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Bitter sweeteners: tetrazole derivatives of arylsulfonylalcanoids – synthesis, structure and comparative study

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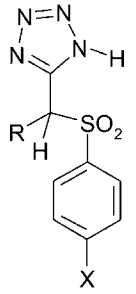
Within a research project aimed at the design of new sweeteners, the tetrazole moiety was introduced to arylsulfonylalkanoic acids (ASA) as a bioisostere of the carboxyl group. The crystal structures of four newly synthesized tetrazole derivatives and one intermediate product of the reaction were determined in order to explain the bitter taste of these compounds. Three chiral compounds crystallize as racemic mixtures in centrosymmetric space groups of the monoclinic system, whereas the non-chiral compound, with a higher dipole moment, crystallizes in the polar space group *Cc*. Intermolecular N—H···N hydrogen bonds between tetrazole moieties were observed in all four structures and are compared with the analogous interactions observed in tetrazole derivatives deposited in the Cambridge Structural Database (CSD). Specifically, the typical N1—H···N4 as well as N1—H···N3 interactions, which are less abundant in the CSD, are described. The formation of the latter interaction type can be hypothetically explained by an asymmetry of π-electron distribution in the tetrazole rings caused by the crystalline environment. Important features of the crystal architecture are the chains of molecules linked by N—H···N bonds. A possible reason for the lack of a sweet taste of the tetrazoles investigated may be the improper position of the tetrazole H atom, and the mutual orientation of the proton donor and acceptor in their molecules. This orientation does not allow the tetrazoles to interact with the sweet-taste receptor in a way similar to that of ASA. The bitter taste of the investigated compounds needs further study.

1. Introduction

Effective artificial sweeteners are still sought after in many research laboratories. Since a considerable number of available sweeteners are not harmless for some populations, especially for those who suffer from certain diseases (*e.g.* aspartame for people with phenylketonuria, diabetes, Parkinson's disease), the search for new, better sweeteners is still important. Moreover, there is much inconsistent information about the safety of sweetening compounds which are used in the food industry at the moment. Developing a new synthetic sweetener is a complex problem, since such compounds need to have excellent taste quality, comparable to that of sucrose, and their use should be completely safe (Polański *et al.*, 2000). Another difficulty is the design of syntheses: sometimes bitter compounds are obtained instead of new potential sweeteners (Polański & Jarzembek, 2002; Owens *et al.*, 1991), which is not surprising because bitter and sweet tastes are strongly related (Shallenberger, 1996).

Table 1
Taste quality of described tetrazoles.

Compound number	*X*	*R*	Taste quality	Formula moiety
(1)	CH_3	CH_3CH_2	Bitter	
(2)	Cl	CH_3	Bitter	
(3)	CH_3O	CH_3CH_2	Bitter	
(4)	H	H	Bitter	

Sweet-taste chemoreception requires an interaction between a sweetener and the sweet-taste receptor located in the taste buds spread on the surface of the tongue. The classical theories (Shallenberger, 1996; Kier, 1972) postulate the occurrence of three specific sites in the molecule of the sweetener: proton donor (*A*H), proton acceptor (*B*) and a hydrophobic group (*X*). More sophisticated models were also proposed (Nofre & Tinti, 1996; Walters, 1995; Barker *et al.*, 2002), but the relationship between the molecular structure and the taste of sweeteners is still not elucidated. In the past few years, during which the human genome has being gradually described, the gene Tas1R3, which most probably encodes the G-protein-coupled receptors T1R1, T1R2 and T1R3, was discovered (Montmayeur & Matsunami, 2002). These receptors exist in a taste cell as heterodimers, from which the T1R2/3 heterodimer was recognized as a sweet-taste receptor (Margolskee, 2002; Temussi, 2002). The bitter taste is still less well understood since it is less interesting from a commercial point of view. However, the recognition of a bitter taste is very important because it is a signal that the substance can be a poison (Chandrashekar *et al.*, 2000; Margolskee, 2002). This protective function might explain the existence of a large family of selective T2Rs that were recognized as bitter-taste receptors, their number being estimated at 40–80. All these proteins differ from each other in the sequence of amino acid residues.

According to the recent studies of taste receptors, the binding pockets for sweet compounds are localized in the extracellular part of a protein and some of them can be in the membrane part (Morini *et al.*, 2005). For bitter compounds the binding pocket is probably localized only in the membrane part of the receptor (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Margolskee, 2002).

Until now neither the sweet- or bitter-taste receptor nor their complexes with any sweet or bitter molecule, respectively, have been obtained in the crystalline form. This is the reason why the glucophore and picrophore and the manner of their interactions with the active site of the receptor is still a matter of speculation.

In previous papers a series of sweet-tasting arylsulfonylalkanoic acids (ASA) were described (Łysiak *et al.*, 2005). This prompted us to further investigate related compounds and their taste quality. Recently we described a taste–structure study of different compounds containing the sulfonyl group (Polański & Jarzembek, 2002; Polański & Ratajczak, 1993).

An interesting group of compounds with potential sweetening properties are tetrazoles, which are known bioisosteres of carboxylic acids (Herr, 2002; Kraft *et al.*, 2002; Tinti & Nofre, 1991) employed in the syntheses of new drugs and sweeteners. This method has already been practised in sweetener chemistry for a couple of years, but some of the tetrazole surrogates of sweet-tasting molecules unexpectedly appeared to be bitter (Nofre *et al.*, 1987; Owens *et al.*, 1991).

Our investigations presented in this paper are aimed at an explanation as to why selected tetrazoles, designed as sweet compounds, are bitter. To this end we have undertaken the synthesis, taste quality test, crystal structure analysis, a structural data search and molecular modelling of tetrazole analogues of arylsulfonylalkanoic acids (ASA).

Tetrazole derivatives are very interesting compounds also because of the acidity, basicity and complex formation features of their heteroatomic ring. Among their properties important for medicine are antibacterial, anti-allergic, anti-inflamatory, angiotensine II antagonistic, hormone-like and other activities (Castro *et al.*, 1996; Herr, 2002; Kurup *et al.*, 2001; May & Abell, 2001). A search of the CSD (Allen, 2002) indicates two main periods during which there have been an increasing number of deposited tetrazole structures, namely 1990–1999 and 2002 until the present (Fig. 1).

