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Spacer conformation in biologically active molecules. Part 1. Structure and conformational preferences of 2-substituted benzoxazoles

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Abstract

The mutual position of two pharmacophoric elements in flexible biologically active molecules depends on the spacer conformation. This is true even for a two-atomic chain put to use as a spacer. It was established for 2-substituted-benzoxazoles containing two aromatic centres joined by $-CH_2-X-$ (X = S or O). From crystallographic studies of four molecules it was found that the role of heteroatom is essential for the whole molecule conformation. The spacer with X = S adopts the (−)synclinal conformation while for X = O the (+)antiperiplanar one. Such preferences were also found in the statistical data from Cambridge Structural Database (CSD).

Keywords: Crystal structure; HOMA; Phenyl rings; X-ray structures; Spacer conformation; Benzoxazole moiety

1. Introduction

The structure and conformation of biologically active molecules constitute a signifcant position in current crystallographic and theoretical studies. The main goal of these studies is focused on the search for molecule conformations, which would successfully fulfil conditions in the ligand–receptor complex formation. There are two profitable tools for such study: receptor binding model and/or pharmacophoric points for potential ligands and 3-D structure of the ligand.

Despite the biological activity profile, from structural viewpoint, all ligands can be arranged into two subsets. The first one comprises conformationally inflexible molecules, for example kynurenic acid molecule [1]. The establishing of biologically active conformation in these specific objects is usually evident. However, in the second subset of ligands, pharmacophoric groups (aromatic rings or H-bond participants) are joined by a flexible aliphatic chain containing two or even more (practically maximum four) atoms (for example see arylopiperazines [2]). That chain is briefly named as a spacer. In general, that class of spacer could consist of only carbon atoms or an additional heteroatom, mostly sulphur, oxygen or nitrogen in the form of the NH group. The total amount of possible low energy conformations for the elastic chain depends on the number and nature of the bonds. Theoretically each bond possesses rotational privilege. So, even if only two bonds (i.e. three atoms) are taken into consideration, the molecule conformation can vary in broad range,

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Table 1
Experimental details for **1–4**

Crystal data	**1**	**2**	**3**	**4**
Chemical formula	$C_{15}H_{13}ONS$	$C_{15}H_{13}ONS$	$C_{15}H_{13}O_2N$	$C_{15}H_{13}O_2N$
Chemical name	5-Methyl-2-(phenylthiomethyl) benzoxazole	6-Methyl-2-(phenylthiomethyl) benzoxazole	5-Methyl-2-(phenoxymethyl) benzoxazole	6-Methyl-2-(phenoxymethyl) benzoxazole
Chemical formula weight	255.32	255.32	239.26	239.26
Cell setting	Monoclinic	Triclinic	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P\bar{1}$	$C2/c$	$C2/c$
a (Å)	11.337(2)	4.5390(10)	29.501(6)	21.221(4)
b (Å)	5.690(10)	11.718(2)	4.052(8)	4.9800(10)
c (Å)	20.237(4)	12.043(2)	23.140(5)	23.342(5)
α (°)	90	89.99(3)	90	90
β (°)	96.80(3)	85.21(3)	117.73(3)	90.58(3)
γ (°)	90	85.95(3)	90	90
V (Å^3)	1296.3(4)	636.7(2)	2448.4(8)	2466.7(9)
Z	4	2	8	8
D_x (mg m^{-3})	1.308	1.332	1.298	1.289
Radiation type	CuK_α	CuK_α	CuK_α	CuK_α
Wavelength (Å)	1.54178	1.54178	1.54178	1.54178
θ range (°)	55	55	55	55
μ (mm$^{-1)}$	2.100	2.137	0.699	0.693
Temperature (K)	293	293	293	293
Crystal size (mm)	0.21 × 0.11 × 0.09	0.21 × 0.11 × 0.09	0.17 × 0.09 × 0.07	0.18 × 0.08 × 0.07
Data collection				
Diffractometer	Four-cycle KM4	Four-cycle KM4	Four-cycle KM4	Four-cycle KM4
Data collection method	ω–2θ scan	ω–2θ scan	ω–2θ scan	ω–2θ scan
No. of measured reflections	3676	2985	5486	8224
R_{int}	0.0244	0.0629	0.0285	0.0657
θ_{max} (°)	80.12	80.23	80.3	80.97
Range of h, k, l	$-14 \rightarrow h$rarr;14 $1 \rightarrow k \rightarrow 7$ $-25 \rightarrow l \rightarrow 1$	$-5 \rightarrow h \rightarrow 5$ $-14 \rightarrow k \rightarrow 14$ $-15 \rightarrow l \rightarrow 1$	$-37 \rightarrow h \rightarrow 26$ $0 \rightarrow k \rightarrow 5$ $-26 \rightarrow l \rightarrow 27$	$-27 \rightarrow h \rightarrow 27$ $-6 \rightarrow k \rightarrow 5$ $-16 \rightarrow l \rightarrow 1$
Refinement				
Refinement on	F^2	F^2	F^2	F^2
$R[F^2 > 2\sigma(F^2)]$	0.0442	0.0457	0.0557	0.0479
$wR(F^2)$	0.1108	0.2515	0.1590	0.0940
S	0.974	0.944	1.004	0.973
No. of reflections used in refinement	2605	2625	2331	1924
No. of parameters used	164	164	164	164
Weighting scheme	$w = 1/[\sigma^2(F_0)^2 + (0.0921P)^2]$ where $P = (F_0^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_0)^2 + (0.0632P)^2]$ where $P = (F_0^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_0)^2 + (0.1262P)^2]$ where $P = (F_0^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_0)^2 + (0.0875P)^2]$ where $P = (F_0^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	0.000	0.000	0.000	0.000
$\Delta\rho_{max}$ (e Å^{-3})	0.293	0.214	0.281	0.230
$\Delta\rho_{min}$ (e Å^{-3})	−0.230	−0.279	−0.337	−0.229

