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Protonation and anion-binding properties of aromatic sulfonylurea derivatives†

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In this work the anion-binding properties of three aromatic sulfonylurea derivatives in acetonitrile and dimethyl sulfoxide were explored by means of NMR titrations. It was found that the studied receptors effectively bind anions of low basicity (Cl^- , Br^- , I^- , NO_3^- and HSO_4^-). The stoichiometry of the complexes with receptors containing one binding site was 1 : 1 exclusively, whereas in the case of the receptor containing two sulfonylurea groups 1 : 2 (receptor : anion) complexes were also detected in some cases. The presence of strongly basic anions (acetate and dihydrogen phosphate) led to the deprotonation of the sulfonylurea moiety. This completely hindered its anion-binding properties in DMSO and only proton transfer occurred upon the addition of basic anions to the studied receptors. In MeCN, a complex system of equilibria including both ligand deprotonation and anion binding was established. Since ionisation of receptors was proven to be a decisive factor defining the behaviour of the sulfonylurea receptors, their $\text{p}K_a$ values were determined using several deprotonation agents in both solvents. The results were interpreted in the context of receptor structures and solvent properties and applied for the identification of the interactions with basic anions.

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Introduction

The class of sulfonylurea receptors received relatively low attention, despite advantageous features of these compounds regarding anion coordination making them prospective candidates as selective and efficient supramolecular hosts. The pharmaceutical relevance of this class of compounds provides added value to corresponding fundamental research as it provides deeper insight into their properties and unlocks new potential in their application. In the process of rational design of new active pharmaceutical ingredients, it is of great importance to understand and predict their interactions with diverse species encountered in living organisms. Thus, the study of supramolecular complexes of derivatives belonging to an important class of pharmaceuticals facilitates the development of new, more potent drugs.

Sulfonylurea (SU) derivatives have played a crucial role in the treatment of type II diabetes for several decades as the first oral hypoglycemic agents. Hyperglycemia in type II diabetes is the consequence of defects in insulin secretion from pancreatic β -cells and insulin sensitivity in peripheral tissues such as liver, muscle, and fat.¹ SU-based drugs stimulate insulin release from

the β -cells of the pancreas thereby lowering the level of glucose in the blood.² Some of the SU agents were also shown to improve insulin sensitivity. Metabolism of SU derivatives occurs both in the liver and the kidneys, which makes them suitable for patients with hepatic or renal dysfunction. The lower costs of SU derivatives with respect to other drugs make them more accessible to patients worldwide.³ In addition, SU derivatives have been used as diuretic agents,⁴ anticancer drugs,^{5,6} anti-malarial drugs,⁷ and agents active against tuberculosis.⁸ It should be pointed out that the application of sulfonylureas is not limited to the pharmaceutical industry and these derivatives have been applied as catalysts in organic synthesis.⁹ Further, SUs are also common structural motifs in agrochemicals, most frequently used as herbicides.¹⁰

To understand the properties of SU derivatives, it is important to perform a detailed study of their acidity and potential to establish non-covalent interactions resulting in supramolecular complexes. A large number of neutral receptors possessing NH groups that interact through hydrogen bonds with the anionic guests, such as amides,^{11,12} peptides,^{13,14} pyrroles,^{15,16} indoles,¹⁷ sulfonamides,¹⁸ and (thio)urea derivatives,^{19–28} have been successfully implemented in anion recognition.²⁹ Considering the extensive knowledge regarding the anion coordination chemistry in solution, it is obvious that sulfonylurea moiety features several key attributes for efficient anion coordination. This includes high affinity as a hydrogen-bond donor (acidity), its simple incorporation into different molecular scaffolds, and the fact that it possesses two directed hydrogen bond-donating NH groups which could enhance the stability of the complexes

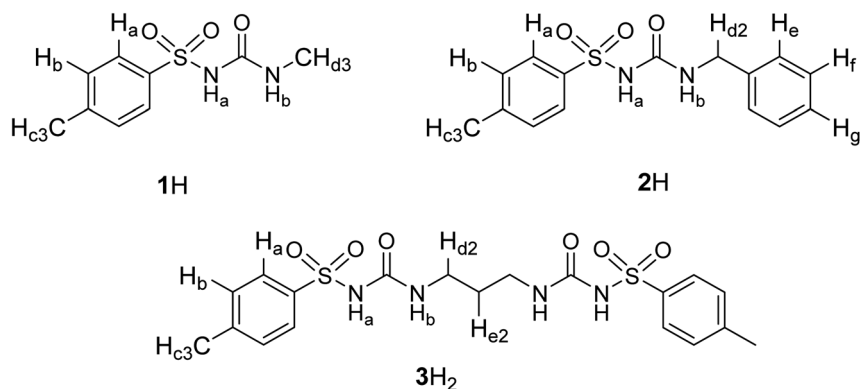
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Scheme 1 Sulfonylurea receptors studied in this work.

and introduce a basis for selective recognition. Interactions of some SU-based pharmaceuticals with methacrylate anion have been studied, employing the concept of molecular imprinting for their extraction.^{30–32} Still, the potential of sulfonylureas as anion receptors remained almost completely unexplored.

