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**Original article**

# Isosterism among analogues of torasemide: conformational, electronic and lipophilic properties

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**Abstract** – The structures, electronic (charges, molecular electrostatic potential, molecular orbitals) and lipophilic properties of three isostere analogues of torasemide were determined and the influence of the replacement of the sulfonyl urea group on the conformation and electronic properties of the molecules is discussed. Lipophilicity of the compounds seems to be the most discriminating property along the series and affects their pharmacological activities. © 2000 Éditions scientifiques et médicales Elsevier SAS

**isosterism / sulfonyl(thio)urea / steroelectronic properties / lipophilicity**

## 1. Introduction

Nonclassical bioisosteric molecules are characterised by a different number of atoms, by similar physicochemical parameters, and by a broadly similar biological activity [1]. This concept of nonclassical bioisosterism has been applied to the sulfonyl-urea function of torasemide (**1**), a loop diuretic [2]. This strategy led to the design and synthesis of the corresponding sulfonyl-thiourea (**2**), -cyanoguanidine (**3**), and -diaminonitroethylene (**4**) (*figure 1*). In order to better understand the influence of the replacement of the (thio)urea function by a cyanoguanidine or 1,1-diamino-2-nitroethylene group, a series of structural, electronic (charges, molecular electrostatic potential, molecular orbitals) and lipophilic properties of isostere analogues of torasemide were determined. The parameters that were studied are similar to those extensively applied to determine QSAR parameters among sulfonamides, some of which possessing diuretic properties [3, 4]. In the present study, a qualitative interpretation of the influence of the replacement of the sulfonylurea group on the properties of the molecules was also discussed. Due to the limited number of compounds investigated, no quantitative correlations between the biological data and the calculated properties were performed.

## 2. Chemistry

The sulfonyl-urea (**1**) and -thiourea (**2**) were obtained by the reaction of the sodium salt of the sulfonamide with isoproyl-isocyanate or -isothiocyanate, respectively (*figure 1*) [5, 6]. Addition of *N*-isopropyl *N*-cyano-*S*-carbamidothiolate or 1-isopropylamino-1-methylthio-2-nitroethylene gave the sulfonylcyanoguanidine (**3**) [7] and the sulfonamidonitroethylene (**4**) [8], respectively.

*Abbreviations:* HOMO, highest occupied molecular orbital; MEP, molecular electrostatic potential; MES, maximum electroshock seizures test; RP-HPLC, reverse-phase high performance liquid chromatography.

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## 3. Results and discussion

In the present work, the crystal structures of **2** and **4** (*figure 2*) have been determined by X-ray crystallography and compared to the crystal structures of the analogues **1** and **3** [9] (*table I*). For torasemide (**1**) four conformations are observed in different crystal environments [9]. A total of seven experimental conformations are thus observed for molecules **1**–**4** (*figure 3*). Among those crystal structures, and according to previous studies [7, 10], four different conformations (α, β, γ, δ) were defined, based on the values of the torsion angles ϕ1–ϕ4 (*table I*). In particular, torasemide (**1**) can adopt three of those theoretical conformations (α, β, γ). Compounds **2** and **4** adopt conformations accessible to **1,** while **3** adopts a