from the folded to the extended one. At the end, the mutual position of all pharmacophoric points-in fact-is going to be a function of the spacer conformation.

For this reason, from the structural viewpoint, the spacer seemed to be a particularly interesting element of the structures from the second subset of biologically active molecules. Based on our

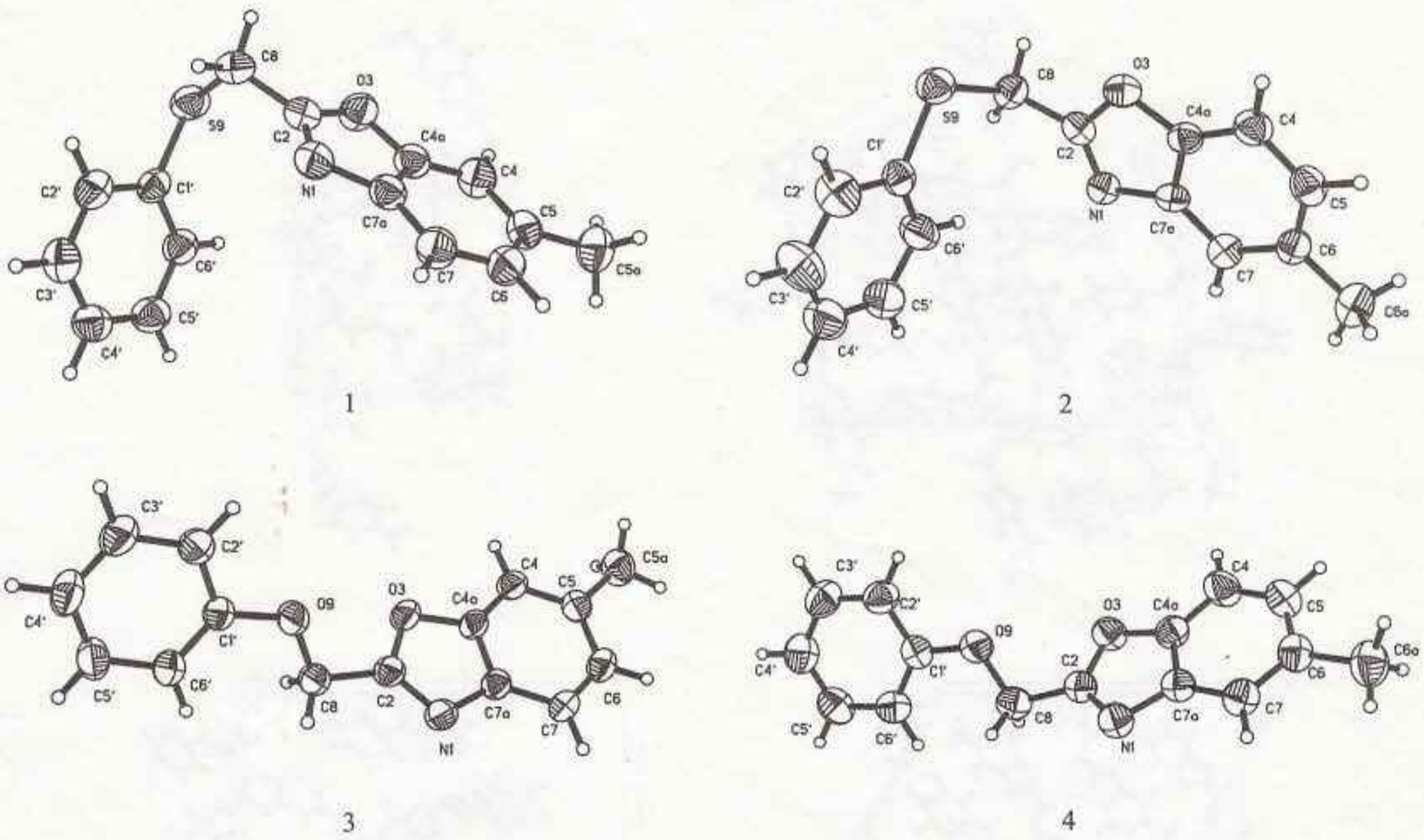

Fig. 1. ORTEP drawing for molecules **1–4**.

contemporary studies with the structures of biologically active molecules, we decided to conduct a thorough examination of the conditions and consequences of spacer conformation. We elected to utilise our own X-ray data, computation chemistry methods, and statistical data from the Cambridge Structural Database (CSD) [3]. In the planned series of the papers, we decided to begin with benzoxazole derivatives of confirmed antifungal activity. This choice is not accidental and the main reason for selecting these compounds is that the molecules contain the simplest two-atomic spacer conformation in their structures (Scheme 1). It should be mentioned that the spacer conformation seems to be important for the antifungal activity of benzoxazoles, as the previously structurally studied inflexible analogues without having any spacer were found to be less active [4–6].

2. Experimental

2.1. Source of the compounds

The title compounds (5-methyl-2-(phenylthiomethyl)benzoxazole **1**, 6-methyl-2-(phenylthiomethyl)benzoxazole **2**, 5-methyl-2-(phenoxymethyl) benzoxazole **3**, 6-methyl-2-(phenoxymethyl)benzoxazole **4**) were prepared at Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry and all of them exhibited antifungal activity[7].

2.2. Data collection, structure solution and refinement

Crystals for X-ray experiment were obtained by slow evaporation from mixtures of methanol–ethanol solutions. Preliminary data were obtained using a KM4 four-cycle diffractometer. The accurate cell dimensions were determined by the least-squares refinement method from the angular settings of 99 reflections located within $5 < \Theta < 55°$. Diffraction data were collected on a KM4 diffractometer at room temperature using graphite monochromated CuK_α radiation; $\omega/2\Theta$ scans were made for $\Theta < 80°$; no absorption correction was applied; the intensities of two standard reflections monitored every 100 reflections showed no significant fluctuations. Details of cell data, data collection and refinement are summarised in Table 1. For compounds **3** and **4**, the space group was $C2/c$ and $Z = 8$. For **1**, the