As mentioned above, due to their enhanced acidity, SU derivatives are expected to form stronger hydrogen bonds with anions, compared to their urea analogues. However, in aprotic

solvents this feature can also lead to proton transfer (receptor to anion) in the presence of basic anions like dihydrogen phosphate or carboxylates. Such behaviour of NH-based anion receptors has been reported in numerous cases in recent literature.^{18,23,33,34} Manesiotis *et al.* clearly demonstrated that proton transfer from SU to carboxylate occurs in solutions.³² By employing interactions between methacrylate and sulfonylurea drugs as the basis for molecular imprinting, the authors

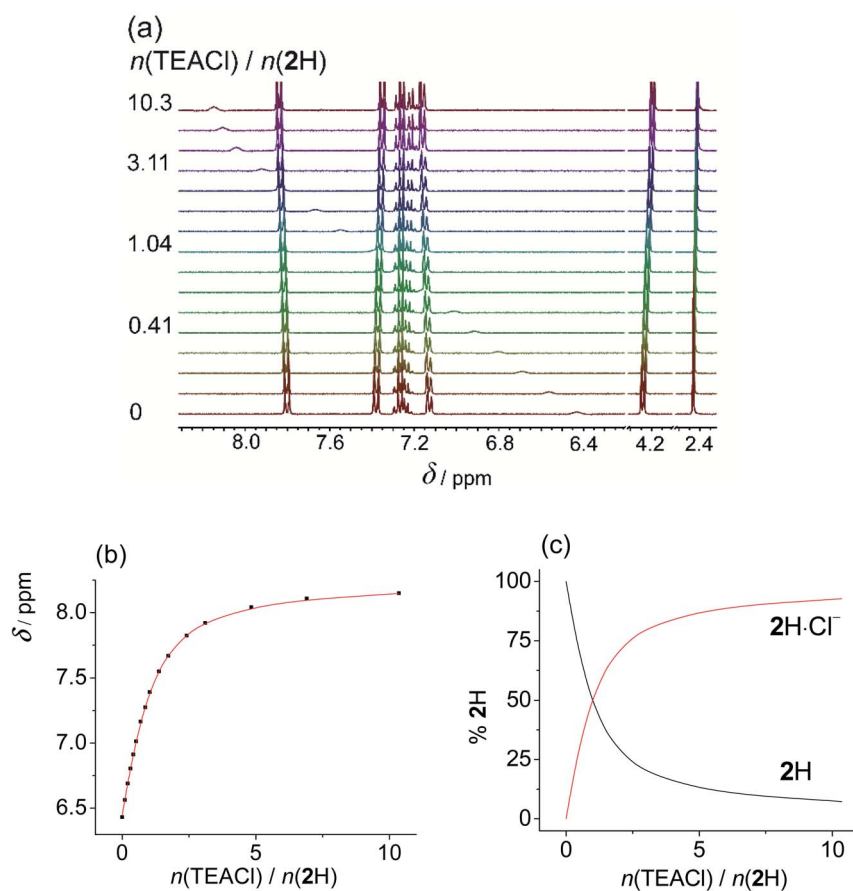


Fig. 1 (a) ^1H NMR titration of 2H ($c = 1.15 \times 10^{-3} \text{ mol dm}^{-3}$) with TEACl ($c = 2.10 \times 10^{-2} \text{ mol dm}^{-3}$) in MeCN-d_3 at $(25.0 \pm 0.1)^\circ\text{C}$, $V_0 = 0.53 \text{ mL}$. (b) Dependence of NH_b proton chemical shift on $n(\text{TEACl})/n(2\text{H})$ molar ratio. ■ Experimental, – calculated. (c) Distribution of species during the titration of 2H with TEACl.



encountered deactivation of binding sites due to the exchange of protons between the SU drug and methacrylate, showcasing the importance of understanding the interplay between anion binding and deprotonation.

In this work we studied three aromatic sulfonylurea derivatives (Scheme 1) and provided valuable insight into their anion-binding and protonation properties in non-aqueous solutions. A detailed investigation of the correlation between the receptor structures and the relevant properties, which will enable fine-tuning of their characteristics was carried out. We strongly believe that the results presented will endorse the development of the anion receptor chemistry of sulfonylureas, possibly enhancing the understanding of their pharmacokinetic behaviour.

Results and discussion

Synthesis

The studied receptors (1H, 2H and 3H₂) were prepared from tosyl isocyanate by reaction with amines in dichloromethane (DCM).³⁵ Upon reaction, the target products precipitated from the reaction mixture and filtration yielded pure compounds. By using DCM as the solvent pure products were obtained and the system did not show any tendency to form a gel, unlike when several other solvents were employed (benzene, toluene, THF).

Anion binding – weakly basic anions (Cl⁻, Br⁻, I⁻, NO₃⁻, HSO₄⁻)

The binding of a series of anions including halides, nitrate, and hydrogen sulphate (added as tetraalkylammonium salts) was investigated by means of ¹H NMR titrations in deuterated DMSO and acetonitrile (Fig. 1 and S7–S35 in the ESI†). Titration curves of receptors 1H and 2H could be processed by assuming 1 : 1 complex stoichiometry (Fig. 1), whereas compound 3H₂ formed 1 : 1 and 1 : 2 (receptor : anion) complexes since this receptor possesses two binding sites. As expected, the most pronounced changes in the chemical shift were detected for NH_b protons which exhibited a significant downfield shift (Fig. 1b). This finding affirmed sulfonylurea groups as the binding sites and hydrogen bond formation between the SU moieties and the anion as the main interaction providing

stabilisation of the complexes. The NH_a proton signal could not be detected in the NMR spectrum which can be attributed to its high acidity leading to the coalescence of the corresponding signal.