The crystal structures of four bitter tetrazole analogues of ASA and one intermediate product of the synthesis, as well as

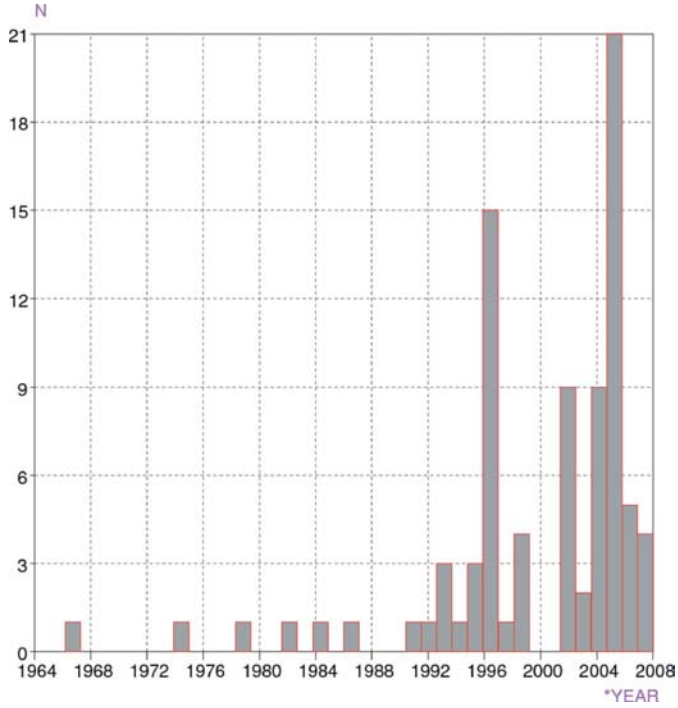


Figure 1
Number of tetrazole-containing structures deposited in the CSD from 1964 until the present.

Table 2
Crystal data and parameters of intensity data collection and refinement for the structures of (1)–(4).

	(1)	(2)	(3)	(4)
Crystal data				
Chemical formula	$C_{11}H_{14}N_4O_2S$	$C_9H_9ClN_4O_2S$	$C_{11}H_{14}N_4O_3S$	$C_8H_8N_4O_2S$
M_r	266.32	272.71	282.32	224.24
Cell setting, space group	Monoclinic, $P2_1/c$	Monoclinic, $P2_1/n$	Monoclinic, $P2_1/c$	Monoclinic, Cc
Temperature (K)	293 (2)	293 (2)	293 (2)	293 (2)
a, b, c (Å)	10.9886 (3), 12.6952 (5), 9.6299 (2)	9.7121 (2), 19.1953 (7), 13.4438 (4)	9.8188 (2), 12.5801 (2), 21.5687 (5)	22.8887 (7), 5.0315 (2), 9.1377 (3)
β (°)	104.535 (2)	108.737 (2)	99.339 (1)	105.387 (2)
V (Å^3)	1300.40 (7)	2373.46 (12)	2628.89 (9)	1014.62 (6)
Z	4	8	8	4
D_x (Mg m^{-3})	1.360	1.526	1.427	1.468
Radiation type	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$
μ (mm^{-1})	0.25	0.49	0.26	0.31
Crystal form, colour	Block, white	Plate, white	Plate, white	Prism, yellow
Crystal size (mm)	0.35 × 0.15 × 0.13	0.14 × 0.11 × 0.02	0.25 × 0.24 × 0.05	0.33 × 0.25 × 0.1
Data collection				
Diffractometer	KappaCCD	KappaCCD	KappaCCD	KappaCCD
Data collection method	CCD	CCD	CCD	CCD
Absorption correction	Multi-scan†	Multi-scan†	Multi-scan†	Multi-scan†
T_{min}	0.92	0.94	0.94	0.91
T_{max}	0.97	0.99	0.99	0.97
No. of measured, independent and observed reflections	15 274, 2939, 2338	16 855, 5416, 2925	21 761, 7607, 4077	14 160, 4333, 3214
Criterion for observed reflections	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$
R_{int}	0.033	0.047	0.040	0.031
θ_{max} (°)	27.4	27.5	30.0	34.9
Refinement				
Refinement on	F^2	F^2	F^2	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.049, 0.124, 1.08	0.054, 0.136, 1.02	0.058, 0.139, 1.01	0.045, 0.116, 0.95
No. of reflections	2939	5416	7607	4333
No. of parameters	168	316	352	139
H-atom treatment	Mixture‡	Mixture‡	Mixture‡	Mixture‡
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.0528P)^2 + 0.4433P]$, where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0539P)^2 + 0.1761P]$, where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.047P)^2 + 0.747P]$, where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0572P)^2 + 0.3771P]$, where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	< 0.0001	< 0.001	< 0.0001	< 0.001
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å−3)	0.27, −0.37	0.36, −0.37	0.21, −0.29	0.27, −0.29
Extinction method	*SHELXL*	*SHELXL*	*SHELXL*	*SHELXL*
Extinction coefficient	0.034 (4)	0.0026 (7)	0.0031 (7)	–
Absolute structure	–	–	–	Flack (1983)
Flack parameter	–	–	–	−0.03 (7)

Computer programs used: *COLLECT* (Nonius BV, 1997–2000), *HKL DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997), *SHELXS*97 and *SHELXL*97 (Sheldrick, 2008), *ORTEP*III for Windows (Farrugia, 1997), *WinGX* publication routines (Farrugia, 1999). † Multi-scan based on symmetry-related measurements. ‡ Mixture of independent and constrained refinement.

the most important types of intermolecular interactions are described. Interpretation of all the short contacts observed should help to predict the molecular fragments which will most probably interact with the active site of the putative taste receptor.

2. Experimental and computational methods

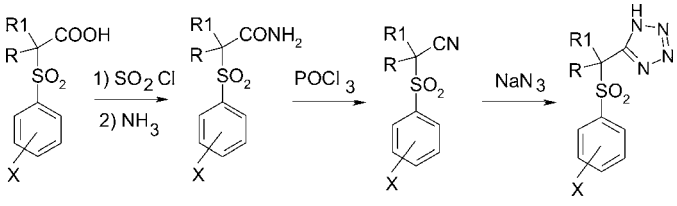
2.1. Synthesis and toxicological tests

The synthesis of novel arylsulfonylmethyltetrazole derivatives was carried out in order to obtain compounds which might be used in the structure–taste investigations.

Tetrazoles can be obtained from the corresponding carboxylic acids by reaction of amidrazones with nitrous acid (Pinner, 1897), by reaction of diazonium salts with acylhydrazides or *S*-diacylhydrazines (Dimroth & de Montmollin, 1910), and by condensation of phenyl azides with the phenylhydrazones of aldehydes in alcoholic sodium alkoxide solutions (Dimroth & Merzbacher, 1910). The synthesis of some arylsulfonylmethyltetrazoles was described previously by MacManus (1969). We slightly modified this procedure by changing the reaction sequence (Polański & Jarzembek, 2002), as shown below. We have found that this method is a facile synthetic route giving a variety of target compounds.