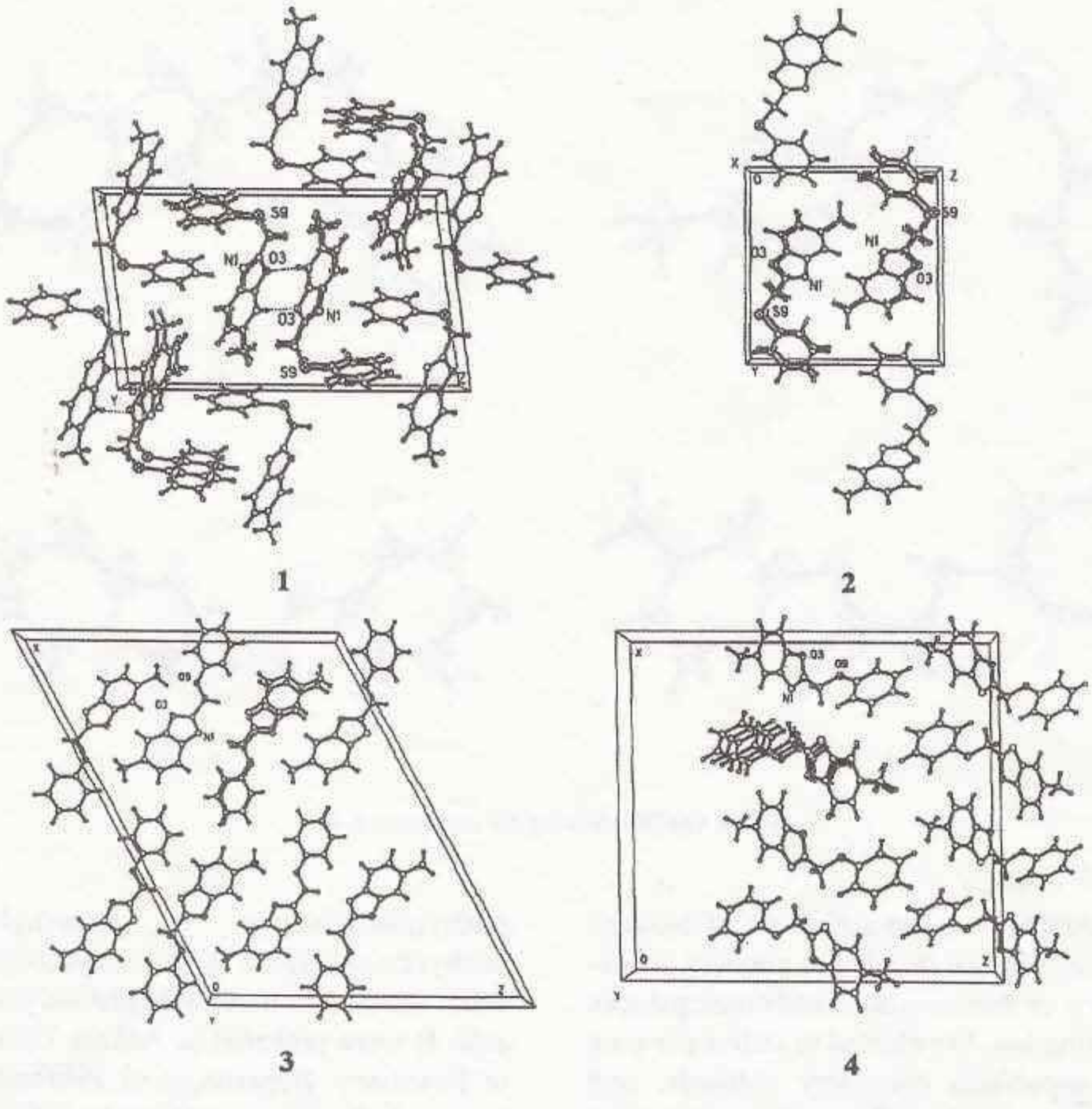

Fig. 2. Unit cells packing for **1**–**4**. In structure **1** H-bonds are marked by dotted lines.

space group was $P2_1/n$. Compound **2** was triclinic $P\bar{1}$ and diffracted weakly, with ca 32% of the reflections observed at the 2σ level. The structures were solved by direct methods using SHELXS97 [8] and refined with all data on F^2 using SHELXL97 [8]. A weighting scheme based upon $P = [F_0^2 + 2F_c^2]/3$ was employed in order to reduce statistical bias [9]. All H atoms were located geometrically. The isotropic temperature factors for all H atoms were held at 1.5 times the respective values for the parent carbon atom, and