The stability constants obtained by multivariate non-linear regression analysis of the titration data are listed in Table 1. Among the tested anions, the studied receptors form the most stable complexes with chloride (approximately one order of magnitude higher stability constant compared to that with Br⁻). This finding is in line with the highest basicity of Cl⁻ in terms of hydrogen-bond formation. Consequently, the prepared sulfonylureas can be regarded as moderately selective receptors of chloride. Compounds 1H and 2H exhibit similar binding properties with the aromatic derivative being slightly better anion receptor, most likely stemming from weak resonance effects of the benzyl group. The anion binding affinity of compound 3H₂ was found to be significantly higher than that of 1H and 2H (comparing stabilities of 1 : 1 complexes). This is partly the result of an additional binding site that statistically favours complexation, but cooperative interactions of both binding sites with the anion are also possible. A significant difference in the stability constants obtained for 1 : 1 and 1 : 2 complexes (the latter being much less stable) supports the assumption of cooperative interaction of both sulfonylurea groups in the 1 : 1 complex. Great differences in the binding constants determined in the two solvents are in line with the competitive nature of the DMSO molecules which act as strong H-bond acceptors in contrast to MeCN which does not significantly compete for hydrogen bonds. Consequently, the anion complexes with nitrate, hydrogen sulphate, and iodide were not detected in DMSO at the experimental conditions used, most likely due to their very low stability.

The comparison of the herein studied hosts with previously reported ones belonging to urea or thiourea families is not straightforward since many effects (sterical, inductive, *etc.*) govern the performance of the anion hosts.^{29,36} Within the available literature, most data has been collected for dihydrogen phosphate and carboxylate complexes.²⁷ These data cannot be compared to the presented results since deprotonation of SU group is favoured over anion coordination occurred (see next chapter). However, the effect of SU moiety in terms of

Table 1 Stability constants (log *K*) of anion complexes with receptors 1H, 2H, and 3H₂ in MeCN and DMSO determined by ¹H NMR spectroscopy at 25 °C^a

	MeCN				DMSO			
	1H	2H	3H ₂		1H	2H	3H ₂	
	1 : 1	1 : 1	1 : 1	1 : 2	1 : 1	1 : 1	1 : 1	1 : 2
Cl ⁻	3.22(1)	3.27(1)	3.96(1)	1.76(4)	1.09(1)	1.16(1)	1.72(2)	0.71(4)
Br ⁻	2.28(1)	2.41(1)	2.97(1)	1.28(2)			0.54(4)	—
I ⁻	1.21(1)	1.30(1)	1.76(2)	0.74(8)				
HSO ₄ ⁻	2.06(1)	2.04(1)	2.45(1)	<1 ^b				
NO ₃ ⁻	1.72(1)	1.89(1)	2.24(9)	1.07(5)				

^a Uncertainties are given in parentheses as standard deviation. ^b Estimated.



complex stability can be assessed if chloride binding properties of non-macrocyclic urea or thiourea derivatives in MeCN or DMSO are considered. For instance, fluorescent thiourea reported by Gunnlaugsson did not exhibit any change in fluorescence spectrum upon the addition of chloride.³⁷ An elaborate, preorganised thiourea receptor reported by Johnson *et al.* was able to bind chloride and bromide with comparable affinity but still lower than SU derivatives **1H** or **2H**.³⁸ In our previous study, it was shown that non-aromatic urea derivatives of dehydroacetic acid also do not bind chloride in neither DMSO nor MeCN.¹¹ The same result was obtained for aromatic mono- and bis-urea derivatives in DMSO.²³ The aromatic urea derivatives bearing adamantane moiety reported by Blažek *et al.* also featured a rather low affinity for chloride in DMSO or MeCN.²⁶ It can thus be unambiguously argued that sulfonylurea moiety provides stronger stabilisation of the chloride complex stemming from enhanced acidity. Moreover, the fact that the sulfonylurea group can be deprotonated by the addition of base, provides a possibility of controlled anion release. Namely, the deprotonated form of anion would not be active as an anion host and complex dissociation would occur upon the addition of a base.

Protonation properties and interactions with basic anions

Upon addition of more basic anions, (acetate and dihydrogen phosphate), rather different spectral changes were detected

Table 2 pK_a values of **1H**, **2H** and **3H₂** in DMSO determined by ¹H NMR spectroscopy at 25 °C using different deprotonating agents^a

Base	1H	2H	3H₂	
	$pK_{a,1}$	$pK_{a,1}$	$pK_{a,1}$	$pK_{a,2}$
DIPEA	9.69(1)	9.41(1)	9.2(1)	10.65(6)
OAc ⁻	9.74(4)	9.6(1)	—	10.4(1)
H ₂ PO ₄ ⁻	9.72(1)	9.58(3)	9.5(1)	10.39(2)

^a Uncertainties are given in parentheses as standard deviation.

than those described above. In DMSO the obtained titration curves were monotonous but featured a decrease in chemical shift of the NH_b proton. Such spectral change is the opposite of the behaviour expected for anion coordination, *i.e.*, downfield shift as was observed by the addition of non-basic anions. Increased shielding of NH_b can be accounted for by dissociation of the sulfonylurea moiety (NH_a proton) caused by the addition of basic anions. The acquired data was processed assuming the proton transfer, treating the anions as bases, and omitting the formation of anion complexes from the model. All additional processes affecting the protonation equilibria (dimerization and homoassociation of anions and their conjugated acids) were taken into account in the course of data analysis using the values determined previously (more details are given in the