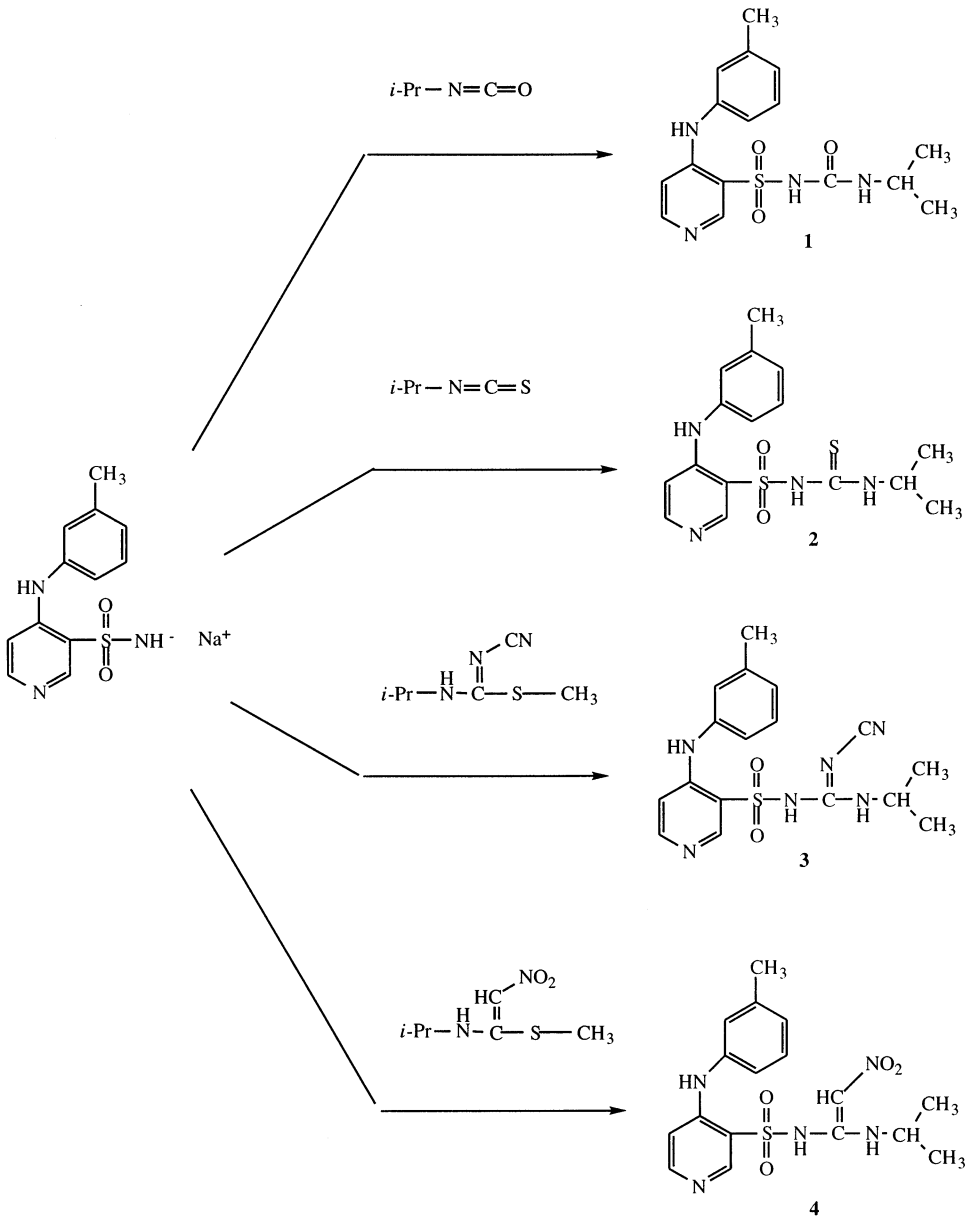



**Figure 1.** Synthesis and structure of torasemide and its bioisosters.

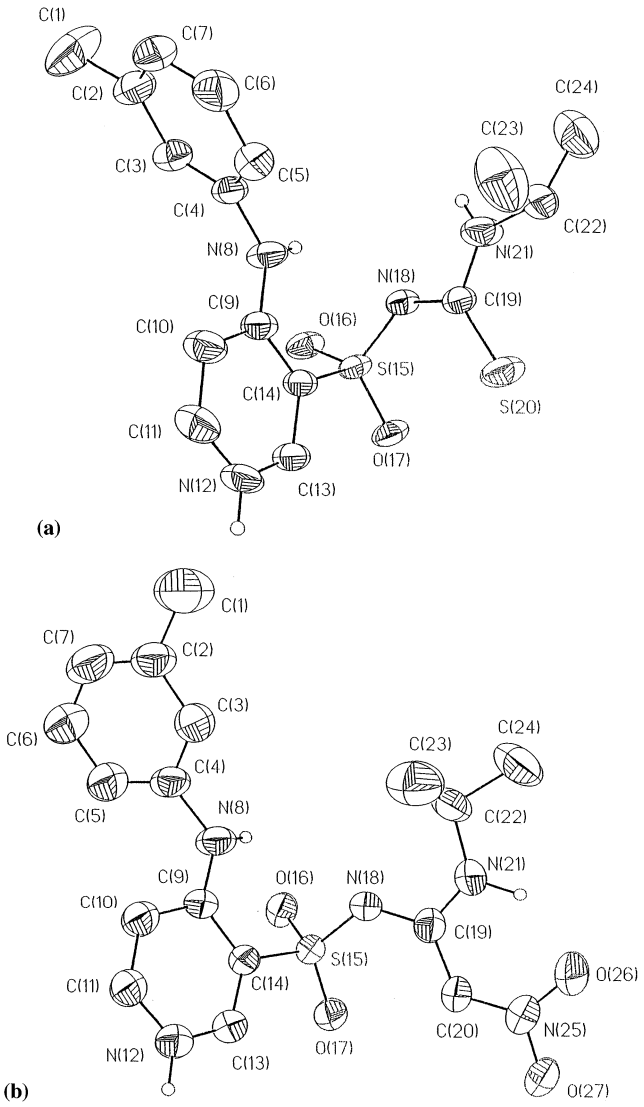



**Figure 2.** Crystal structure conformations (ORTEP diagram) of molecules **2** and **4**. Observed hydrogens on nitrogens are included in order to underline the zwitterionic character of both compounds.

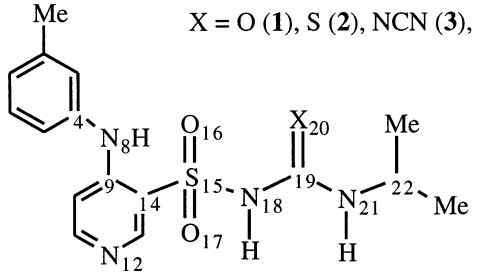
distinct conformation (δ, *table I*). It has been postulated [7] that **3** adopts this distinct conformation for steric reasons (hindrance of the N–CN group).

In the solid state, the four compounds **1**–**4** adopt a zwitterionic form resulting from the transfer of the sulfonamide proton to the nitrogen atom of the pyridine ring. Indeed, the acidity ($pK_a$) of the $-SO_2NH-$ function is ranging from 6.0 to 6.7 (*table II*). Analysis of the intermolecular interactions, in particular the fact that the protonated nitrogen in the pyridinium ring serves as a hydrogen donor in strong H bonds in the crystal structures is in agreement with the