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Synthesis of sulfanilamide derivatives and investigation of *in vitro* inhibitory activities and antimicrobial and physical properties

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ABSTRACT

Novel sulfanilamide derivatives were synthesized and evaluated for carbonic anhydrase inhibitory activity as a target for the treatment of glaucoma, and antibacterial properties for use in chemotherapy. Synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and photoluminescence. *In vitro* inhibitory activities were measured by UV–Vis and some of the compounds were found have greater inhibitory effects than the lead compound sulfanilamide. The correlation between inhibitory activity, biological properties and the physicochemical properties of water solubility and partition coefficients was also investigated. Sulfanilamide derivatives gave intense emissions upon irradiation by UV light and a dimethyl substituted compound and a cyclic analog have photoluminescence quantum yields 42% and 31% and long excited-state lifetimes of 3.92 and 2.91 ns, respectively.

1. Introduction

The zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) has been the target for drug design for numerous biological applications that include diuretics, antiglaucoma and antitumor effects [1–3]. Sulfonamides of the general formula RSO_2NH_2, are well known to have powerful CA inhibitory activity and in particular its use in treatment of glaucoma as a result of the inhibition of CA, and thus causing the eventual reduction ophthalmic intraocular pressure (IOP). Also sulfonamides exhibit antibacterial properties playing an important role in chemotherapy [4,5]. The design and development of new sulfanilamide derivatives can help determine any structural requirements for improved biological activity.

The focus of this study was the synthesis and evaluation of sulfanilamide derivatives as a target for glaucoma therapy and antimicrobial activity for potential use in chemotherapy; the general structure of antibacterial sulfonamides (**1**), where sulfanilamide (**2**) is the parent sulfonamide and is shown in Fig. 1. The design of the derivatives synthesized aimed to analyze the effect of side chain length of the tail originating from the nitrogen of the amide function and the resulting effect of dimethyl substitution on this chain. Further, a cyclic analog was examined for any apparent changes in biological activity. Sulfanilamide derivatives were tested for antimicrobial activity against several bacteria and yeast and were compared with one another for structure–activity relationships. The correlation between biological properties and various physicochemical properties was also examined. The results of these investigations may offer further insight into the treatment of glaucoma and may offer promise for antibiotic developments.

In this study sulfanilamide derivatives were examined for CA inhibition, antimicrobial activity and structure–activity relationships. The photoluminescence properties of these derivatives were also studied. The chloro-substituted amides 4-(chloroalkanoylamino)-benzenesulfonamides **3**–**5** were synthesized according to the procedure described elsewhere [6], and is shown here in Scheme 1. The synthesis of derivative 4-(3-chloro-2,2-dimethylpropanoylamino) (**6**) is also shown.

Scheme 2 illustrates the synthesis of the derivative 4-(2-oxopyrrolidinyl)-benzenesulfonamide (**7**). Intramolecular cyclization of 4-(chlorobutanoylamino)-benzenesulfonamide (**5**), in the presence of potassium carbonate, gave the derivative 4-(2-oxopyrrolidinyl)-benzenesulfonamide (**7**).

2. Materials and method

2.1. In vitro inhibition assays

Bovine carbonic anhydrase (CA) enzyme (Bovine type II, Boehringer Mannheim) was purchased from Aldrich. The enzyme inhibitory activities of compounds were expressed as IC_{50} values, where IC_{50} is the molar concentration required to inhibit 50% of enzyme. In order to determine the inhibitory activities of synthesized compounds, the effect of the enzyme on 4-nitrophenyl acetate hydrolysis, with changes in concentration, was examined. The hydrolysis of 4-nitrophenyl acetate to 4-nitrophenol catalyzed by

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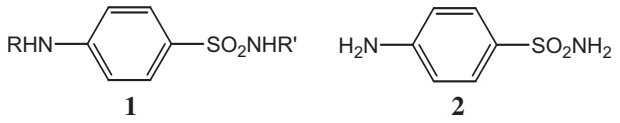


Fig. 1. General structure of antibacterial sulfonamides (**1**) and parent sulfonamide sulfanilamide (**2**).

carbonic anhydrase was monitored spectrophotometrically at 400 nm at pH 8.3 with a Hitachi U-1900 UV–Vis Spectrophotometer, interfaced with an IBM compatible PC [7], using a modified literature procedure [8]. The control (blank) was without inhibitor (compound) present. Sulfanilamide was used as the standard for the enzymatic assays.