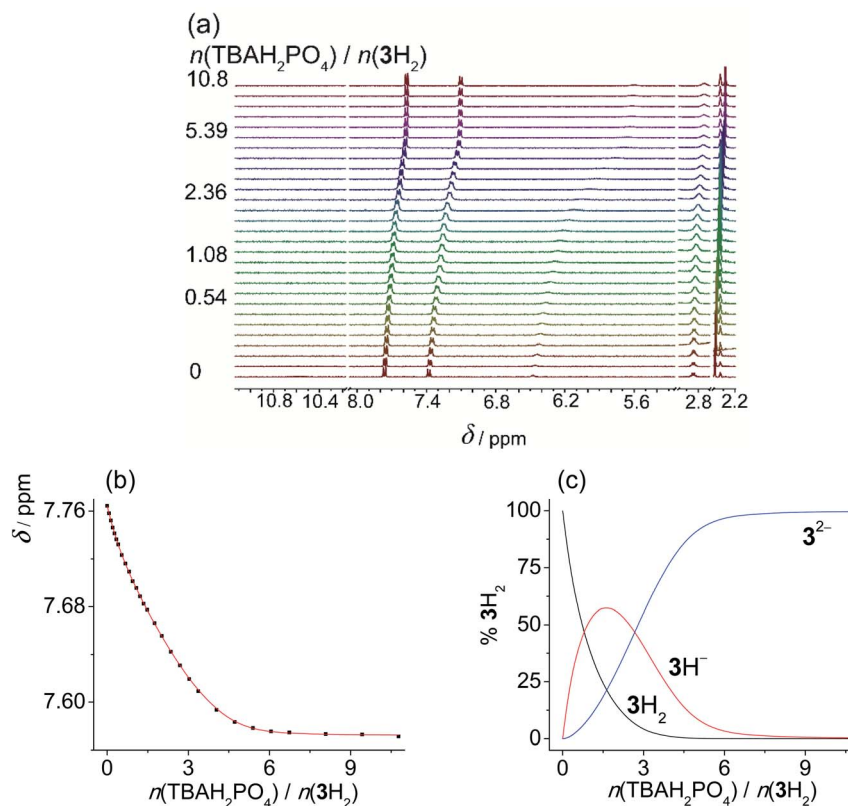


Fig. 2 (a) ¹H NMR titration of **3H₂** ($c = 1.20 \times 10^{-3} \text{ mol dm}^{-3}$) with TBAH₂PO₄ ($c = 8.56 \times 10^{-3} \text{ mol dm}^{-3}$) in DMSO-*d*₆ at $(25.0 \pm 0.1) \text{ }^\circ\text{C}$, $V_0 = 0.53 \text{ mL}$. (b) Dependence of H_b proton chemical shift on $n(\text{TBAH}_2\text{PO}_4)/n(\text{3H}_2)$ molar ratio. ■ Experimental, – calculated. (c) Distribution of protonation species of **3H₂** during the titration of **3H₂** solution with TBAH₂PO₄.



experimental part).³⁹ Excellent agreement of the experimental and calculated data was obtained in this way. This procedure yielded very similar pK_a values using both $H_2PO_4^-$ (Fig. 2 and S36 and S37 in the ESI†), and OAc^- (Fig. S38–S40 in the ESI†) as bases (Table 2). Due to the high basicity of acetate, the corresponding titration curve featured a sharp break at 1 : 1 molar ratio for 1H and 2H. In the case of $3H_2$ the NMR spectrum continued to change up to 2 : 1 molar ratio since both sulfonylurea moieties could undergo deprotonation. In the case of dihydrogen phosphate, the curves were smoother, reflecting the lower basicity of dihydrogen phosphate. Still, the chemical shifts measured after adding an excess of the base were the same regardless of the anion added. Moreover, analogous results were obtained by using *N,N*-diisopropylethylamine (DIPEA) as the base (Fig. S42–S44 and S37 in the ESI†).[‡] This tertiary amine with bulky substituents is able to deprotonate the sulfonylurea group but it is not expected to interact with the studied receptors in any other way. Titrations of studied compounds with DIPEA yielded rather similar pK_a values (Table 2) and characteristic spectra of deprotonated forms as in the cases when OAc^- and $H_2PO_4^-$ were used as bases (Tables S7–S9 in the ESI†). This confirmed that in DMSO no anion complexation occurred and that reliable pK_a values were measured.

Dissociation of 1H, bearing aliphatic sidearm was the least favourable, while the introduction of benzyl moiety resulted in stabilisation of the anionic form, due to delocalisation of the negative charge. Receptor $3H_2$ was more prone to release the first proton compared to monoprotic derivatives. This could, in great part, be ascribed to the statistical factor. The second deprotonation of $3H_2$ was significantly less favourable, as the negative charge generated by first proton dissociation hindered the following deprotonation reaction.

The primary reason for the dominance of proton transfer over potential anion coordination is a large difference in pK_a values of acetic and phosphoric acid compared to those of SU derivatives (SU being much stronger acids). The apparent basicity of the studied anions is further increased by homo-association processes (AH_2 formation). The lack of anion binding affinity of the deprotonated receptors is not surprising as these species become poor H-bond donors and their negative charge introduces unfavourable electrostatic interactions with anions. Further on, DMSO molecules compete strongly for the H-bonds which are the origin of the stability of most anion complexes. In general, it can be concluded that in DMSO highly basic anions will only cause deprotonation of sulfonylurea NH_a groups, whereas only the anions of low basicity are coordinated by this class of receptor molecules.