zwitterionic structures. For compound **2**, an intramolecular H bond involves nitrogen $N_8$ and the unprotonated $N_{18}$ nitrogen atom: $N_8$–H…$N_{18}$ ($N_8$...$N_{18}$ = 2.956(3) Å, H…$N_{18}$ = 2.329 Å, ∠$N_8$–H…$N_{18}$ = 130.0°). A second intermolecular H bond connects the protonated nitrogen $N_{12}$ of the pyridinium ring to an oxygen of the sulfonamido moeity: $N_{12}$–H...$O^i_{17}$ ($N_{12}$...$O^i_{17}$ = 2.763(3) Å, H...$O^i_{17}$ = 1.991 Å, ∠$N_{12}$–H...$O^i_{17}$ = 148.9° with $i = 3/2 - x, -1/2 + y, 1/2 - z$). In compound **4**, the protonated nitrogen of the pyridinium ring interacts with the diaminonitroethene group through an intermolecular H bond: $N_{12}$–H...$O^{ii}_{27}$ ($N_{12}$...$O^{ii}_{27}$ = 2.726(5) Å, H...$O^{ii}_{27}$ = 1.768 Å, ∠$N_{12}$–H...$O^{ii}_{27}$ = 170.7° with $ii = -x, 1/2 + y, -1/2 - z$). An intramolecular H bond between $N_{21}$ and $O_{26}$ (of the nitro group) imposes to **4** a β-conformation ($N_{18}$–$C_{19}$–$N_{21}$–$C_{22}$ close to 0°) which is not adopted by **2** (*figure 2*, *table I*).