2.2. Physical measurement

2.2.1. Determination of the partition coefficient

The ether–water partition coefficients of sulfanilamide (**2**) and synthesized compounds **3**–**7** were measured as described by Hansch [9,10]. The calculations for the measurements were carried out using the equation below:

$$P = C_o/C_w(1-\alpha)$$

where P is the ether–water partition coefficient, C_o is the concentration of the compound in the ether phase, C_w is concentration of the compound in the water phase and α is the degree dissociation compound in the water phase. The value of ether–water partition coefficient should be at least 0.01 in order to get good dispersion in the internal eye tissue [11,12].

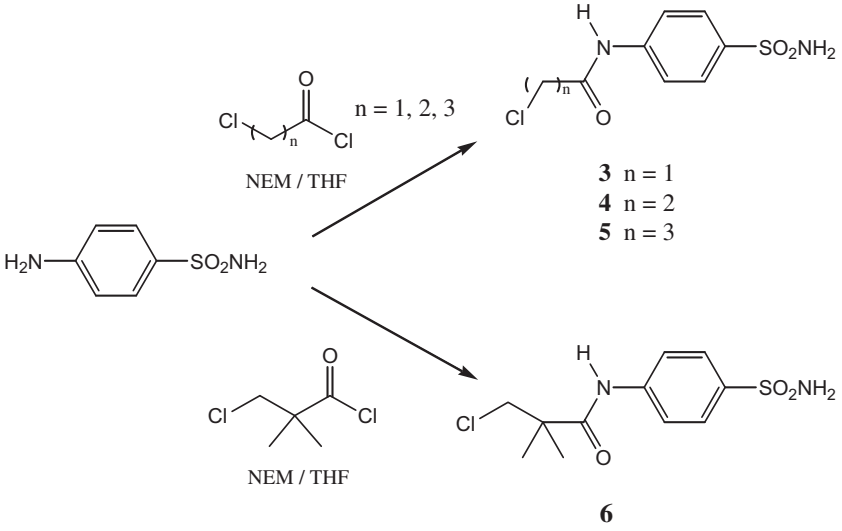
A calibration curve was plotted using differing concentrations of sample solutions. The absorbance and wavelength at these concentrations were determined spectrophotometrically by UV–Vis. A 0.01% sample solution was prepared to calculate the division coefficient of the synthesized compound. An aliquot (5 mL) of this known concentration of sample was taken and same volume of diethyl ether (5 mL) was added. This mixture was shaken for at least 5 min and the two phases were separated. The absorbance of the water phase was measured and after the solvent (diethyl ether) was evaporated, water was added to dissolve any remaining sample and then the absorbance of this solution was measured. The concentration of compounds in ether phase and water phase was calculated using the absorbance values obtained from UV–Vis measurements using the previously prepared calibration curve.

2.2.2. Determination of solubilities in water

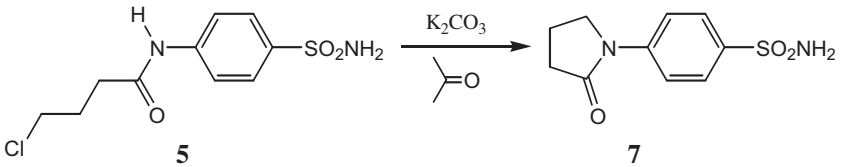
A sample as an effective CA inhibitor should have good solubility in water. 1% sample solution in deionized water was prepared and its solubility was calculated. The sample solution was shaken well in water for at least 30 min at 25 °C. The undissolved sample was isolated by filtration and the solubility was calculated and given as g/100 mL.

2.3. Determination of antimicrobial activity

The agar diffusion method using sterile filter paper discs was used for the screening of antibacterial and antifungal activities of synthesized sulfanilamide derivatives [13]. Inhibition zones formed on Mueller Hinton Agar (MHA) and Sabourand Dextrose Agar (SDA) (expressed as percentage (%) growth inhibition) were evaluated. Standard paper discs of the antibiotic streptomycin served as the positive antibacterial controls. Negative controls were done using paper discs loaded with DMSO. Experiments were



Scheme 1. Synthesis of 4-(2-chloroethanoylamino)-benzenesulfonamide (**3**, $n = 1$), 4-(3-chloropropanoylamino)-benzenesulfonamide (**4**, $n = 2$), 4-(4-chlorobutanoylamino)-benzenesulfonamide (**5**, $n = 3$) and 4-(3-chloro-2,2-dimethylpropanoylamino)-benzenesulfonamide (**6**).