In MeCN the titration curves obtained by the addition of acetate or dihydrogen phosphate salts to SU derivatives featured a much more complex shape (Fig. S45–S50 in the ESI†). Again, the NH_b proton underwent the most significant change in chemical shift providing the most valuable information about the reactions taking place in the investigated solutions. In the

initial part of titration, a downfield shift was detected, suggesting that in MeCN anion binding is favoured at a low anion : ligand ratio (up to 1 equivalent). Upon further addition of anions, shielding of the NH_b proton was enhanced, which could again be ascribed to ligand deprotonation coupled with anion protonation (and other secondary processes). Rather similar results and qualitatively almost identical titration curves were previously reported by Gale *et al.* studying other compounds bearing acidic NH group.¹⁸ In an effort to study the underlying equilibria in a quantitative manner, we again performed the titrations of sulfonylureas with amine bases in MeCN (Fig. 3, S52 and S53 in the ESI†). DIPEA was again used to deprotonate 1H₂ and 2H₂, whereas diethylamine (DEA) was applied in the case of $3H_2$ due to lower pK_a values of $3H_2$ and intermediate exchange kinetics (signal coalescence) encountered by the addition of DIPEA. In this way, we were able to study the proton dissociation independently of anion binding and obtain reliable pK_a values in MeCN (Table 3).

As expected, the sulfonylurea moiety is much less acidic in MeCN compared to DMSO with the corresponding difference $pK_a(\text{MeCN}) - pK_a(\text{DMSO}) \approx 9$. In both studied solvents 2H is somewhat more prone to dissociation compared to 1H. Diprotic ligand $3H_2$ is again the most acidic SU derivative with the first pK_a value lower compared to the other two sulfonylureas.

With the dissociation constants at hand, we were able to include these data in the fitting procedure for titration curves obtained for OAc^- and $H_2PO_4^-$. Unfortunately, we could still not achieve satisfactory agreement of the experimental and calculated data by including only anion binding and proton transfer in the model. In spite of our best effort, we could not identify and quantitatively describe any other processes possibly taking place in the solution. As already mentioned, Gale *et al.* reported almost identical shape of the titration curves with several systems.^{18,40} In the case of diamidopyrrole derivatives this was rationalised by a “narcissistic dimer” formation. On the other hand, sulfonamide receptors were found to form anion complexes, but receptor deprotonation caused its dissociation as the anion concentration was increased. With the aim of resolving the underlying reactions in the present system we performed a DOSY NMR titration, measuring the diffusion coefficient of 1H in the presence of an increasing amount of DEA, OAc^- , or $H_2PO_4^-$ at different molar ratios (Fig. S56 in the ESI†). Upon addition of DEA approximately 10% decrease in diffusion coefficient at 1 : 1 molar ratio was observed for all three bases which would correspond to $\approx 50\%$ increase in molecular volume.^{41,42} Although this change is not negligible, it does not provide firm proof that dimeric species of receptors are formed in solution. Deprotonation itself might also cause the increase in effective molecular volume *via* changes in solvation or conformation of the molecule upon ionisation. As described above, in the case of DEA addition, the diffusion coefficient dropped continuously throughout the titration. In contrast, during titration with acetate or phosphate, D_{eff} decreased at a low molar ratio, but a change in the trend was detected as an excess of acetate or phosphate was added, *i.e.*, D_{eff} started to increase. This feature revealed that at least two processes occurred during the titration.

[‡] The protonation equilibrium constant for DIPEA in both solvents was measured by means of UV-Vis titration using bromochresol green or bromothymol blue as the indicator in MeCN and DMSO, respectively (Fig. S41 and S51 in the ESI†).



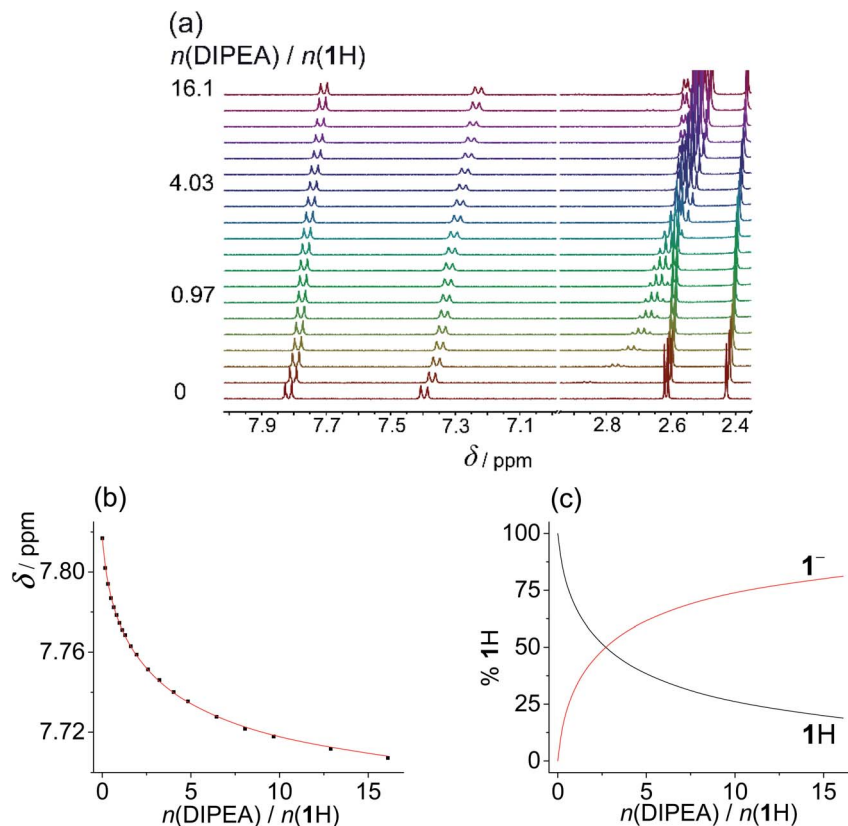


Fig. 3 (a) ^1H NMR titration of 1H ($c = 1.16 \times 10^{-3} \text{ mol dm}^{-3}$) with DIPEA ($c = 1.97 \times 10^{-2} \text{ mol dm}^{-3}$) in $\text{MeCN}-d_3$ at $(25.0 \pm 0.1)^\circ\text{C}$, $V_0 = 0.53 \text{ mL}$. (b) Dependence of H_b proton chemical shift on $n(\text{DIPEA})/n(1\text{H})$ molar ratio. ■ Experimental, – calculated. (c) Distribution of protonation species of 1H during the titration of 1H solution with DIPEA.