Table 2
Selected geometrical details for **1**–**4**. Bond length in (Å) and all angles in (°)

	1	**2**		**3**	**4**
C1′–S9	1.777(3)	1.755(5)	C1′–O9	1.384(3)	1.380(3)
C8–S9	1.817(3)	1.811(5)	C8–O9	1.419(3)	1.408(3)
C1′–S9–C8	100.4(3)	104.5(3)	C1′–O9–C8	116.3(2)	117.6(2)
C6′–C1′–C2′	119.7(3)	117.5(5)	C6′–C1′–C2′	121.1(3)	120.4(3)
N1–C2–C8–S9	110.3(3)	111.5(5)	N1–C2–C8–O9	123.0(3)	138.5(3)
C2–C8–S9–C1′	–68.2(2)	–74.7(4)	C2–C8–O9–C1′	175.8(2)	177.2(2)
C8–S9–C1′– C6′	115.3(2)	–8.0(5)	C8–O9–C1′–C6′	6.2(4)	– 3.5(3)
Dihedral angle fenyl/benzoxazole	127.8	76.1		54.1	47.6

their positions were refined in riding model. Fig. 1 shows the molecular units in compounds, and Fig. 2 shows details of the crystal structures. Selected geometrical data are collected in Table 2.

2.3. Computational procedure

The calculations were done with application of the packet MSI Insight® II 98.0. The Discover® module was used for conformational analysis calculation [10]. The calculations were performed in the range of torsion angle from −180 to 180° with 10° increments. As a basic conformation crystallographically obtained structures were put to use.

3. Results and discussion

3.1. Molecular structures

In each molecule out of four 2-substituted benzoxazoles (Scheme 1), two cyclic parts (bicyclic benzoxazole and phenyl) are connected by a two-atomic spacer $-CH_2-X-$, where X = O or S. Taking X-atom as the main guideline, in fact, we solved the structures of two pairs of isomers differing in the position of the CH_3 substituent at the benzoxazole moiety (Fig. 1). The main bicyclic benzoxazole moiety is planar with endocyclic atoms deviating from the least-squares plane by less than 0.005 Å. Besides this, all exocyclic benzoxazole substituents, e.g. a carbon atom in methyl groups (at C5 or C6) and C8 (first spacer atom), were detected also in bicyclic ring plane. All bonds in benzoxazole are conjugated ones.

As was shown in our previous paper, the Harmonic Oscillator Model of Aromaticy (HOMA) [11,12] index — quantitative measure of aromaticity — can be used also for heterocycles. The HOMA index is based on structural criterion[13–16]. The value of the index is equal to one for fully aromatic structure. Therefore, having in view planarity and bond coupling in benzoxazole, we decided to estimate both ring aromaticities applying HOMA indices. Review of the CSD (CSD, version of April 2000) [3] gave us 51 structures containing the benzoxazole bicycle. The average HOMA indices are equal to 0.943 and 0.799 for six- and five-membered rings, respectively. Those values clearly proved aromaticity of the six-membered ring. Similarly, the five-membered ring, exhibiting lower HOMA index, can be labelled as aromatic as well. The lowering of HOMA is a direct outcome of the presence of endocyclic oxygen [11]. It is worth noticing, that even for highly aromatic five-membered rings with one nitrogen, the HOMA index decreases when an additional oxygen (as the second heteroatom) is introduced [12]. The average HOMA indices for benzoxazole rings in four solved structures are similar to the values given above equalling 0.959 and 0.762 for both the rings, respectively.

Benzoxazole and phenyl rings are connected via C8–X9 spacer. In the studied molecules X-atom is bonded to phenyl ring (Fig. 1). From the selected geometrical data collected in Table 2, it is clear that the conformation of molecule **1** (X = S) differs significantly in the proximity of the phenyl ring, which is in contrast to the remaining three structures. In those three molecules **2**, **3** and **4** the torsion angles C8–X9–C1′–C6′ adopt values of about 10°. At the same time, the corresponding angle in **1** is significantly higher reaching 115°. So, as C8 atom lies in benzoxazole moiety plane, the contrasted values of torsion angle C8–X9–C1′–C6′ suggested the presence of two conformations from the viewpoint of phenyl ring rotation. In fact, these forms diverge in inclination of phenyl and benzoxazole rings. In **1**, both rings are inclined at 127.8°, while in the remaining three structures the inclinations assume significantly lower values (76.1, 54.1 and 47.6°, respectively, for **2**, **3**, **4**). This observation is confirmed by the packing details of the molecules in the crystals (see below).