Scheme 2. Synthesis of 4-(2-oxopyrrolidinyl)-benzenesulfonamide (**7**).

performed in triplicate, and the developing inhibition zones were compared with those of reference parent compound sulfanilamide.

The following collection of microbes was used: three Gram-positive (*Bacillus cereus* EU, *Bacillus megaterium* DSM 32, *Enterococcus faecalis* A10), one Gram-negative (*Escherichia coli* DM) and one yeast (*Saccharomyces cerevisiae*). All microorganisms were provided by the Microbiology Laboratory Culture Collection, the Department of Biology, Kahramanmaras Sutcu Imam University, Turkey.

All the bacteria mentioned above were incubated at 37 ± 0.1 °C for 24 h by inoculation into Nutrient Broth (Difco), and the yeast studied was incubated at 25 °C in Sabouraud Dextrose Broth (SDB) (Difco) for 24 h. Mueller Hinton Agar (MHA) (Oxoid) and Sabourand Dextrose Agar (SDA) sterilized in a flask and cooled to 40–45 °C was distributed over sterilized petri dishes having a diameter of 9 cm (15 mL) after injecting (0.1 mL) of bacteria and yeast (10^5/mL for bacteria and 10^4/mL for yeast), ensuring homogeneous distribution of the food medium over the petri dishes. Sterile filter paper discs (6 mm) injected with 10 μL solutions in DMSO (80 μg/disc) at a concentration of 8.0 mg/mL were placed on the surface of the solid agar medium and were slightly pressed. Petri dishes prepared as described above were kept for 2 h at 4 °C to enable prediffusion of the samples into the agar, and subsequently samples inoculated with bacteria were incubated at 37 ± 0.1 °C for 24 h and those with yeast were incubated at 25 °C for 24 h. The inhibition zones formed on the medium were measured and expressed as percentage (%) growth inhibition. These studies were performed in triplicate.

3. Experimental

Commercially available and/or reagent grade solvents and reagents were purchased from Aldrich Co. or Merck and were used without purification unless otherwise stated. Carbonic anhydrase enzyme (Bovine type II, Boehringer Mannheim) was purchased from Aldrich and used as the standard for IC_{50} measurements. Analytical thin-layer chromatography (TLC) was performed using aluminum-coated Merck DC Alufolien Kieselgel 60 F 724 plates.

All reactions were carried out under an atmosphere of nitrogen gas. Reaction temperatures were measured either externally, or by a thermometer inserted into the reaction mixture. Melting points was recorded using a Stuart Scientific Melting Point Apparatus and are reported uncorrected.

NMR spectra were recorded on a Bruker AC 300. pH was measured using a Crison pH Meter, Basic 20. UV–Vis spectra were recorded using a Hitachi U-1900 UV–Vis Spectrophotometer and 1 cm optical path quartz cuvette. FTIR spectra were obtained using a PerkinElmer RXI FTIR Spectrometer. FTIR spectra were obtained using samples prepared as KBr pellets; samples were dry ground using a pestle and mortar and then were pressed into transparent pellets.

The single-photon fluorescence spectra were collected on a PerkinElmer LS55 luminescence spectrometer. All the samples were prepared in spectrophotometric grade DMSO and analyzed in a 1 cm optical path quartz cuvette. The solution concentration of the compounds in DMSO was 1.0×10^{-5} mol L^{-1} and the samples were excited at 257 nm wavelength. The photoluminescence quantum efficiencies of the compounds and their derivatives were calculated using 9,10-diphenylantracene as the standard [14].

3.1. 4-(3-Chloro-2,2-dimethylpropanoylamino)-benzenesulfonamide (6)