Considering all results gathered in this study and previously reported research, we conclude that anion binding does occur in parallel to receptor deprotonation. It should be stressed out that the receptors are roughly 1000 times more acidic than acetic acid. In spite of this, anion complexation is detected, which suggests that sulfonylureas form very strong hydrogen bonds with OAc^- and H_2PO_4^- in MeCN, and the stability of the corresponding anion complex hinders their ionisation to some extent. However, due to the high acidity of the NH_a proton, its dissociation cannot be avoided, and a complex system of equilibria is established, which prevented us from describing this system quantitatively. Interestingly, in DMSO such behaviour was not detected and only proton dissociation occurred, showcasing a distinct solvent effect on the reaction equilibria. Namely, in DMSO the dissociation is strongly favoured over

anion complexation since DMSO is polar and acts as a strong H-bond acceptor. MeCN, on the other hand, is not an H-bonds acceptor, which allows the anion complexes to be formed. Hence, the systems studied in this work represent an interesting example of solvent-control over receptor behaviour.

Conclusion

In this study three aromatic sulfonylurea derivatives were prepared and characterized. Their anion binding and proton dissociation reactions were studied in detail in acetonitrile and DMSO. The gathered results affirmed SU derivatives as good anion binders and provided insight into their structure–reactivity relationship. The performed NMR titrations indicated that strongly basic anions deprotonate SU derivatives, inhibiting their anion-binding potential. These findings revealed a striking difference in solvent effect on the underlying chemical equilibria. The presented work could serve as a strong foundation for the further development of sulfonylurea anion-receptor chemistry.

Experimental part

Synthesis

General procedure. *p*-Toluenesulfonyl isocyanate (1 mmol) and dry DCM (10 mL) were added to a round bottom flask. The

Table 3 pK_a values of 1H , 2H and 3H_2 in MeCN determined by ^1H NMR spectroscopy at 25°C using DIPEA (1H , 2H) or DEA (3H_2) as deprotonation agents^a

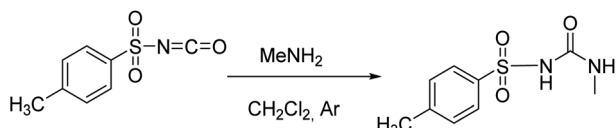
	1H	2H	3H_2
$pK_{a,1}$	19.28(1)	18.70(3)	18.01(9) ^b
$pK_{a,2}$			18.67(9) ^b

^a Uncertainties are given in parentheses as standard deviation. ^b DEA was used as base.



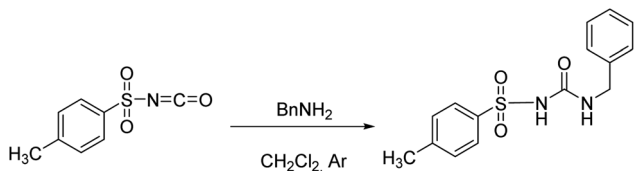
mixture was cooled to 0 °C and kept under argon. Corresponding amine (1 mmol) was added dropwise, and the reaction mixture was stirred for 24 h at RT. The product was filtered off, washed with DCM, and was used without further purification.

Synthesis of *N*-(methylcarbamoyl)-4-methylbenzenesulfonamide (**1H**)⁴³



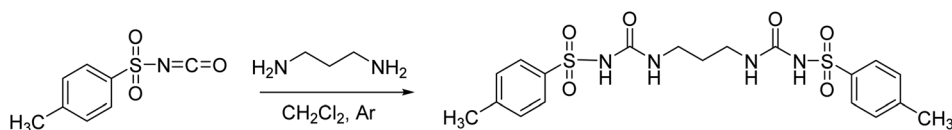
Following the general procedure, **1H** was prepared from TsNCO (774 μ L, 5.07 mmol) and methylamine (2.6 mL, 5.2 mmol, 2 M in THF). Pure product **1H** (0.4 g, 1.75 mmol, 35%) was isolated as a white powder. ¹H NMR (400 MHz, DMSO-*d*₆) δ /ppm: 10.63 (s, 1H), 7.78 (d, *J* = 8.3 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 2H), 6.37 (q, *J* = 4.7 Hz, 1H), 2.50 (s, 3H), 2.39 (s, 3H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ /ppm: 152.34, 143.99, 137.98, 129.87, 127.68, 26.66, 21.49. HRMS (ESI⁺) *m/z*: C₉H₁₂N₂O₃S [M + H]⁺ calcd: 229.0647, found: 229.0638.