Table 3
Selected bond distances (Å) and torsion angles (°) for (1)–(4).

	(1)	(2)		(3)		(4)	Mean value of bonds and angles for tetrazoles in CSD (total observations: 85)
	Mol. *R*	Mol. *S*	Mol. *R*	Mol. *S*	Mol. *R*		
S1—O1	1.434 (2)	1.435 (2)	1.431 (2)	1.439 (2)	1.435 (2)	1.437 (1)	
S1—O2	1.436 (2)	1.439 (2)	1.441 (2)	1.435 (2)	1.437 (2)	1.439 (1)	
S1—C1	1.764 (2)	1.759 (3)	1.755 (3)	1.750 (2)	1.757 (2)	1.757 (2)	
S1—C7	1.803 (2)	1.818 (3)	1.812 (3)	1.802 (2)	1.801 (2)	1.786 (2)	
C7—C8	1.483 (2)	1.480 (4)	1.483 (4)	1.479 (3)	1.481 (3)	1.484 (3)	
N1—C8	1.335 (2)	1.328 (3)	1.336 (3)	1.332 (2)	1.327 (2)	1.325 (3)	1.333 (1)
N4—C8	1.316 (2)	1.315 (3)	1.320 (3)	1.323 (2)	1.321 (2)	1.318 (3)	1.321 (1)
N1—N2	1.343 (2)	1.337 (3)	1.346 (3)	1.336 (2)	1.341 (2)	1.344 (3)	1.348 (1)
N2—N3	1.293 (2)	1.293 (3)	1.290 (3)	1.295 (2)	1.295 (2)	1.290 (3)	1.289 (1)
N3—N4	1.355 (2)	1.356 (3)	1.363 (3)	1.359 (2)	1.358 (2)	1.361 (3)	1.361 (1)
C7—C8—N1	125.77 (14)	125.5 (2)	124.6 (2)	126.74 (15)	126.31 (15)	125.66 (18)	125.55 (25)
C8—N1—N2	109.35 (14)	109.8 (2)	109.5 (2)	110.03 (15)	109.77 (15)	108.8 (2)	108.82 (11)
N1—N2—N3	105.44 (15)	105.7 (2)	106.0 (2)	105.65 (16)	105.28 (16	106.4 (2)	106.35 (11)
N2—N3—N4	111.42 (14)	110.7 (2)	110.7 (2)	110.91 (15)	111.41 (15)	110.4 (2)	110.70 (11)
N3—N4—C8	105.76 (14)	106.2 (2)	106.3 (2)	105.96 (15)	105.42 (15)	105.86 (19)	105.92 (13)
N4—C8—N1	108.03 (16)	107.7 (3)	107.5 (3)	107.45 (17)	108.12 (17)	108.50 (19)	108.20 (13)
N4—C8—C7	126.14 (16)	126.8 (3)	127.9 (3)	125.79 (17)	125.54 (17)	125.78 (18)	126.22 (30)
C1—S1—C7—C8	62.4 (1)	84.4 (2)	−82.7 (2)	−57.5 (2)	59.7 (2)	−170.3 (1)	
C1—S1—C7—C9	−172.8 (1)	−43.4 (2)	46.1 (2)	177.1 (1)	−175.4 (1)	–	



Toxicological tests were performed by investigations of fish growth, as recommended by OECD 201 and 203 standards (Organization for Economic Cooperations and Development, 1993). The results indicated no toxicological effect on the populations tested. The taste study results are presented in Table 1.

2.2. X-ray structure determination

Suitable crystals of (1), (3) and (4) were obtained by slow evaporation of their ethanol solutions at room temperature. Crystals of (2) were grown in a mixture of acetone and water. The crystallographic data as well as the data collection and refinement parameters are summarized in Table 2.[1] All non-H atoms were refined anisotropically. The positions of the H atoms bonded to C atoms were calculated, while those bonded to the N atoms in the tetrazole groups were located from difference-Fourier maps. The H atoms were refined using a riding model with the isotropic displacement parameter set equal to 1.2 or 1.5 (methyl group) times that of the parent atom. Asymmetric units of (1)–(4) are presented in Figs. 1–4 (*ORTEP*III; Farrugia, 1997).

[1] Supplementary data for this paper are available from the IUCr electronic archives (Reference: BS5063). Services for accessing these data are described at the back of the journal.

Table 4
Hydrogen-bond parameters (Å, °).

D—H···*A*	*d*(*D*—H)	*d*(H···*A*)	*d*(*D*···*A*)	∠(*DHA*)
(1)				
N1—H1N···N3^{i}	0.92 (2)	1.98 (2)	2.884 (2)	166 (2)
(2)				
N11—H11N···N24^{ii}	0.88 (3)	1.98 (3)	2.849 (3)	168 (2)
N21—H21N···N14^{iii}	0.92 (3)	1.92 (3)	2.817 (3)	166 (3)
(3)				
N11—H11···N23	0.82 (2)	2.16 (2)	2.959 (2)	166 (2)
N21—H21···N13^{iv}	0.81 (2)	2.15 (2)	2.951 (2)	172 (2)
(4)				
N1—H1N···N4^{v}	1.00 (3)	1.90 (3)	2.890 (3)	170 (3)

Symmetry codes: (i) $x, -y-\frac{1}{2}, z+\frac{1}{2}$; (ii) $-x+\frac{1}{2}, y-\frac{1}{2}, -z+\frac{1}{2}$; (iii) $-x+\frac{3}{2}, y+\frac{1}{2}, -z+\frac{1}{2}$; (iv) $x-1, y, z$; (v) $x, y+1, z$.

Selected bond lengths and torsion angles are shown in Table 3. Hydrogen-bond parameters are reported in Table 4.

2.3. CSD search criteria

The search of tetrazole-containing structures deposited in the CSD (release 2007; Allen, 2002) was performed for the main tetrazole moiety, with an unsubstituted N atom in the tetrazole ring (equivalent of N1 in the presented molecules) and any substituent bonded to the C atom of the ring (equivalent of C8). A set of 75 hits was found from which nine structures were excluded because their atomic coordinates were not available. For the remaining 66 structures the parameters of the statistical distributions of bond distances and angles were calculated for the tetrazole rings (Table 3). The total number of observations is 85 for each distance and angle

because in 16 of the selected structures deposited in the CSD there was more than one tetrazole ring in the asymmetric unit. From all structures ten were defined as complexes with the tetrazole groups being chelating agents – the only exception is WAKWAH (Astakhov *et al.*, 2004), where tetrazoles are not in the coordinating sphere of the cation (Na^+). In three structures, HAVBEM (Jin *et al.*, 2005), TESDOL (Ma *et al.*, 2004) and TESDOL01 (von Denffer *et al.*, 2005), the tetrazole moiety was protonated and positively charged.