To a solution of 3.00 g (17.42 mmol) sulfanilamide in 50.0 mL THF, 4.41 mL (34.84 mmol) NEM was added. A solution of 4.50 mL (34.84 mmol) 3-chloro 2,2 dimethylpropanoylchloride in 20.0 mL THF was added with stirring. A white precipitate of NEM·HCl salt was immediately observed. The reaction mixture was stirred at room temperature for 24 h, the progress of which was monitored by TLC (ethyl acetate/methanol 6/1 v/v). The precipitate was filtered out and the filtrate collected was evaporated *in vacuo* to leave a residue. The residue was dissolved in ethyl acetate. The organic extract was washed with 3 M hydrochloric acid and then saturated sodium bicarbonate solution and then finally with brine. The extract was dried ($MgSO_4$) and concentrated by evaporation in vacuo to give a residue. Re-crystallization (ethanol) afforded 2.84 g (56%) of product as a white solid and was stored in the refrigerator (4 °C) when not in use: m.p. 183–184 °C; ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.31 (s, 6H), 3.88 (s, 2H), 7.15–7.28 (s, 2H), 7.70–7.85 (m, 4H), 9.67 (s, 1H); ^{13}C NMR (75 MHz, d_6-DMSO) δ 23.4, 44.1, 51.5, 120.3, 126.8, 139.1, 142.4, 173.8; FTIR (KBr) 3347, 3320, 3110, 2939, 1435, 1666, 1592, 1525, 1435, 1370, 1185, 792 cm^{-1}.

3.2. 4-(4-Chlorobutanoylamino)-benzenesulfonamide (5)

4-(Chlorobutanoylamino)-benzenesulfonamide (**5**) was prepared as described previously [6], where 3.66 g (76%) was obtained as a white solid from sulfanilamide (3.00 g, 17.42 mmol) in 50.0 mL THF, NEM (4.41 mL (34.84 mmol)) and 4-chlorobutanoylchloride (3.90 g, 34.84 mmol) in 20.0 mL THF. The reaction mixture was stirred at room temperature for 3 h, the progress of which was monitored by TLC (ethyl acetate/methanol 6/1 v/v). The solid was recrystallized in ethanol, dried in vacuo and stored in the refrigerator (4 °C) and kept in the dark when not in use: m.p. 194–195 °C.

3.3. 4-(2-Oxopyrrolidinyl)-benzenesulfonamide (7)

To a solution of previously prepared 1.50 g (5.42 mmol) 4-(chlorobutanoylamino)-benzenesulfonamide (**5**) in 100.0 mL acetone, 1.00 g (7.24 mmol) K_2CO_3 was added with stirring. The reaction mixture was heated at reflux for 24 h, the progress of which was monitored by TLC (ethyl acetate/methanol 6/1 v/v). The precipitate was filtered out and the filtrate collected was evaporated *in vacuo* to leave a residue. The residue was dissolved in ethyl acetate. The organic extract was washed with 3 M hydrochloric acid and then saturated sodium bicarbonate solution and then finally with brine. The extract was dried ($MgSO_4$) and concentrated by evaporation in vacuo to give a residue. Re-crystallization (ethanol) afforded 0.69 g (53%) of product as a white solid and was stored in the refrigerator (4 °C) when not in use: m.p. 245–246 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00–2.18 (m, 2H), 2.45–2.60 (m, 4H), 7.25–7.33 (m, 2H), 7.70–7.80 (m, 4H) ppm; ^{13}C NMR (75 MHz, d_6-DMSO) 17.7, 32.8, 48.4, 119.1, 126.8, 139.0, 144.9, 175 ppm; FTIR (KBr) 3324, 2977, 2888, 1675, 1460, 1392, 1420, 1334 cm^{-1}.

4. Results and discussion

4.1. Carbonic anhydrase inhibition

The inhibitory activity against the CA enzyme of derivatives chloro- substituted amides 4-(chloroalkanoylamino)-benzenesulfonamides **3**–**5**, 4-(3-chloro-2,2-dimethylpropanoylamino)-benzenesulfonamide (**6**) and 4-(2-oxopyrrolidinyl)-benzenesulfonamide (**7**) are reported herein and compared with that of the parent sulfonamide sulfanilamide. The inhibitory activities are given in Table 1. Compounds **3** and **6** showed inhibitory activity similar to that of sulfanilamide, the lead structure for this class of compounds, and showed the greatest inhibitory activity for these compounds. The number of aliphatic carbon atoms separating the amino-benzenesulfonamide carbonyl moiety from the terminal

chlorine in derivatives **3**, **4** and **5** seems to be important as derivative **3** being one carbon away from the carbonyl function showed the greatest enzyme inhibitory activity. Hence a receptor interaction model may include the lipophilic chain length minimum of one carbon. One carbon chain length showed the greatest activity and thus this was exploited further in terms of multi-substitution at this position, though keeping this chain length to a minimum. The di-substituted derivative **6** fitting this criteria and having a two carbon chain length was able to show similarly higher activity than the lead compound sulfanilamide. Replacement of both the lipophilic chain and chlorine atom with a rigid a 2-oxopyrrolidinyl-unit greatly reduced the inhibitory activity to about half that of sulfanilamide.