The main bond lengths and valence angles are compared in *table I*. Those geometric parameters are comparable among all four compounds and compatible with values reported in the literature for acyclic amides and ureas [11]. Thus, the sulfonyl thiourea (**2**), sulfonyl cyanoguanidine (**3**), or sulfonamido nitroethylene (**4**) groups influence the geometry of the molecules in a way similar to that of the sulfonyl urea function of torasemide (**1**). The short $S_{15}$–$N_{18}$, $N_{18}$–$C_{19}$, $C_{19}$–$N_{21}$, $C_{19}$–$X_{20}$ bond lengths (*table I*) suggest electronic delocalization for all the substituted sulfonyl amido functions investigated. Delocalization within the pyridyl ring is not excluded although the observed $C_{14}$–$S_{15}$ bonds are rather long in comparison with those reported in arylsulfonamide ($C_{Ar}$–$SO_2NX_2$ = 1.758(18) Å) [10]. The greater acidity of the proton on $N_{18}$ versus $N_{21}$ can be partially explained by the stability of the corresponding basis: $N_{18}$ is conjugated to the sulfone group that presents a greater attractive effect. Moreover, in compound **4**, $N_{21}$ is implied in an intra-molecular H bond with the nitro group, requiring the presence of the proton on this nitrogen.

The experimental structures of **1**–**4** have been used as input to theoretical calculations in order to further study their conformations. Theoretical conformational analysis (molecular mechanics optimisations using the Discover program (MSI, San Diego) with the cvff Force Field) showed that a similar geometry corresponding to conformation β is accessible to all

**Table I.** Comparison of bond lengths (Å) and angles (°) in molecules **1**–**4** (standard deviations in parentheses).




$\phi1 = C9\text{-}C14\text{-}S15\text{-}N18$; $\phi2 = C14\text{-}S15\text{-}N18\text{-}C19$ ;
$\phi3 = S15\text{-}N18\text{-}C19\text{-}N21$ ; $\phi4 = N18\text{-}C19\text{-}N21\text{-}C22$

Theoretical conformations [7, 10]:

| | | | | |
|---|---|---|---|---|
| α : | $\phi1$=-90° | $\phi2$=+90° | $\phi3$=180° | $\phi4$=180°; |
| β : | $\phi1$=+90° | $\phi2$=+90° | $\phi3$=180° | $\phi4$=0°; |
| γ : | $\phi1$=+90° | $\phi2$=+90° | $\phi3$=180° | $\phi4$=180°; |
| δ : | $\phi1$=+90° | $\phi2$=+90° | $\phi3$=0° | $\phi4$=180° |

| | **1 (TIA)**[a] | **1 (TIB)**[a] | **1 (TIIA)**[a] | **1 (TIIB)**[a] | **2** | **3** | **4** |
|---|---|---|---|---|---|---|---|
| $C_4$–$N_8$ | 1.421(5) | 1.428(5) | 1.447(7) | 1.432(7) | 1.440(3) | 1.407(3) | 1.448(5) |
| $N_8$–$C_9$ | 1.355(4) | 1.347(5) | 1.342(7) | 1.333(7) | 1.337(3) | 1.359(3) | 1.338(5) |
| $C_{14}$–$S_{15}$ | 1.763(4) | 1.792(4) | 1.773(6) | 1.784(5) | 1.779(3) | 1.783(2) | 1.757(4) |
| $S_{15}$–$N_{18}$ | 1.641(3) | 1.574(3) | 1.567(4) | 1.562(5) | 1.563(2) | 1.576(2) | 1.578(3) |
| $N_{18}$–$C_{19}$ | 1.386(5) | 1.345(5) | 1.370(7) | 1.381(7) | 1.368(3) | 1.375(3) | 1.339(5) |
| $C_{19}$–$N_{21}$ | 1.344(6) | 1.348(6) | 1.345(7) | 1.342(8) | 1.332(3) | 1.322(3) | 1.322(5) |
| $C_{19}$–$X_{20}$ | 1.228(5) | 1.272(5) | 1.251(7) | 1.244(7) | 1.686(2) | 1.334(3) | 1.434(6) |
| $C_4$–$N_8$–$C_9$ | 128.1(3) | 126.8(3) | 123.6(5) | 124.3(5) | 123.8(2) | 133.1(2) | 125.0(3) |
| $S_{15}$–$N_{18}$–$C_{19}$ | 121.2(3) | 119.1(3) | 117.7(4) | 117.9(4) | 123.8(2) | 124.8(2) | 127.7(3) |
| $N_{18}$–$C_{19}$–$N_{21}$ | 113.8(4) | 116.2(4) | 111.9(4) | 113.4(5) | 110.5(2) | 122.8(2) | 115.7(3) |
| $C_{19}$–$N_{21}$–$C_{22}$ | 121.9(4) | 125.6(4) | 122.2(5) | 123.5(6) | 127.3(2) | 125.0(2) | 125.8(3) |
| Conformation | α* | β | γ | γ | γ | δ | β |
| $\phi_1$ | −105.7(3) | 77.5(3) | 47.7(4) | 51.3(4) | 46.2(2) | 70.6(2) | 66.5(4) |
| $\phi_2$ | 65.4(3) | 71.7(3) | 60.8(4) | 62.3(4) | 65.7(2) | 88.4(2) | 108.4(3) |
| $\phi_3$ | −179.8(3) | 172.0(3) | 162.7(4) | −163.1(4) | −166.7(2) | 21.8(3) | 176.6(3) |
| $\phi_4$ | 177.3(5) | −14.3(5) | 170.1(6) | −178.2(6) | 172.1(3) | 179.2(2) | −1.8(6) |