In the comparison study the hydrogen-bond formation was considered to be the most interesting feature. Due to the variety of structures found in the CSD (large number of possible donors and acceptors in some of them), many different types of the selected interactions were observed. To avoid complications, attention was focused only on the structures with different hydrogen bonds between two tetrazole rings. Thus, only 23 (excluding complexes and protonated tetrazoles) of all the tetrazole-containing structures found in the CSD were chosen and used for the structure comparison with the tetrazole analogues of ASA studied by us.

The CSD refcodes of selected structures are presented in Table 5.

3. Results and discussion

The taste study performed for the compounds presented in this paper and for previously reported analogues (Polański *et al.*, 2000; Polański & Jarzembek, 2002) showed that almost all the tetrazole derivatives of ASA obtained are bitter (Table 1).

The *R* enantiomers of (1)–(3) and the molecule of (4) are presented in Figs. 2–4 and in Fig. 5, respectively. The investigated tetrazole analogues of ASA crystallize in the monoclinic system. All of them share a very similar molecular structure with a common moiety consisting of an aromatic ring, sulfonyl group and tetrazole group, separated from the S atom by one C atom (C7). This atom in molecules (1)–(3) is the stereogenic centre. Both enantiomers of molecules (1)–(3) are present in their crystals, which belong to the centrosymmetric space groups. It is interesting that the crystal structure of (4), with a *Cc* polar space group, is noncentrosymmetric although its molecule is not chiral. In this structure the molecular dipole moments are ordered parallel to each other (Fig. 6*b*). The packing pattern indicates that the macropolarization direction is [100].

All the tetrazoles described differ in the type of substituents linked to the C7 atom and to C4 of the benzene ring (*para*

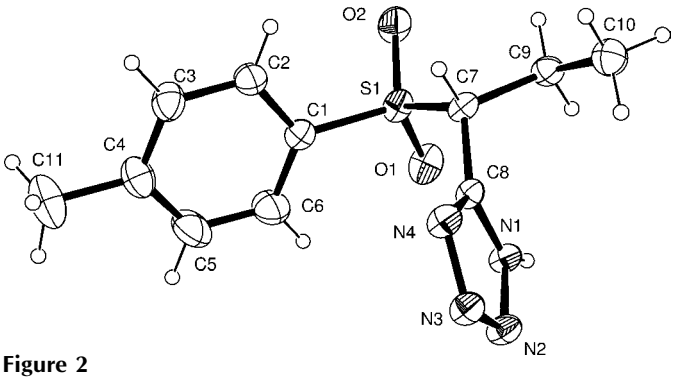


Figure 2
*ORTEP*III (Farrugia, 1997) view and atom numbering for (1), showing the asymmetric unit (*R* enantiomer). The ellipsoids are drawn at 30% probability.

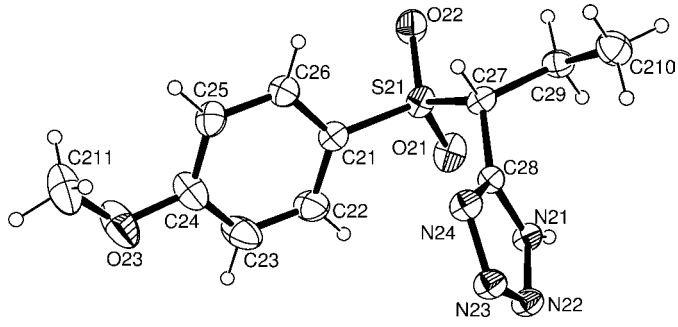


Figure 4
*ORTEP*III (Farrugia, 1997) view and atom numbering for (3), showing one molecule from the asymmetric unit (*R* enantiomer). The ellipsoids are drawn at 30% probability.

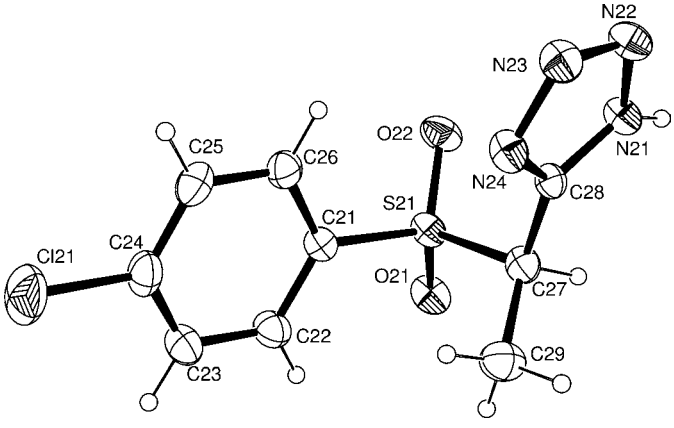


Figure 3
*ORTEP*III (Farrugia, 1997) view and atom numbering for (2), showing one molecule of the asymmetric unit (*R* enantiomer). The ellipsoids are drawn at 30% probability.

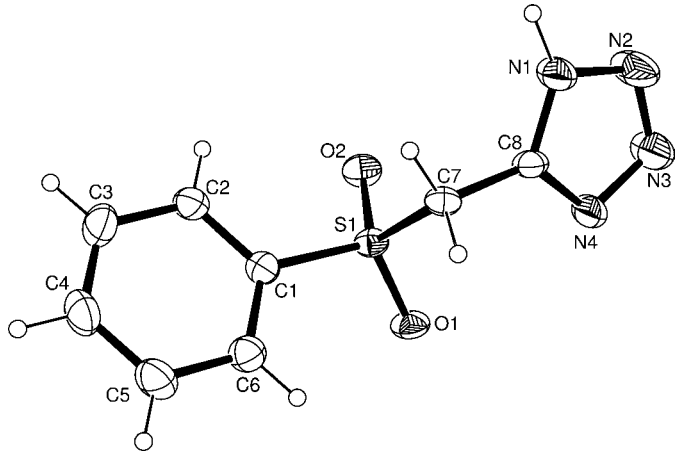


Figure 5
*ORTEP*III (Farrugia, 1997) view and atom numbering for (4). The ellipsoids are drawn at 30% probability.

Table 5
Results of CSD search structures with tetrazole–tetrazole hydrogen bonds: selected parameters of tetrazole ring interactions.