Synthesis of *N*-(benzylcarbamoyl)-4-methylbenzenesulfonamide (**2H**)³⁵



Following the general procedure, **2H** was prepared from TsNCO (774 μ L, 5.07 mmol) and benzylamine (553 μ L, 5.07 mmol). Pure product **2H** (0.98 g, 3.22 mmol, 63%) was isolated as a white powder. ¹H NMR (400 MHz, DMSO-*d*₆) δ /ppm: 10.68 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.2 Hz, 2H), 7.31–7.19 (m, 3H), 7.16–7.11 (m, 2H), 6.99 (t, *J* = 6.0 Hz, 1H), 4.16 (d, *J* = 6.0 Hz, 2H), 2.40 (s, 3H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ /ppm: 151.98, 144.11, 139.62, 137.83, 129.91, 128.72, 127.72, 127.46, 127.33, 43.17, 21.51.

Synthesis of 1,1'-trimethylenebis[3-(*p*-tolylsulfonyl)-urea] (**3H₂**)



Following the general procedure, **3H₂** was prepared from TsNCO (774 μ L, 5.07 mmol) and 1,3-diaminopropane (211 μ L, 2.53 mmol). Pure product **3H₂** (0.98 g, 2.09 mmol, 41%) was isolated as a white powder and additionally triturated with MeOH. ¹H NMR (400 MHz, DMSO-*d*₆) δ /ppm: 10.30 (s, 2H), 7.77 (d, *J* = 8.4 Hz, 4H), 7.37 (d, *J* = 8.1 Hz, 4H), 6.51 (t, *J* = 6.0 Hz, 2H), 2.86 (q, *J* = 6.4 Hz, 4H), 2.37 (s, 6H), 1.36 (p, *J* = 6.7 Hz, 2H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ /ppm: 152.30, 143.84, 138.17,

129.82, 127.60, 36.94, 30.15, 21.48. HRMS (ESI⁺) *m/z*: C₁₉H₂₄N₄O₆S₂ [M + H]⁺ calcd: 469.1216, found: 469.1199.

Solution studies

Materials. The solvents used (acetonitrile (MeCN, J. T. Baker, HPLC Grade), deuterated acetonitrile (MeCN-*d*₃, Eurisotop, >99.8%), dimethyl sulfoxide (DMSO, Sigma-Aldrich, spectro-photometric grade) and deuterated dimethyl sulfoxide (DMSO-*d*₆, Eurisotop, >99.8%)) were used as received. The salts used were tetrabutylammonium dihydrogen phosphate (TBAH₂PO₄, Sigma-Aldrich, >97%), tetrabutylammonium acetate (TBAOAc, Sigma-Aldrich, >97%), tetraethylammonium chloride (TEACl, Sigma-Aldrich, >98%), tetrabutylammonium bromide (TBABr, Sigma-Aldrich, >98%), tetrabutylammonium iodide (TBAl, Sigma-Aldrich, >99%), tetrabutylammonium hydrogensulfate (TBAHSO₄, Sigma-Aldrich, >97%) and tetrabutylammonium nitrate (TBANO₃, Sigma-Aldrich, >99%). Bromocresol green (BCGH₂, Kemika, >95%), bromothymol blue (BTBH₂, Kemika, >95%), diethylamine (DEA, Sigma-Aldrich > 98%) and *N,N*-diisopropylethylamine (DIPEA, Sigma-Aldrich > 99.0%) solutions were standardised prior to use by means of potentiometric titrations. A known amount of titrant was dissolved in water and titrated with the standardised solution of hydrochloric acid (in the case of DIPEA and DEA) or sodium hydroxide (in the case of BCGH₂ and BTBH₂). The concentration was calculated using the inflection point of the obtained titration curves.

NMR titrations. NMR spectra were recorded using a Bruker Avance III HD 400 MHz/54 mm Ascend spectrometer. The temperature was kept constant at 25 °C. Chemical shifts are reported in ppm and referenced to the residual solvent signal. Calibrated syringes (Hamilton) were used for the addition of titrant and titrant solutions. All NMR titration data were processed by nonlinear regression analysis using the HypNMR program.⁴⁴

In all cases the fitting procedure was performed in a multivariate fashion, and all proton signals which exhibited significant changes and could be monitored throughout the titration were included in the data processing. Concentration dependencies of ¹H NMR spectra of all investigated receptors in MeCN-*d*₃ and DMSO-*d*₆ at 25 °C were acquired to dismiss the

possibility of ligand aggregation. The concentration was varied by stepwise addition of receptors stock solutions to MeCN-*d*₃ and DMSO-*d*₆ covering the range $8.0 \times 10^{-5} < c$ (receptors)/mol dm⁻³ < 2.0×10^{-2} .

Protonation properties of receptors. Protonation constants of investigated receptors in MeCN and DMSO were determined by means of ¹H NMR titrations with DIPEA or with DEA in the case of **3H₂** in MeCN. Considering titrations in MeCN, solution



of DIPEA ($c \approx 2.0 \times 10^{-2} \text{ mol dm}^{-3}$) was added to solutions of 1H or 2H ($c \approx 1.2 \times 10^{-3} \text{ mol dm}^{-3}$, $V_0 = 0.53 \text{ mL}$) in MeCN- d_3 or in the case of 3H₂ solution of DEA ($c = 7.7 \times 10^{-3} \text{ mol dm}^{-3}$) was added to solution of 3H₂ ($c = 1.1 \times 10^{-3} \text{ mol dm}^{-3}$, $V_0 = 0.50 \text{ mL}$) in MeCN- d_3 at 25 °C. Considering titrations in DMSO, solution of DIPEA ($c \approx 1.5 \times 10^{-2} \text{ mol dm}^{-3}$ in the case of 1H and 2H and $c = 6.6 \times 10^{-2} \text{ mol dm}^{-3}$ in the case of 3H₂) was added to solutions of receptors ($c \approx 1.2 \times 10^{-3} \text{ mol dm}^{-3}$, $V_0 = 0.53 \text{ mL}$ or $V_0 = 0.50 \text{ mL}$) in DMSO- d_6 at 25 °C.