4.2. Partition coefficients (P) and solubility

The ether/water partition coefficients (*P*) for the compounds synthesized were investigated using the method of Hansch [9,10], and are given in Table 1. The partition coefficients of synthesized compounds varied between 0.254 and 1.200, and were well above 0.01 known as the minimum required for good dispersion in internal eye tissue [11,12]. In order for a drug/compound to be effective for treating glaucoma, it should have good partition coefficient values together with high inhibitory activity. It can be seen from Table 1 that all the synthesized compounds had reasonably good partition coefficient values.

The correlation between partition coefficients (*P*) and IC_{50} was examined. The results showed that for all the synthesized compounds there was no linear relationship between *P* and IC_{50} values. Sulfanilamide has an IC_{50} value of 72.2 nM. The synthesized compounds, in general, had IC_{50} values lower than sulfanilamide; only compounds **3** and **6** had higher IC_{50} values. It can be seen that the inhibitory activities and partition coefficients had no bearing on each other. For example, derivative **7** had IC_{50} value of 119.9 nM and a partition coefficient value of 0.990 showing that it had low inhibitory activity and a high partition coefficient, and likewise derivative **3** had a IC_{50} value of 66.6, hence good inhibitory activity and a low partition coefficient value of 0.436.

The correlation between solubility in water and IC_{50} was also examined. The results showed that for all the synthesized compounds there was no linear relationship between solubility in water and IC_{50} values. A compound with good inhibitory activity may have either high solubility or low solubility in water. For example, compound **6** has lower inhibitory activity than derivative **3**, however for the solubility in water, the reverse was observed. Further derivative **6** has a higher inhibitory activity and solubility in water than **7**.

4.3. Antimicrobial activity

The test solutions were prepared in DMSO. The inhibition zones on the medium were measured and expressed as percentage (%) growth inhibition. The results of the antimicrobial activities are summarized in Table 2.

The synthesized compounds were found to have interesting antimicrobial activity. The antimicrobial activity observed for all synthesized compounds were, in general, greater than the reference compound sulfanilamide. Antimicrobial activity was best observed for Gram-positive bacteria (*B. cereus*, *B. megaterium* and *E. faecalis*). Only the derivative 4-(chloroethanoylamino)-benzenesulfonamide (**3**) showed activity against Gram-negative bacteria (*E. coli*) along with the reference compound sulfanilamide. Additionally the synthesized compounds were not effective against the yeast (*S. cerevisiae*).

A comparison of the antibacterial activity of the chloro-substituted amides 4-(chloroalkanoylamino)-benzenesulfonamides **3**–**5** revealed that for Gram-positive bacteria (*B. cereus*, *B. megaterium* and *E. faecalis*), the shorter the chain length from the carbonyl group to the chlorine atom the greater the activity, thus activity decreased with increasing chain length. Only the small chain congeneric derivative 4-(chloroethanoylamino)-benzenesulfonamide (**3**) showed activity against Gram-negative bacteria (*E. coli*) and was the same as that obtained for the parent sulfanilamide.

The dimethyl substituted compound 4-(3-chloro 2,2 dimethylpropanoylamino)-benzenesulfonamide (**6**) had higher antibacterial activity than the straight chain chloro-substituted amides 4-(chloroalkanoylamino)-benzenesulfonamides **4** and **5**, but had lower activity than small chain congeneric derivative 4-(chloroethanoylamino)-benzenesulfonamide (**3**).

When a γ-lactam ring replaced the chloroalkanoylamino chain as for 4-(2-oxopyrrolidinyl)-benzenesulfonamide (**7**) antibacterial activity was retained for Gram-positive bacteria (*B. cereus*, *B. megaterium* and *E. faecalis*), being lower than the small chain chloro-substituted amides 4-(chloroalkanoylamino)-benzenesulfonamide **3** and higher than two larger congeneric derivatives chloro-substituted amides 4-(chloroalkanoylamino)-benzenesulfonamides **4** and **5**. This γ-lactam derivative (**7**) had higher antibacterial activity against Gram-positive than the dimethyl substituted compound (**6**). Further no antimicrobial activity was observed for this compound γ-lactam derivative (**7**) against Gram-negative bacteria (*E. coli*).