The heteroatoms either S or O, as the straight substituent at phenyl ring, affected on endocyclic valence angles. In molecules with X = S, valence angles in phenyl ring C6′–C1′–C2′ were found to be 119.7(3) and 117.5(5)° in **1** and **2**, respectively. Corresponding angles in the case of X = O are considerably higher, equalling 121.2(3) and 120.4(3)° for **3** and **4**, respectively. So, high electronegativity and strong electron 'withdrawing' from the phenyl ring decreasing that angle for X = S.

The geometrical data, selected for Table 2, mainly visualise the differences in the geometry and conformation of the two spacers. First of all, C–S bond lengths in **1** and **2** are much longer than the analogous C–O bonds in **3** and **4**. Secondly, the valence angle C1′–X9–C8 for X9 = S9 in structures **1** and **2** is

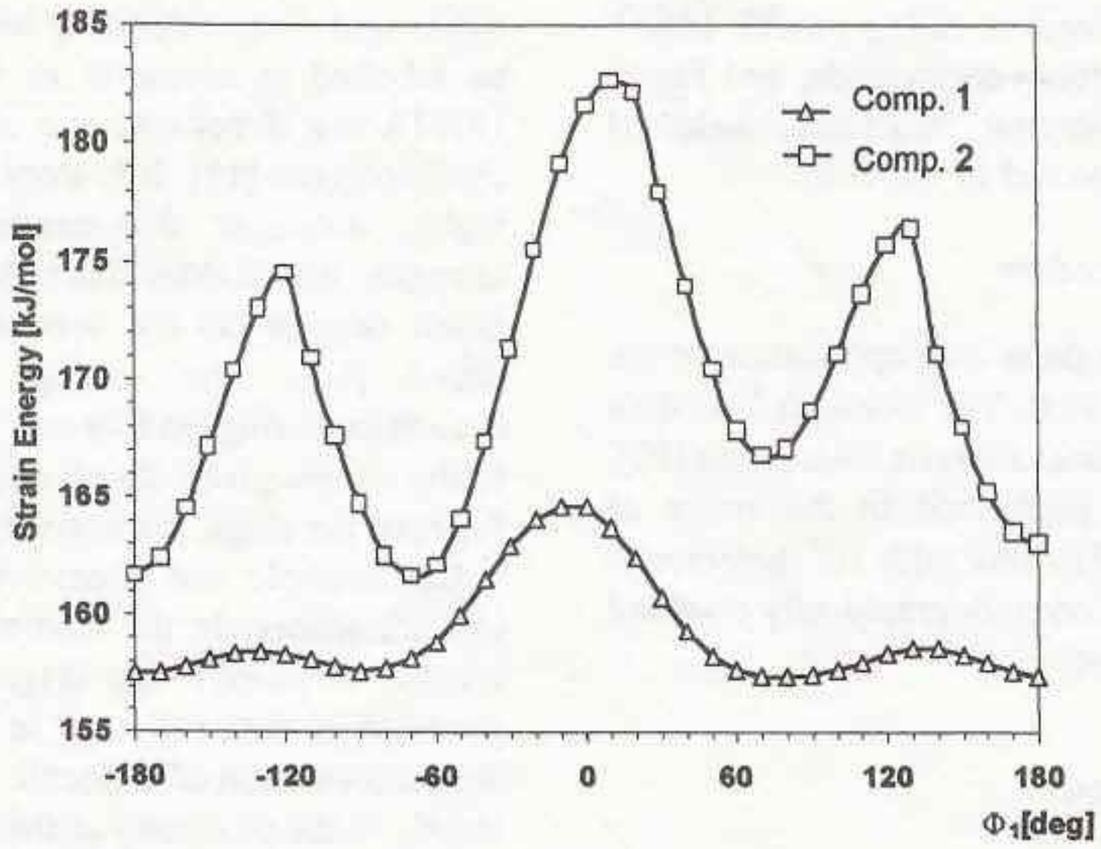

Fig. 3. The strain energy distribution of **1** and **3** as a function of spacer conformation with rotation round C8–X9 bond.