Refcode	Tetrazole–tetrazole hydrogen-bond type	Number of tetrazole rings in asymmetric unit	Hydrogen-bond parameters: $d(D\cdots A)$ < $(D-H\cdots A)$ (Å, °)	Angle (°) between planes of tetrazole rings forming hydrogen bonds, and distance (Å) between ring centroids	
AMTETZ[a]	N1—H···N4	1	2.76; 164.7	41.4	4.9
AMTETZ01[b]	N1—H···N4	1	2.75; 166.0	41.7	4.9
BINROF[c]	N1—H···N3	1	2.87; 153.9	42.3	4.9
EJEHEG[d]	N1—H···N4	1	2.80; 161.7	86.3	5.0
FELPIV[e]	N1—H···N4	1	2.81; 160.5	65.2	5.0
FIZZOD[f]	N1—H···N4	1	2.81; 173.4	5.7	5.0
HUKROU[g]	Mixture of N1—H···N4 (I) and N1—H···N3 (II)	4 (2 molecules with 2 tetrazoles each)	(I): 2.88; 165.2 2.90; 153.8 (II): 2.81; 159.2 2.81; 163.4	(I): 21.6 20.0 (II): 79.3 74.6	5.1 5.1 4.9 5.0
HUKRUA	Mixture of N2—H···N4 (I) and N1—H···N3 (II)	3 (in 1 molecule)	(I): 2.79; 177.8 (II): 2.79; 168.6	(I): 9.7 (II): 5.2	4.9 5.0
NAGVAS[h]	N1—H···N4	2 (in 1 molecule)	2.81; 169.7	0.0	4.9
NAYKOO[i]	N1H···N4	2 (2 molecules)	2.83; 151.6	4.6	4.9
NISGEB[j]	Mixture of N1—H···N4 (I) and N1—H···N3 (II)	3 (in 1 molecule)	(I): 2.78; 171.5 2.86; 166.8 (II): 2.90; 173.1	(I): 27.5 9.8 (II): 13.9	4.9 5.1 5.1
OMOPAH[k]	N1—H···N4	1 (disorder of tetrazole proton position)	2.80; 170.0 (149.29)	0.0	4.9
OMOPAH01	N1—H···N4	1 (disorder of tetrazole proton position)	2.89; 154.4 (156.0)	0.00	5.0
RATJOM[l]	N1—H···N3	1	2.90; 168.6	24.0	5.1
SISYIC[m]	N1—H···N2 (dimer)	1	3.00; 134.1	0.0	4.8
TETZOL[n]	N1—H···N4	1	2.83; 148.1	0.0	4.9
TETZOL02[o]	N1—H···N4	1	2.80; 158.3	0.0	4.9
TOSJOA[p]	N1—H···N4	1	2.82; 165.0	85.7	5.0
TOSJOA01[q]	N1—H···N4	1 (disorder of tetrazole proton position)	2.82; 165.8	85.7	5.0
VEBXEG[r]	N1—H···N4	2 (2 molecules)	2.87; 163.7 2.87; 164.6	0.0	5.0
ZOWDEU[s]	N1—H···N3	1	2.83; 154.2	0.0	4.9
ZOWDIY	N1—H···N4	1	2.80; 165.5	47.6	5.0
ZOWDOE	N1—H···N4	1	2.80; 173.5	67.5	5.0

References: (*a*) Britts & Karle (1967); (*b*) Bray & White (1979); (*c*) Sake Gowda *et al.* (1982); (*d*) Lyakhov *et al.* (2003); (*e*) Geisenberger *et al.* (1987); (*f*) Ohno *et al.* (1999); (*g*) Diop *et al.* (2002); (*h*) Steel (1996); (*i*) Astakhov *et al.* (2005); (*j*) Zubarev *et al.* (1997); (*k*) Fridman *et al.* (2004); (*l*) Weigand *et al.* (2005); (*m*) Parvez *et al.* (1991); (*n*) van der Putten *et al.* (1974); (*o*) Goddard *et al.* (1997); (*p*) Krygowski & Cyranski (1996); (*q*) Marsh (2004); (*r*) Yatshirajan *et al.* (2006); (*s*) Castro *et al.* (1996).

position with respect to the sulfonyl group). The bond lengths, compared in Table 3, are almost identical and close to the mean value of the corresponding bond lengths in the tetrazoles found in the CSD. Other bond lengths and angles as well as those not shown in the table are in agreement with the values given in the literature (Bürgi & Dunitz, 1994).

In the case of (2) and (3), the chosen asymmetric units contain both enantiomers *R* and *S*. The atom numbers in the molecules are preceded by 1 (for the *S* enantiomer) or 2 (for the *R* enantiomer). The molecules in the asymmetric units of these compounds are related *via* non-crystallographic symmetry operations. In (2) it is a pseudo-inversion centre at the position (0.51, 0.53, 0.25), whereas in (3) it is a pseudo-glide plane perpendicular to the direction [010] and crossing the *b* axis at $y = 0.25$.

The differences between conformations of the compared structures seem to result from a possible steric hindrance between substituents at C7 as well as from the short intramolecular contacts between partial negative and positive charges at the O and N atoms, respectively. The molecule of (4) is almost linear while those of (1), (2) and (3) have shapes similar to hairpins (see selected torsion angles, Table 3). The mutual positions of the two aromatic systems with respect to the S1—C7 bond are similar for molecules (1) and (3), which adopt synclinal conformations, while the conformation of (2) is intermediate between synclinal and anticlinal (see the compared torsion angles in Table 3). In contrast, (4) has an antiperiplanar conformation. The latter is most probably caused by the fact that the H atoms do not introduce any steric hindrance and can be closer to the benzene ring than the larger tetrazole group.

While the molecular conformations of (1) and (3) seem to be a result of steric hindrance between the ethyl substituent and the benzene ring, in the case of (2) as well as (2*a*) (structural data in the supplementary material) the situation is not so obvious. It is worth noticing that the two bulky substituents of C7 [methyl and tetrazole, or the nitrile group in the case of structure (2*a*)] in these molecules are located closer

to the benzene ring while the H atom is in the antiperiplanar position. This suggests that this arrangement of the substituents, involved in many intramolecular short contacts, may be energetically favourable despite the steric hindrance between benzene ring and both larger substituents of C7.