The results showed that the synthesized compounds possessed antibacterial activity against Gram-positive and only one compound against Gram-negative bacteria. Antifungal activity was not observed for any of these compounds. Aliphatic chain length was an important component being more effective when kept to minimum length as for compound **3**. Dimethyl substitution in this chain as in compound **6** allowed for high activity to be retained though lower than that observed for the shortest chain (**3**). The introduction of a γ-lactam ring in place of this chain, as for compound **7**, did not adversely affect the antibacterial activity, but in fact kept the activity to a high level. These observations may be used to further develop antimicrobial agent.

Table 1
Inhibitory activities of carbonic anhydrase by different sulfanilamide derivatives.

Compound	IC_{50}[a] (nM)	Partition coefficient (*P*) at pH = 7.2	Solubilities in water (g/100 cm^3)
2	72.2	0.082	0.75
3	66.6	0.436	0.30
4	78.3	0.370	0.10
5	89.1	0.254	0.05
6	69.4	1.200	0.50
7	119.9	0.990	0.15

[a] *In vitro* studies as effects on carbonic anhydrase isozyme II (CA-II).

4.4. Structure–activity relationships

Lipophilicity of compounds has an important effect on their biological activity [12,15]. It is expressed as log *P*, the octanol/water partition coefficient, and high values indicate good permeation of compounds through lipid layers of cell membranes. The prediction of lipophilicity and other physicochemical properties such as molar weight (MW), molecular volume (MV), molecular refractivity (MR) for compounds **2**–**7** was calculated using HyperChem Software in an attempt to correlate physicochemical properties of the compounds with their antimicrobial activity [16]. The physicochemical properties MW, MV, MR and log *P* of the compounds studied are presented in Table 2.

Table 2
Antimicrobial activity data and selected physiochemical properties for sulfanilamide and their derivatives.

Entity[a]	Microorganism[c]				Physiochemical properties			
	B. cereus	*B. megaterium*	*E. fecalis*	*E. coli*	MW	MV (Å^3)	MR (Å^3)	log *P*
Streptomycin[b]	16	20	18	11	NA	NA	NA	NA
2	24	21	19	24	172.20	498.44	47.76	−1.98
3	35	31	38	24	248.68	658.22	61.96	−1.56
4	26	19	28	–	262.71	704.62	66.66	−1.38
5	24	16	26	–	276.74	767.60	71.41	−1.13
6	31	24	31	–	290.76	793.09	75.73	−0.15
7	33	28	33	–	240.28	675.07	64.99	−1.76

[a] Entity refers to compounds.
[b] Standard compound.
[c] % growth inhibition; – denotes no activity; *S. cereviciae* (not shown here) was also used, however all entities showed no antifungal activity against *S. cerevisiae.*

Interestingly, of the chloro-substituted amides 4-(chloroalkanoylamino)-benzenesulfonamides **3**–**5**, compound **3** has the highest lipophilicity, and among all synthesized compounds the rigid 2-oxopyrrolidinyl- compound **7** has the highest lipophilicity. The antimicrobial activity for these two compounds (**3** and **7**) showed a similar trend where antimicrobial activities were also the highest for these compounds. Compounds **3** and **7** have both comparable lipophilicity and antimicrobial activity.

The chloro-substituted amides 4-(chloroalkanoylamino)-benzenesulfonamides **3**–**5** showed that as lipophilicity decreased, antimicrobial activity decreased. Further, antimicrobial activity against *E. coli* was only observed for the smallest and most lipophilic compound **3**.

The dimethyl substituted compound **6** had high antimicrobial activity but low lipophilic character, though the rigid 2-oxopyrrolidinyl-analogue **7** follows the trend of increasing antimicrobial activity with increasing lipophilic character; compound **7** is more lipophilic and had greater antimicrobial activity than the smaller compound **6**. In general, the results showed there was a dependency between biological activity and lipophilicity, where antimicrobial activity increased with increasing lipophilicity. Additionally antimicrobial activity increased with decreasing MV with the exception of compound **6**; for compound **6** molecular size as volume appears to play an adverse role on antimicrobial activity, where for this compound size has a dominating role on antimicrobial activity. MR as with lipophilicity, is also a molecular descriptor used to relate chemical structure to observed behavior. It can be seen from Table 2 that, in general, MR decreased with increasing lipophilicity, and thus, antimicrobial activity increased with decreasing MR.