significantly lower than for X9 = O9 in structures **3** and**4**. Much more informative, however, are torsion angles. Angle Φ = C2–C8–X9–C1′ for X9 = O9 in **3** and **4** is close to 180° (Table 2). On the other hand, for X9 = S9 values of Φ are close to −70°. Therefore, with respect to the rotational angle in the spacer under discussion, Φ = C2–C8–X9–C1′, the conformation of the spacer in the molecules with sulphur is (−)synclinal, while with oxygen (+)antiperiplanar.

Therefore the conformation of a simple two-atomic spacer –CH_2–X– strongly depends on the X-heteroatom in benzoxazole derivatives under discussion which reflected on the mutual position of two aromatic parts in the molecule. This position could be determined by the distance between the centres of two aromatic fragments of the molecule (here abbreviated as Δ). Therefore, when the spacer adopts synclinal conformation in derivatives with X = S, the distance Δ equals about 6 Å. At the same time, when X = O, and the antiperiplanar spacer conformation was observed, Δ — distance is increasing to about 8 Å.

3.2. Crystal structures

The main motif in the crystal structures of four benzoxazole derivatives is comparable. Both aromatic moieties are stacked to analogous fragment of the molecule located over and below (Fig. 2). The spacing between stacked rings equals motifs about 5.5 Å in all. In the crystals, the stacking direction more or less follows the shortest unit cell orientation.

The crystal of the compound **1** is especially interesting. There was an identified intermolecular weak interaction of C4–H4···O3 = 3.401 Å (2 − x, −y, −z) putting together the molecules in the dimer. This phenomenon, not observed for remaining three crystals, is in tandem with mutual orientation of benzoxazole–phenyl rings in **1** (Table 2). The observed conformation, far from coplanarity, induces the conditions favourable to the formation of shorter distance between proton acceptors and proton donors. At the same time, for biological activity even the formation of a weak H-bond can be crucial in practice. For benzoxazole with two-atomic spacer, the conditions required for such interaction can be created simply by phenyl ring free rotation.

3.3. Conformational analysis

It is of wider interest to compare conformations of the spacer from the crystal with calculated minimum energy conformations. Therefore, the conformational analysis with the Φ = C2–C8–X9–C1′ rotation was performed starting from crystallographic forms. The strain energy distributions in the function of Φ for

