the different intermolecular N–H···N and N–H···O=S hydrogen bondnetworks found in the crystal lattice of **7b**. d) Comparison between the H···O=S hydrogen bond networks observed in **7a** and **7b**.

3.4 Biological evaluation

3.4.1 Antiproliferative activity

The synthesized compounds (**7a-7c**) were tested for their *in vitro* antiproliferative activity against two cancer cell lines (MDA-MB-231 and MCF7) and the results were summarized in Table 3. The tested compounds exhibited a remarkable cytotoxicity against MDA-MB-231 cell line.

Among these compounds, electronegative fluoro substituted compound **7b** has shown the most potent activity with IC_{50} of 5.44 µg/mL and the activity observed was more than the standard drug Cisplatin (IC_{50}= 5.61µg/mL). Chloro (**7a**) and methyl (**7c**) derivatives exhibited moderate inhibitory activities with IC_{50} of 17.83 and 19.56 µg/mL respectively. The tested compounds have not shown any significant cytotoxicity against MCF7 cell line even at higher concentrations (IC_{50}> 500 µg/mL). The structural evaluation of similar compounds from the literature revealed that the fluoro substituted quinazoline derivatives have been reported to possess an excellent range of antiproliferative activities. It was observed from the reports that, the fluorophenyl substitution at 4-aminoquinazoline ring can enhance the anticancer activity [40, 41].

Table 3. *In vitro* antiproliferative activity studies of compound **7a-7c**

Comp. No.	R	Antiproliferative activity (IC_{50} in µg/mL)	
		MDA-MB-231	MCF7
7a	4-Cl	17.83	>500
7b	4-F	5.44	>500
7c	4-CH_3	19.56	>500
Cisplatin	-	5.61	6.78

3.4.2 Antimicrobial activity

The *in vitro* antibacterial activity was tested against two Gram-positive (*B. subtilis and S. aureus*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains by broth dilution method. The results are displayed in Table 4. The compounds showed toxic effects against all the tested bacterial strains and the toxicity is more pronounced against Gram-negative bacterial strains than Gram-positive bacterial species. Compound **7c** with methyl substitution showed less toxicity profile against all the tested bacterial strains. The replacement of the methyl group with halo substitutions enhanced the activity. Compounds **7a** and **7b** showed a moderate to good activity against the Gram-negative bacterial strains (*E. coli* and *P. aeruginosa*). It is significant that 4-chloro derivative **7a** was found to be more active against Gram-negative bacteria than Ciprofloxacin, which is a known antimicrobial drug. The compound **7a** also showed moderate activity against the tested Gram-positive bacteria. Therefore, compound **7a** can be considered as a potent antibacterial candidate against the Gram-negative bacteria. The antibacterial activity of compounds is mainly due to the presence of sulfonamide moiety and the synthesized phenylsulfonamide derivatives were observed as less active compared to heterocyclic sulfa drugs

[42, 43]. The compounds were also tested against a fungal strain *A. niger*, but did not show any remarkable activity. They were active only at higher concentration (MIC 50 µg/mL) compared to the standard drug Fluconazole.

Table 4. Antimicrobial activity studies of compound **7a-7c**.

Comp. No.	Antimicrobial activity, MIC in µg/mL				
	Gram-positive Bacteria		Gram-negative bacteria		Fungal strain
	B. S[a]	***S. A***[b]	***E. C***[c]	***P. A***[d]	***A. N***[e]
7a	12.5	6.25	1.6	3.12	50
7b	50	12.5	6.25	3.12	50
7c	25	6.25	50	50	50
Cisplatin	NT	NT	NT	NT	NT
Ciprofloxacin	2.0	2.0	4.0	2.0	NT
Fluconazole	NT	NT	NT	NT	4.0

'NT' Indicates not tested; a, *B. subtilis*, b. *S. aureus*, c, *E. coli*, d, *P. aeruginosa*, e. *A. niger*.

4 Conclusion

Three novel quinazoline derivatives containing phenylsulfonamide moiety were synthesized and single crystals of each compound were grown by a slow evaporation method in acetone. X-ray single crystal structures were succesfully determined for all three quinazoline derivates. Compounds **7a** and **7c** showed to be isostructural whereas the fluoro derivative **7b** manifested clearly different molecular packing and hydrogen bond network in the crystal lattice. Even though the molecular packing scheme between **7a**/**7c** and **7b** proved to be different, the three compounds exhibited only two different types of molecular conformations: two types (A and B) in case of **7a** and **7c** and type B in case of **7b**. The *in vitro* anticancer activity of the compounds was tested against two breast cancer cell lines (MDA-MB-231 and MCF7). The fluoro derivative **7b** displayed excellent activity against MDA-MB-231 with IC_{50} value of 5.44 µg/mL, which was more than the standard drug Cisplatin. Compound **7a** with chloro substitution revealed the highest antimicrobial activity against the Gram-negative bacterial strain, *E. coli* out of the three tested compounds, whereas no significant activity was shown against the fungal strain by any of the compounds.

Electronic Supplementary Information (ESI) available: NMR data, additional figures and crystallographic data tables. CCDCs 1857204, 1563909 and 1857205 contain the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

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Synthesis, characterization, crystal structures and biological screening of 4-amino quinazoline sulfonamide derivatives

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Highlights

- Three quinazolin-4-ylamino derivatives containing phenylbenzenesulfonamides (**7a-7c**) were synthesized.
- Structural properties were examined by single crystal X-ray diffraction.
- Two different types (A and B) of molecular conformations of crystals were studied.
- *In vitro* antiproliferative and antimicrobial activity studies were performed.