The synthesized compounds all possessed carbonyl groups and possible hydrogen bonding with the active cell components may be influential for antimicrobial activity, resulting in the interference and disruption of normal cell processes.

4.5. Fluorescence measurements

Absorption and photoluminescence spectra were studied for solutions compounds excited at 257 nm. The most striking feature was that compounds and their derivative gave an intense emission upon irradiation by UV light. The photoluminescence spectrum of compounds in DMSO is shown in Fig. 2. Maximum luminescent intensity was observed at 381 nm and the full width at half maximum was 117 nm for compound **2**. Compound **2** exhibited a photoluminescence quantum yield of 33% and a long excited-state lifetime of 3.06 ns. Addition of more electron donating groups at the para-position of the derivatives caused an increase in the intensity of the main peaks and shifted to higher emission wavelength. Also, addition of more electron withdrawing groups at the para-position of the derivatives caused a decrease in the intensity of the main peaks and shifted to lower emission wavelength.

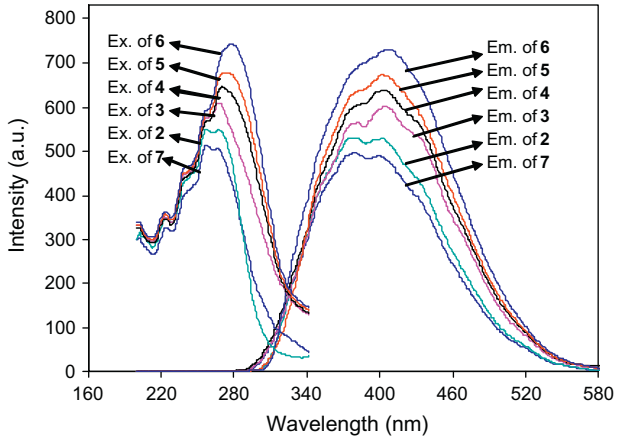


Fig. 2. Photoluminescence spectra of compounds in DMSO; samples were excited at 257 nm.

Table 3
Photoluminescence data for compounds.

Entity	λ_{max} Ex (nm)	In Ex	λ_{max} Em (nm)	In Em	ϕ_f (%)	τ_f (ns)
2	261 (229;241;271)	538	381 (360;406;435)	531	33	3.06
3	266 (231;242;255)	607	406 (379;434)	599	36	3.37
4	272 (232;243;256)	639	408 (382;435)	631	38	3.50
5	278 (233;244;257)	673	410 (384;437)	664	39	3.64
6	282 (234;245;258)	741	412 (386;439)	731	42	3.92
7	258 (226;237;269)	504	378 (358;402;433)	498	31	2.91

λ_{max} Ex: maximum excitation wavelength; In Ex: maximum excitation intensity.
λ_{max} Em: maximum emission wavelength; In Em: maximum emission intensity.
ϕ_f: quantum yield; τ_f: excited-state lifetime.

The photoluminescence data are summarized in Table 3. The photoluminescent properties of these compounds may indicate great potential for numerous optical applications and for medicinal biomarkers.

5. Conclusion

In vitro inhibitory activities of the synthesized compounds expressed as IC_{50} were investigated by using a UV–Vis spectrophotometer. Partition coefficients of the synthesized compounds

were also studied. Some of the synthesised compounds were found to be comparable or more potent than sulfanilamide. The partition coefficient values revealed good dispersion but did not follow any clear cut pattern in relation to their inhibitory activities. The solubility in water of the synthesized compounds were examined and correlated with their inhibitory activities. In general, water solubilities were erratic and there was no linear relationship between solubility in water and IC_{50} values. The antimicrobial activity observed for all synthesized compounds were in general greater than the reference compound sulfanilamide. Antimicrobial activity was best observed for Gram-positive bacteria and only one compound showed activity against Gram-negative bacteria (*E. coli*) along with the reference compound sulfanilamide. None of the synthesized compounds were effective against yeast. In general, the results showed there was a dependency between biological activity and lipophilicity, where antimicrobial activity increased with increasing lipophilicity.

In regard to fluorescence properties electron donating groups onto ring at para-position of derivatives caused increase in intensity of main peaks and a shift to higher emission wavelength. Further electron withdrawing groups at para-positions caused a decrease in intensity of main peaks and a shift to lower emission wavelength.

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