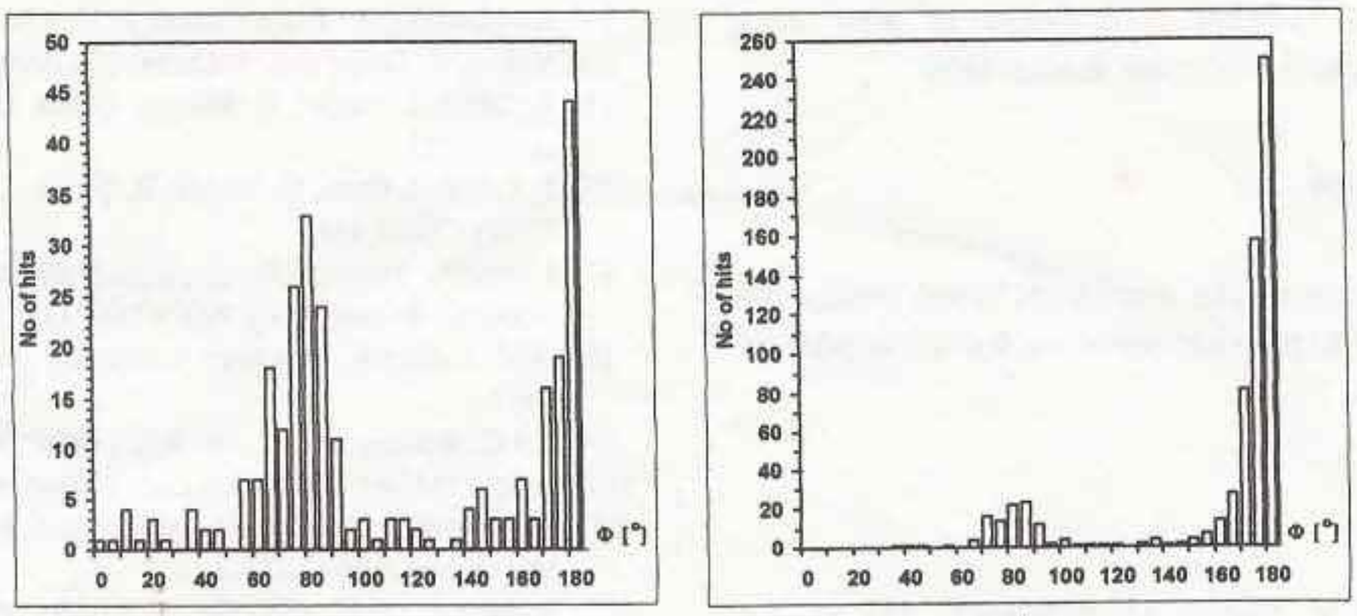

Fig. 4. Histograms of torsion angles Csp^2 (endocyclic) $-Csp^3-X-C$ (aromatic), for: (a) X = S, (b) X = O.

isomeric molecules were similar. So, in Fig. 3 graphical presentation the diagrams are given only for two derivatives with different heteroatom in the spacer, e.g. for **1** and **3**.

Independent of the X atom, two energy minima are observed in both diagrams: one at $\phi = \pm 80°$ (synclinal spacer conformation) and the other at $\phi = \pm 180°$ (antiperiplanar one). However, for X = S, the energy barrier between both minima is relatively low. From Fig. 3a one can see that the barrier between synclinal and antiperiplanar form equals just about 1 kJ mol^{-1}. For spacer with X = O (Fig. 3b) this barrier is about 10 times higher. Additionally, the minimum at $\Phi = \pm 180°$ is slightly deeper in Fig. 3b than the other one.

Conclusions from conformational calculation are equivalent with experimentally obtained structures characterised by crystallographically obtained torsion angle values presented in Table 2.

3.4. Statistical data from CSD

As the final step of our studies on two atomic spacer conformations, statistical data from CSD were employed. Review of the CSD was done for four-atomic chains as follows: Csp^2(endocyclic)–Csp^3–X–C (aromatic), where X = S and/or O. In the case of X = S additionally participation in only two bonds were restricted. Moreover, only structures having $R \leq 0.09$ were accepted.

Finally, 203 structures with X = S were selected while the set with X = O included 413 hits. Firmly incorporating two sets of selected data, the histograms for torsion angle $|\phi|$ = C(aromatic)–X–Csp^3–Csp^2(endocyclic) were obtained. As it is visible from Fig. 4a, spacer containing sulphur with almost equal probability adopts conformations with $|\phi| \sim 80°$ (49% of hits) and with $|\Phi| \sim 180°$ (51% of hits). For structures with spacer containing oxygen, the conformations found with respect to $|\Phi|$ are similar. However, almost 80% of all chains showed $|\Phi| \sim 180°$. So, both spacer conformations, synclinal and antiperiplanar, are possible for both heteroatoms. Nevertheless, for structures collected in the CSD, the antiperiplanar conformation dominates in derivatives with $-CH_2-O-$. However, $-CH_2-S-$ can be either synclinal or antiperiplanar. It should be noticed that the excellent agreement was found between the conformational calculation and statistical data for the structure supplied by CSD.

4. Concluding comments

As it was established in the discussion, the conformation of a simple two-atomic spacer $-CH_2-X-$ strongly depends on the X-heteroatom. In consequence the mutual position of two aromatic parts in the molecules of varying conformation is dramatically different. This observation is important for future work on designing the new derivatives for pharmacological screening. Moreover, it can prove to be helpful in modelling the ligand–receptor interaction on molecular level. The crystallographically

confirmed weak H-bond interaction is also very significant for ligand–receptor interaction.

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