[a] Four distinct conformations have been reported for torasemide (**1**). X=O (**1**), S (**2**), N (**3**), C (**4**).

four compounds and that it could correspond to their bioactive conformation [7, 8]. It has been suggested [7] that the differences in biological properties could be partially related to the facility of the compounds of adopting the bioactive conformation.

Many studies have been published recently regarding molecular quantum and QSAR calculations of sulfonamides. For example, it has been demonstrated that the carbonic anhydrase inhibitory properties of sulfonamides are also determined by charge factors such as the charges on the atoms of the sulfonamide group and the dipole moment [3, 4]. Calculation of electronic properties such as charges, molecular electrostatic potential, molecular orbitals have been calculated for molecules **1**–**4** (*table III*). Due to the limited number of compounds investigated in the present work, no quantitative correlations between the biological data and those calculated properties can be developed. Some qualitative trends can however be extracted among the different isosters.

In particular, similar charge distributions are observed among our series (*table III*) consistent with the experimental geometries (bond lengths and angles) and suggesting a conserved delocalization profile among the series.

On all fragments, the highest occupied molecular orbital (HOMO) is of π type with a high density on $C_{19}$, $X_{20}$ (X = O, S, N–CN, CH–$NO_2$), and $N_{21}$. The energies of both frontier orbitals are given in *table III*. They do not correlate with the biological data.

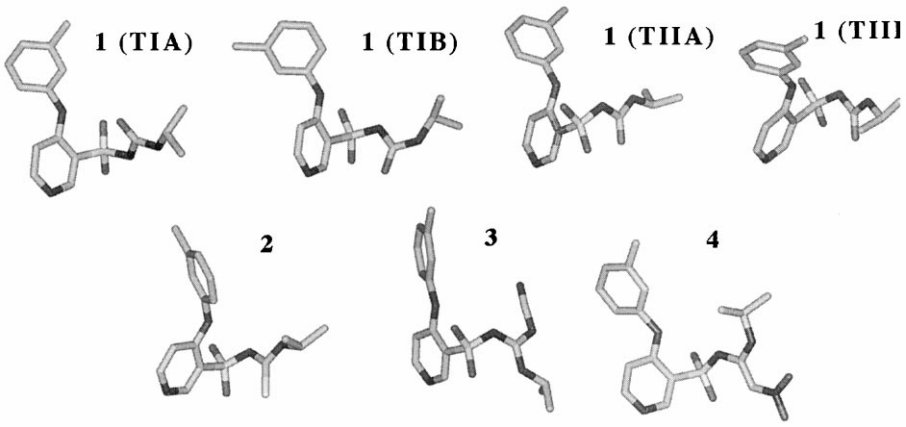



**Figure 3.** Molecular conformations of **1–4**. All conformations were deduced from crystallographic studies.

**Table II.** Experimental physicochemical properties (lipophilicity and acidity) and biological data (diuretic and anticonvulsive activities) associated to molecules **1–4**

| | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| log $P$[a] | $+0.45^{16}$ | $+0.61$ | $+0.86^{7}$ | $+1.12^{8}$ |
| p$K_a$[b] | 6.68 | 6.74 | 6.00 | 6.25 |
| | $\pm 0.01^{14}$ | $\pm 0.01$ | $\pm 0.01^{7}$ | $\pm 0.02$ |
| OD2x (mg/kg)[c] | 1.6 | 4.4 | 9.1 | >30 |
| MES test protection[d] | 0/6 | – | 0/6 | 2/6 |