Protonation constants of receptors in DMSO were also studied by means of ¹H NMR titrations with TBAOAc and TBAH₂PO₄. Solution of TBAOAc ($c \approx 1.2 \times 10^{-2} \text{ mol dm}^{-3}$ in the case of 1H and 2H or $c = 8.2 \times 10^{-3} \text{ mol dm}^{-3}$ in the case of 3H₂) or TBAH₂PO₄ ($c \approx 1.0 \times 10^{-2} \text{ mol dm}^{-3}$ in the case of 1H and 2H or $c = 8.6 \times 10^{-3} \text{ mol dm}^{-3}$ in the case of 3H₂) was added to solutions of receptors ($c \approx 1.1 \times 10^{-3} \text{ mol dm}^{-3}$, $V_0 = 0.53 \text{ mL}$ or $V_0 = 0.50 \text{ mL}$) in DMSO- d_6 at 25 °C.

In the data fitting procedure, the protonation constant of DIPEA in MeCN and DMSO was kept fixed at the value determined spectrophotometrically and the protonation constant of DEA in MeCN was kept fixed at the literature value ($\log K^{\text{H}}(\text{DEA}) = 18.8$).^{45,46} Processes defining acid–base properties of acetic and phosphoric acid in DMSO (protonation, homoassociation and dimerisation) were accounted for and their equilibrium constants were kept fixed at the values recently reported by us: ($\log K^{\text{H}}(\text{AcOH}) = 12.82$, $\log K(\text{AcOH} \cdot \text{OAc}^-) = 2.45$, $\log K_{\text{d}}((\text{AcOH})_2) = 1.45$, $\log K^{\text{H}}(\text{H}_3\text{PO}_4) = 10.80$, $\log K_{\text{d}}((\text{H}_2\text{PO}_4^-)_2) = 2.26$, $\log K(\text{H}_3\text{PO}_4 \cdot \text{H}_2\text{PO}_4^-) = 4.23$, $\log K(\text{H}_3\text{PO}_4 \cdot (\text{H}_2\text{PO}_4^-)_2) = 2.92$).³⁹ In the case of titration of 3H₂ with DEA in MeCN- d_3 , ¹H NMR chemical shifts of protons characteristic for a protonated form of 3H₂ were kept fixed at the values acquired prior to the addition of DEA. In the fitting procedure regarding titrations of 3H₂ with TBAOAc in DMSO, $\log K^{\text{H}}(3\text{H}_2)$ was kept fixed at the value determined by ¹H NMR titration with TBAH₂PO₄. The protons chemical shifts of the protonated form of 3H₂ were kept fixed at the values acquired prior to the addition of titrant solutions.

Anion complexation. Anion complexation properties of studied compounds were investigated by performing ¹H NMR titrations of receptors with solutions of TBAOAc, TBAH₂PO₄, TEACl, TBABr, TBAI, TBAHSO₄ and TBANO₃ in MeCN- d_3 and DMSO- d_6 at 25 °C. Solutions of salts were added in a stepwise manner to the solutions of investigated receptors ($c \approx 1.2 \times 10^{-3} \text{ mol dm}^{-3}$, $V_0 = 0.53 \text{ mL}$, or $V_0 = 0.50 \text{ mL}$). The concentration of the titrant solutions differed significantly ($7.7 \times 10^{-3} \text{ mol dm}^{-3}$ to $6.0 \times 10^{-1} \text{ mol dm}^{-3}$), depending on the system, *i.e.* the equilibrium constant of the binding process.

Spectrophotometry

Protonation constants of DIPEA. Protonation constants of DIPEA in MeCN and DMSO were determined at 25 °C by means of spectrophotometric titrations which were carried out by adding a solution of DIPEA ($c \approx 9.2 \times 10^{-4} \text{ mol dm}^{-3}$) to the solution of bromocresol green ($c \approx 4.0 \times 10^{-5} \text{ mol dm}^{-3}$, $V_0 = 2.08 \text{ mL}$) in the case of MeCN or by adding a solution of DIPEA ($c \approx 3.4 \times 10^{-2} \text{ mol dm}^{-3}$) to the solution of bromothymol blue ($c \approx 8.4 \times 10^{-5} \text{ mol dm}^{-3}$, $V_0 = 2.36 \text{ mL}$) in the case of DMSO. Spectrophotometric data were processed by nonlinear regression analysis using the HypSpec program.⁴⁷ In the course of data

analysis, protonation constants of bromocresol green and bromothymol blue were kept fixed at the literature value ($\log K_1^{\text{H}}(\text{BCGH}_2) = 18.5$, $\log K_2^{\text{H}}(\text{BCGH}_2) = 11.0$, $\log K^{\text{H}}(\text{BTBH}_2) = 11.3$).^{48,49}

Spectrophotometric titrations were carried out at (25.0 ± 0.1) °C by means of a Varian Cary 5 spectrophotometer equipped with a thermostating device. The titrant solution was added in stepwise fashion directly into the measuring quartz cell (Hellma, Suprasil QX, $l = 1 \text{ cm}$) using calibrated syringes (Hamilton). The spectral changes were recorded after each addition. Absorbances were sampled at 1 nm intervals with 0.2 s integration time. All titrations were done in triplicate.

Conflicts of interest

There are no conflicts of interest to declare.

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