The most important type of interactions, which strongly influence the crystal architectures, are intermolecular hydrogen bonds of the type N1—H1···N. They are formed by two tetrazole groups of symmetry-related molecules. For structures (1) and (3) the observed hydrogen-bond system is N1—H1···N3 (Fig. 6), whereas for (2) and (4) it is N1—H1···N4 (Fig. 7). In all cases the hydrogen-bonded molecules form chains parallel to one of the crystal axes. In (1) the chains run along the *c* axis [*c* = 9.6299 (2) Å] and are approximately parallel to the (100) plane. The angle between planes of hydrogen-bonded tetrazole rings is 40.14 (6)°. In (2) and (3) the chains are formed by alternate *S* and *R* enantiomers. Linked molecules run along the *a* axes [*a* = 9.7121 (2) and 9.8188 (2) Å, respectively] and are approximately parallel to the (001) plane. The interplanar angles of tetrazole rings form angles of 11.04 (11) and 40.82 (6)° (Nardelli, 1995), respectively for (2) and (3). The cell parameters depend, in the case of structures (1)–(3), on the distances between 'the triplets' of hydrogen-bonded molecules, where the first and the third molecule are separated by the particular lattice translation. In the polar structure (4) chains run along the *b* axis [*b* = 5.0315 (2) Å] and are parallel to the (001) plane. Rings of linked tetrazole groups are parallel to each other. The H atoms bonded to N1 were well defined in the difference-Fourier maps. Inspection of the residual electron-density maps did not show any other possible positions for protons in the tetrazole rings.

The interactions between the chains are mainly weak, 'non-classical' hydrogen bonds of the type: C—H···O and C—H···N. These bonds form complicated systems, often of a centrosymmetric, multi-membered ring character.

All the relevant structures found in the Cambridge Structural Database (Allen, 2002) show interatomic distances in the tetrazole rings which are comparable to those observed in the structures presented here (Table 3). In many CSD structures, excluded from our study, hydrogen-bond formation other than tetrazole–tetrazole was observed owing to the presence of other donors and/or acceptors, which belong to co-crystallizing water molecules, amine, hydroxyl or carboxyl groups. In the selected structures (Table 5), hydrogen bonds corresponding to N1—H1···N4 were normally observed (in 19 of 23 structures), whereas N1—H1···N3 is less abundant (in 6 of 23 structures). In the structures OMOPAH, OMOPAH01 (Fridman *et al.*, 2004) and TOSJOA01 (Marsh, 2004) a disorder of protons linked to N1 or N4 occurs. To simplify the comparison, the atoms in the tetrazole rings of the selected CSD structures are labelled here with the numbers corresponding to those used in this paper.

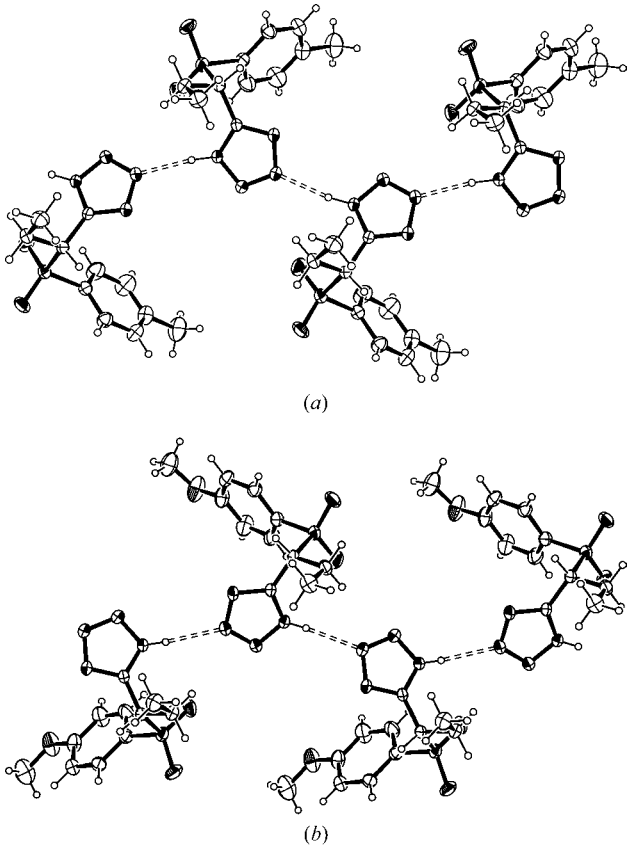


Figure 6
N1—H···N3 hydrogen-bond system of (*a*) (1) and (*b*) (3). The chain runs along the [001] and [100] axes in structures (1) and (3), respectively. Visualization made with *ORTEP*III (Farrugia, 1997); the ellipsoids are drawn at 30% probability.

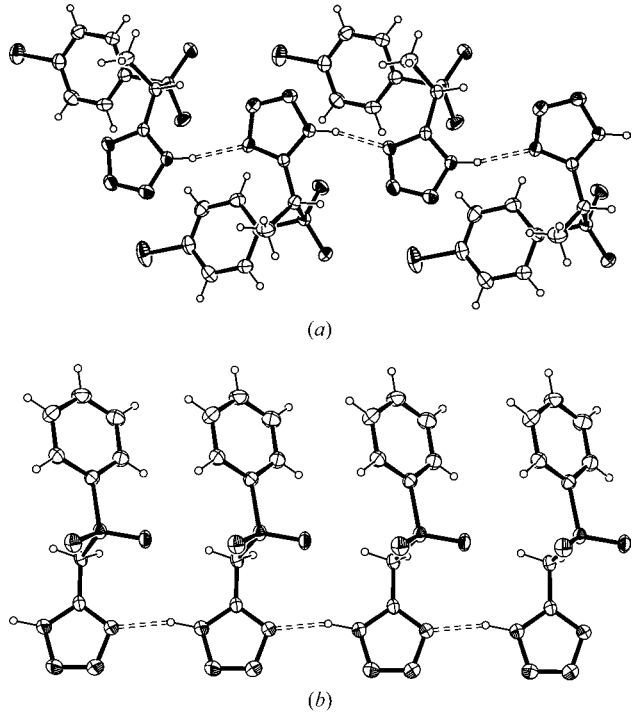


Figure 7
N1—H···N4 hydrogen-bond system in the structures of (*a*) (2) and (*b*) (4). The chain runs along [100] and [010] axes in structures (2) and (4), respectively. Visualization made with *ORTEP*III (Farrugia, 1997); the ellipsoids are drawn at 30% probability.

Table 6
Distances between N2, N3 and surrounding atoms (different than H, C, N) within the sphere of 4 Å radius.