[a] log $P$ is the logarithm of the partition coefficient in 1-octanol/phosphate buffer pH 7.40 [7, 8, 16].
[b] p$K_a$ of the sulfonamido group was determined by titration of the sodium salt [7, 14].
[c] Oral dose that double the urinary volume excreted by control rats over 4 h following administration [7].
[d] Rate of protection of mice against convulsions induced by maximal electroshock (50 mA for 0.2 s) applied 3 h after intraperitoneal administration of 30 mg/kg [17].

Based on the shape of the molecular electrostatic potential (MEP) (data not shown), two topologies emerge; one associated to **1** and **2** and the second to **3** and **4**. Those differences do not seem sufficient to explain entirely the variations in biological properties but do certainly play a role during the molecular recognition of the target receptor(s).

At physiological pH (7.4), the lipophilicity of the isosteres (**1–4**), expressed as the logarithm of the partition coefficient (log $P$) in a $n$-octanol–phosphate buffer system, is ranging as follows (*table II*): **4** > **3** > **2** > **1**, the nitroethylene compound being the most lipophilic. Among all the properties studied, lipophilicity of the compounds seems to be the most discriminating property along the series and certainly affects their pharmacological properties. Indeed, the most lipophilic molecule (**4**) can cross the blood–brain barrier and exert a moderate anticonvulsive activity [8]. In contrast, the poor log $P$ value of **1**, **2** and **3** prevents a central activity. On the other hand, the diuretic activity of these molecules is the result of the inhibition of the $Na^+$ $K^+2Cl^-$ cotransporter located on the luminal membrane of the thick ascending limb of the loop of Henle [2, 7]. Generally, the glomerular filtration is enhanced for hydrophilic compounds and the tubular reabsorption increased for lipophilic compounds [12]. According to these considerations, the diuretic properties of **1–4** are negatively related to their lipophilicity, probably due to their luminal concentration (*table II*).

In conclusion, sulfonylcyanoguanidines and sulfonamidonitroethylenes are original isosteres of the sulfonyl (thio)urea function as they share common geometric and electronic properties. However, they cover a range of lipophilicity that makes them suitable for pharmocomodulation of compounds as illustrated by the diuretic and antiepileptic properties of analogues of torasemide.

## 4. Experimental protocols

### *4.1. Chemistry*

The synthesis of compounds **1** [5], **3** [7] and **4** [8] has been described previously. The sulfonylthiourea **2** was

**Table III.** Atomic charges (hydrogen charges summed on heavy atom), dipole moments (Debye), and frontier orbital energies calculated (ab initio, 3-21G*) on molecules **1**–**4**

| Atomic charges (*e*) | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| S15 | 1.622 | 1.641 | 1.633 | 1.652 |
| O16 | −0.613 | −0.617 | −0.563 | −0.593 |
| O17 | −0.568 | −0.552 | −0.568 | −0.572 |
| N18 | −0.657 | −0.644 | −0.656 | −0.666 |
| N21 | −0.569 | −0.528 | −0.552 | −0.526 |
| C19 | 1.224 | 0.638 | 1.162 | 0.886 |
| X20 | O −0.655 | S −0.205 | N −0.799 | C 0.272 |
| | | | C 0.642 | N 0.194 |
| | | | N −0.504 | O −0.465 |
| Dipole moment (Debye) | 5.993 | 6.147 | 11.201 | 7.597 |
| HOMO energy (ua) | −0.40997 | −0.33655 | −0.36702 | −0.34498 |
| LUMO energy (ua) | 0.20781 | 0.12528 | 0.13714 | 0.07248 |