		N2 surrounding	Distance (Å)	N3 surrounding	Distance (Å)
(1)	Mol. *R*	N2···O1^{i}	3.414 (2)	N3···O1^{ii}	3.396 (2)
		N2···O2^{ii}	3.418 (3)	N3···O2^{ii}	3.609 (2)
(2)	Mol. *S*	–	–	N13···O2^{iii}	3.653 (2)
	Mol. *R*	N22···Cl1^{iv}	3.967 (2)	N23···Cl1^{iv}	3.989 (2)
				N23···C21^{v}	3.614 (3)
(3)	Mol. *S*	N12···O12^{vi}	3.347 (2)	N13···O12^{vi}	3.537 (2)
		N12···O21^{vii}	3.445 (2)	N13···O21^{vii}	3.439 (2)
		N12···O23^{viii}	3.436 (2)		
	Mol. *R*	N22···O11	3.374 (2)	N23···O11	3.367 (2)
		N22···O13^{ix}	3.589 (2)	N23···O22^{ii}	3.673 (2)
		N22···O22^{ii}	3.449 (3)	N23···O23^{viii}	3.764 (2)
(4)		–	–	–	–

Symmetry codes: (i) $x, -y-\frac{1}{2}, z-\frac{1}{2}$; (ii) $-x, y-\frac{1}{2}, -z+\frac{1}{2}$; (iii) $-x+1, -y+1, -z$; (iv) $-x+\frac{3}{2}, y+\frac{1}{2}, -z+\frac{1}{2}$; (v) $x+\frac{1}{2}, -y+\frac{3}{2}, z+\frac{1}{2}$; (vi) $-x+1, y+\frac{1}{2}, -z+\frac{1}{2}$; (vii) $x+1, y, z$; (viii) $-x+1, -y+1, -z+1$; (ix) $-x+1, -y, -z+1$.

In three of the selected structures (HUKROU, HUKRUA and NISGEB), containing more than one tetrazole ring in the asymmetric unit, both N1—H1···N4 and N1—H1···N3 hydrogen bonds were found. In HUKROU (four tetrazoles in the asymmetric unit; Table 5) separated chains of N1—H1···N4 and N1—H1···N3 are observed, forming a kind of a net. One of three tetrazole rings (the rings called here A, B and C) in the asymmetric unit of HUKRUA have the H atom bonded to N2. The triplets of hydrogen bonds form motifs corresponding to: $N2_A—H_A\cdots N4_B—N3_B—N2_B—N1_B—H_B\cdots N3_C—N2_C—N1_C—H_A\cdots O$, which create a ribbon-like theme. The chains with the repeating motif $N1_A—H_A\cdots N4_B—N3_B—N2_B—N1_B—H^B\cdots N4_C—N3_C—N2_C—N1_C—H_A\cdots N3_A—N2_A—N1_A$ are characteristic of the crystal structure of NISGEB (three tetrazoles in the asymmetric unit labelled here A, B and C; Table 5). Two bonds, N1—H···N4, of the motif lie in one line while the additional segment, N1—H···N3, induces the line bend. The three-dimensional structure, formed in this way, can be compared to a waving line.

In three other examples: BINROF, RATJOM and ZOWDEU (Table 5), only the N1—H···N3 hydrogen-bond type is observed. It is caused by the fact that the N4 atom is involved in a different hydrogen bond, N—H···N4, where the amine group is a donor.

In the crystal structures of ten complexes (not given in Table 5) comprising the tetrazole moiety, only in four cases were tetrazole–tetrazole hydrogen bonds observed: EXIGIB (Hill *et al.*, 2004), IBEDUP (Vasiliev *et al.*, 2004), NAWYOA (Weigand *et al.*, 2005) and WAKWAH (Astakhov *et al.*, 2004), with 2, 1, 4 and 2 tetrazole rings in an asymmetric unit, respectively. In the first two chosen examples the N1—H···N4 hydrogen bond was found. The N3 atoms in these structures form coordination bonds with Ti^{4+} and Li^{+} ions, respectively. The crystal structure of the sodium complex NAWYOA is rich with tetrazole moieties: each of the two molecules in the asymmetric unit contains two tetrazole rings. Both molecules, labelled here *A* and *A*′, are in the coordination sphere of the cation with their N atoms, N2 in *A* and N4 in *A*′, engaged in N—Na^{+} bonds. These tetrazoles create a hydrogen-bonded chain: $N1_A—H_A\cdots N4_A—N3_A—N2_A—N1_A—H_A\cdots N3_A$. The other rings, of which one is deprotonated and negatively charged, interact with each other *via* N1—H1···N3 bonds and form additional hydrogen bonds with co-crystallizing water molecules. In the Na^{+} complex WAKWAH, in which the tetrazole is not a coordinating agent, the hydrogen bonds N1—H1···N3 were observed while N4 forms a hydrogen bond with the water molecule.

The formation of the N1—H1···N3 hydrogen bond can be justified based on the theoretical study by Jimenez & Alderete (2006), who performed calculations on the protonation of a free tetrazole molecule. According to their paper, the most stable protonated tetrazole was that with the protonation site at N4 or N3. Thus, both these N atoms could play the role of acceptors in the hydrogen bonds, as exemplified by our results. The presence of these two proton acceptors in one tetrazole ring favours the chain-like arrangements. In this connection, the N1—H2···N2 hydrogen bond in the SISYIC structure, where centrosymmetric dimers are observed, is quite surprising even though this bond is rather weak (N1···N2 = 2.996 Å and N1—H1···N2 = 134.10°).

In five structures (including complexes) selected from the CSD, the N1—H1···N3 interaction as the only tetrazole–tetrazole hydrogen bond is observed when N4 is involved in other types of interactions. In three other structures described 'mixed' hydrogen bonds are present with different N atoms as the proton acceptors. Thus, structures (1) and (3), presented in this paper, in which the N1—H1···N3 hydrogen bond is formed leaving N4 as a 'free' atom, seem to be an exception in view of these observations. A possible explanation is given by the data in Table 6, which suggests that other intermolecular interactions, occurring due to the packing scheme, are most probably responsible for the N1—H1···N3 hydrogen-bond formation in the tetrazoles (1)–(4). The close distance of the electronegative atoms (O or Cl) to N2 rather than to N3, in structures (1) and (3) (Table 6), may be the reason for a repulsion which leads to the asymmetry of the π-electron cloud distribution in the bond N2═N3. Consequently, the π-electrons are shifted in the direction of the N3 atom, resulting in the accumulation of local negative charge on it. This makes N3 more attractive than N4 as an acceptor of hydrogen bonds in the aforementioned structures. An indication of the electron density shift may be the slight change of the N2—N3—N4 angle in comparison to the mean value among the collection of structures (Table 3).

Most of the structures listed in Table 5 show comparatively similar parameters of the N—H···N hydrogen bonds as well as the distances between the centroids of the tetrazole rings connected by these bonds, which lie in the range 4.863–5.101 Å. The influence of the crystal environment manifests itself in the modification of the angles between the planes of the tetrazole rings, their values varying from 0.00 (in planar tetrazole-tetrazole units) to 79.25°.

The problem of the molecular structure–sweet-taste relationship is still a matter of hypotheses. According to the latest way of thinking, it is no longer possible to consider the Kier's

glukophor geometry, mentioned in the Introduction, as the only correct one. The geometry postulated by Nofre and Tinti (Nofre & Tinti, 1996) is too flexible and it does not help in the design of sweeteners.

In the crystal structure of the well known artificial sweetener saccharine (Okaya, 1969), as well as in some cyclamates (Leban *et al.*, 2007), the molecules form dimers *via* hydrogen bonds. The same mode of interaction has also been found in the crystal structure of sweet arylsulfonylalkanoic acids (Polański *et al.*, 1997; Jones *et al.*, 2006). The lack of a dimeric system in the case of the presented tetrazole derivatives could be an explanation of their bitter, instead of sweet, taste. However, this idea is in contradiction to the fact that some, although very few, sweet amino acids and sodium cyclamate (Leban *et al.*, 2007) form chains of molecules linked together *via* hydrogen bonds, as in our case.

At least four different binding pockets on the sweet-taste receptor are postulated (Morini *et al.*, 2005), but the specificity of binding the sweetener molecule by the receptor is still not known. It is, however, possible that compounds sharing similar conformations and approximately similar distributions of potentially active fragments will bind to the same pocket. Thus, the speculation can be made that only one binding pocket is capable of interacting with the arylsulfonylalkanoic acids (ASA) and their bioisosteres. The fact that the synthesized tetrazoles are not sweet seems to show that molecules (1)–(4) are not able to interact with the receptor.

The conformation of tetrazole molecules presented here in the crystalline state is only slightly different from that of the optimized molecule (data not shown). Moreover, molecules of ASA and their tetrazole analogues share similar geometries in their crystal structures (Fig. 8). The similar conformations as well as known bioisosterism of tetrazole and carboxyl groups suggest that designed molecules should be sweet. The lack of their activity can be explained with analysis of the superimposed molecules of active carboxyl acids (Jones *et al.*, 2006; Kalinowska-Tłuścik *et al.*, 2006) and tetrazole, *e.g.* a molecule of (2) (Fig. 8). The orientation of the N—H bond of tetrazole is in strong disagreement with that of the O—H bond of the carboxyl group (by more than 90°).

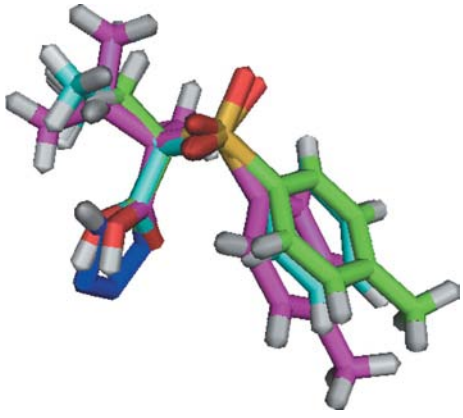


Figure 8
Superposition of a tetrazole derivative of ASA [here (2) – green carbon atoms] with molecules of sweet ASA [Jones *et al.*, 2006 (magenta C atoms), Kalinowska-Tłuścik, *et al.*, 2006 (cyan C atoms)]. The orientation of the N—H bond in the tetrazole molecule is different from the O—H bond in the sweet ASA.

Other features which suggest why tetrazole cannot bind to the sweet-taste receptor may be related to the 'active' acceptors of hydrogen bonds in the tetrazole moiety, which are in most cases the N3 or N4 atoms. These atoms are involved in a rigid ring structure, which may make them unavailable for dimer formation; the preferred motifs observed in the crystal structures are therefore chains. It is known that carboxylic acids often form dimers in their crystal structures. Thus, the lack of dimers in the crystal structures of the tetrazoles could be treated as a symptom of their inability to interact with the sweet-taste receptor. The reason for this inability is most probably the improper mutual arrangement of the proton donor and acceptor in their molecules (in the rigid ring moiety).

On the other hand, the conformation of the presented molecules seems not to hinder their interaction with the sweet-taste receptor. Even though the tetrazoles contain many 'rotatable' bonds, the molecules presented mostly retain the 'hairpin' conformation similar to that of the sweet acids. This suggests that the aromatic ring of the tetrazoles can interact with the receptor *via* hydrophobic interactions.

The above considerations elucidate the lack of sweet taste of the tetrazoles, but, unfortunately, the reason for their bitter taste cannot be given yet. The explanation for this phenomenon requires more detailed study on the binding pockets for bitter- and sweet-taste receptors, which is a very difficult task.

4. Concluding comments

Three bitter derivatives of sweet arylsulfonylalkanoic acids, in which the carboxyl moiety was exchanged for a bioisosteric tetrazole group, are chiral. They crystallize as a racemic mixture in centrosymmetric space groups. The crystal structure of a related nonchiral compound adopts a polar space group. The most probable reason for this behaviour may be the polarity of the investigated molecules, which is connected with their constitution and conformation.

The most important intermolecular interactions are those between the hydrogen-bearing nitrogen atom (N1) of the tetrazole moiety and a nitrogen atom (N4) of the same group of another molecule. This type of tetrazole–tetrazole hydrogen bonding is the most common within structures found in the CSD. Much less representative in the crystal chemistry of tetrazoles is the occurrence of the hydrogen bonds, in which the proton acceptor is the N3 atom. In all the tetrazole structures, where only the N1—H···N3 bond is observed, the N4 atom is engaged in other types of interactions. An interesting exception, in the structures described in this paper, is the occurrence of the N1—H···N3 hydrogen bond in the presence of the 'free' N4 atom. This phenomenon may be explained by the specific influence of electronegative atoms of neighbouring molecules on the distribution of the π-electron density in the tetrazole ring system.

The packing of the molecules in the unit cell is determined by the intermolecular hydrogen bonds. The chains of mole-

cules linked by these interactions in the three centrosymmetric crystals run along the directions of similar lattice parameters: 9.6299 (2), 9.7121 (2) and 9.8188 (2) Å. These parameters depend on the distances between 'the triplets' of hydrogen-bonded molecules, of which the first and the third molecules are separated by the particular lattice translation. In the polar structure the lattice parameter along the chain is much shorter, b = 5.0372 (5) Å, because in this case the molecules, with parallel dipole moments, linked *via* hydrogen bonds, are translationally identical.

The lack of the hydrogen-bond donor disqualified the molecule (2*a*) (supplementary material) as a potential sweet compound. In its crystal structure, as well as in the case of (2), a very interesting mutual orientation of the benzene ring and both larger substituents linked to C7 is observed. This can be caused by many intramolecular van der Waals interactions (short contacts), in which the aforementioned substituents are involved.

The lack of sweet taste of tetrazole bioisosteres of ASA may be the result of the different position of the tetrazole hydrogen from that in the carboxyl group of ASA, and of the improper mutual arrangement of the proton donor and acceptor. These features may impede the tetrazole interaction with the sweet-taste receptor. However, the bitter taste of tetrazoles is as yet difficult to explain.

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