obtained from the reaction of the sodium salt of 4-(3′-methylphenylamino)pyrid-3-yl sulfonamide (2.0 g, 7.6 mmol) [6] and isopropyl isothiocyanate (2 mL, 19.1 mmol) in 50 mL of water–acetone (50:50) at room temperature. At the end of the reaction (2 h), the solvent was removed under reduced pressure, the residue dissolved in water (70 mL) and 2.5 N NaOH (10 mL). The solution was extracted three times with diethyl ether (80 mL) and adjusted to pH 7 with dilute hydrochloric acid. The precipitate was collected by filtration, washed with water, dried and crystallised in ethanol to afford 1.78 g of **2**. (yield: 64.3%). Mp: 182–184 °C; $^1$H-NMR (DMSO-$d_6$, 80 MHz) $\delta$: 1.05 (2s, 6H, C(C$H_3$)$_2$), 2.51 (s, 3H, aryl-C$H_3$), 4.20 (m, $^1$H, C$H$(CH$_3$)$_2$), 7.13–7.21 (m, 4H, phenyl), 7.43 (d, 5$H$-pyridine), 8.22 (d, 1H, 6$H$-pyridine), 8.65 (s, 1H, 2$H$-pyridine), 9.77 (br s, 1H, pyridine–N$H$. For C, H, N, S, the elemental analysis corresponds to the theoretical values of the title compound ($C_{16}H_{20}N_4O_2S_2$).

### 4.2. Crystallographic structures

Crystallographic data for compounds **2** and **4** were collected on single crystals obtained by slow evaporation, using an Enraf Nonius CAD4 diffractometer with a copper anode ($\lambda$ (Cu K$\alpha$) = 1.54178 Å). The following parameters were obtained for **2** and **4**.

Compound **2** : $C_{17}H_{20}N_4O_2S_2$, monoclinic, $P2_1/n$, $a$ = 10.307(2) Å, $b$ = 9.782(2) Å, $c$ = 18.006(4) Å, $\beta$ = 93.54(3)°, $V$ = 1811.9(7) Å$^3$, $Z$ = 4, $\mu$ = 1.72 mm$^{-1}$, $D_x$ = 1.263 g cm$^{-3}$, $F(000)$ = 728, $T$ = 290 K, 2602 unique reflections ($R_{int}$ = 0.017), $R_1$ = 0.0399 for 2378 $F_o > 4\sigma(F_o)$ and $wR_2$ = 0.1104, GOF = $S$ = 1.066.

Compound **4**: $C_{17}H_{21}N_5O_4S$, monoclinic, $P2_1/c$, $a$ = 9.381(2) Å, $b$ = 13.848(2) Å, $c$ = 14.985(4) Å, $\beta$ = 94.38(3)°, $V$ = 1941.0(7) Å$^3$, $Z$ = 4, $\mu$ = 0.20 mm$^{-1}$, $D_x$ = 1.340 g cm$^{-3}$, $F(000)$ = 824, $T$ = 290 K, 3813 unique reflections ($R_{int}$ = 0.069), $R_1$ = 0.0682 for 2058 $F_o > 4\sigma(F_o)$ and $wR_2$ = 0.1881, GOF = $S$ = 0.967.

Full matrix least-squares on $F^2$ using the program SHELXL97 [13] were used for both refinements. Supplementary material has been deposited at the IUCr.

### 4.3. Acidity constants

Acidity of the molecules, expressed in terms of the p$K_a$ of the sulfonamido group, was determined by titration of the corresponding sodium salt [14].

### 4.4. Calculated properties

Electronic properties (atomic charges, dipole moments, molecular electrostatic potential, molecular orbital energies and topologies) were computed by quantum mechanics (ab initio RHF, 3-21G* basis set) using the Gaussian94 suite of programs [15] on methyl sulfonyl urea, thiourea, cyanoguanidine, and sulfonamido nitroethylene fragments. The starting geometries were taken from the crystal structures and optimised ab initio.

### 4.5. Lipophilicity

Lipophilicity of the molecules is given by their log $P$ value, the logarithm of the partition coefficient in 1-octanol/phosphate buffer pH 7.40 [8].

### 4.6. Biological activities

Diuretic activity was expressed in terms of the oral dose (OD2x) that doubles the urinary volume excreted by control rats over 4 h following administration [7].

Maximum electroshock seizures (MES) tests gave access to the rate of protection of mice against convulsions induced by maximal electroshock (50 mA for 0.2 s) applied 3 h after intraperitoneal administration of 30 mg/kg of the compound under study [17].

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