Arylidene Imidazothiazoles. Synthesis, Structure and Benzodiazepine Receptor Binding

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Received June 5, 1998
Revised October 23, 1998

A series of arylidene imidazo[2,1-*b*]thiazoles was synthesized, in order to investigate the influence of different spatial arrangements of the arylidene substituent towards the bicyclic structure of imidazo[2,1-*b*]-thiazole on benzodiazepine receptor affinity. 1,2- And 2,3-cyclized derivatives of *mono*- and *di*-substituted *Z*-5-arylidene-2-thiohydantoins were investigated. As an example of *E* isomers *E*-5-benzylidene-2,3-dihydroimidazo[2,1-*b*]thiazol-6(5*H*)-one was obtained. The spatial arrangement of the arylidene substituent toward the bicyclic structure as well as the character of isomers had little influence on the benzodiazepine receptor affinity of the compounds. It seems that the greatest influence on biological activity has the nature and the number of substituents on the phenyl ring. All investigated imidazo[2,1-*b*]thiazoles were less active than previously described arylidene imidazo[2,1-*b*]thiazepinones.

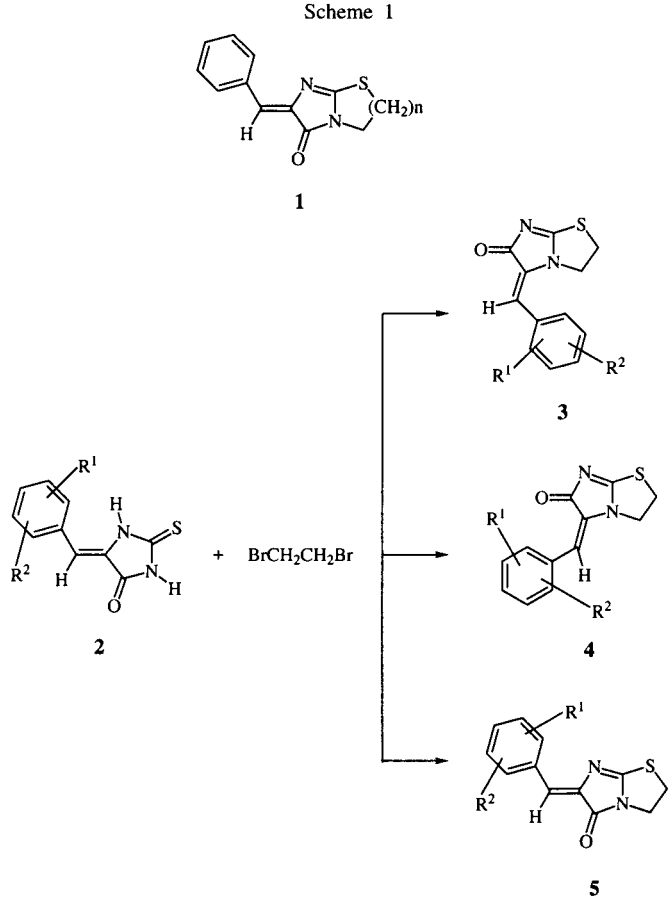
J. Heterocyclic Chem., **36**, 257 (1999).

Introduction.

The γ-amino butyric acid-A receptor is a member of the superfamily of ligand-gated ion channels [1]. The γ-amino butyric acid-A receptor complex carries high-affinity binding sites able to modulate the channel function such as benzodiazepine, picrotoxin, barbiturate, neurosteroids and ethanol sites [2]. Of these, compounds which mediate their actions at the benzodiazepine binding site are the most widely studied. Benzodiazepine receptor ligands elicit a full range of activities from maximal positive allosteric modulation (agonists - anxiolytic, anticonvulsant, sedative-hypnotic, and myorelaxant agents) to maximal negative allosteric modulation (inverse agonists - anxiogenic, somnolytic, proconvulsant or even convulsant agents) [3]. The effects of both classes of ligands are blocked by antagonists which show high affinity for the benzodiazepine site and which exhibit no pharmacological effects.

Typical benzodiazepine mediated side effects are physical dependence, amnesia, oversedation (anxiolytics) and muscle relaxation or in the case of inverse agonists, convulsive activity [4]. For therapeutic use it would be useful to find partial agonists with anxiolytic and anticonvulsant properties in the absence of myorelaxant or sedative-hypnotic activity or inverse agonists which can enhance general memory, learning and block or reverse the effects of barbiturate toxicity but are devoid of proconvulsant or convulsant activity [5]. The benzodiazepine receptor ligands include not only substances with a benzodiazepine [6] structure, but also other classes of organic compounds such as imidazopyridines [7], triazoloquinoxalines [8], imidazoquinoxalines [9], β-carbolines [10] and pyrazoloquinolines [11]. They all are condensed nitrogen contain-

Scheme 1



ing heterocyclic compounds frequently possessing additional aromatic substituents [12].

In the course of our research on annelated 2-thiohydantoin derivatives, it was found that derivatives of benzylidene substituted imidazo[2,1-*b*]thiazole, -thiazine and -thiazepine with the general structure **1** presented on Scheme 1 exhibit affinity for the benzodiazepine binding site [13]. Their activity was strongly dependent on the substitution pattern of the benzylidene residue. Their profile of action was characterized on the basis of the γ-aminobutyric acid shift [14] as partial agonists at the benzodiazepine receptor.

Continuing our research on this class of compounds we have decided to explore the imidazothiazole bicyclic ring system, since it offers the possibility of systematic investigation of the varied benzylidene ring orientation toward the annelated bicyclic structure.

Results.

Chemistry.

The synthesis of compounds of type **3**, **4**, and **5** is outlined in Scheme 1. It was carried out by reaction of substituted *Z*-5-arylidene-2-thiohydantoins **2**, prepared by Knoevenagel condensation of appropriate aldehydes with 2-thiohydantoin [15], with 1,2-dibromoethane under phase transfer catalysis conditions (solid-liquid) in acetone in the presence of potassium carbonate and benzyltriethylammonium chloride as a phase-transfer catalyst. It was previously found in our group [16,17] that under such conditions two isomeric products are obtained: the products of 1,2-substitution (**3**) and 2,3-substitution (**5**) of arylidene 2-thiohydantoins. The alkylations were carried out with mono-, di- or unsubstituted benzylidene 2-thiohydantoins. Products of 2,3-substitution (**5**) were obtained in predominance. The products of 1,2-substitution were isolated in lower yields. In the reaction with unsubstituted 5-benzylidene-2-thiohydantoin, contrary to our earlier described results [16], two products of 1,2-substitution were obtained. On the basis of elemental and spectral analysis (^{1}H nmr) it was stated that *E* isomers **4a** (previously described as *Z* [16]) and *Z* isomers **3a** were obtained. The structure and configuration of both isomers was finally confirmed by X-ray structure analysis of **3a** and **4a** (Figure 1 a and b) as well as for the starting 5-benzylidene-2-thiohydantoin **2a** [18]. In the latter case

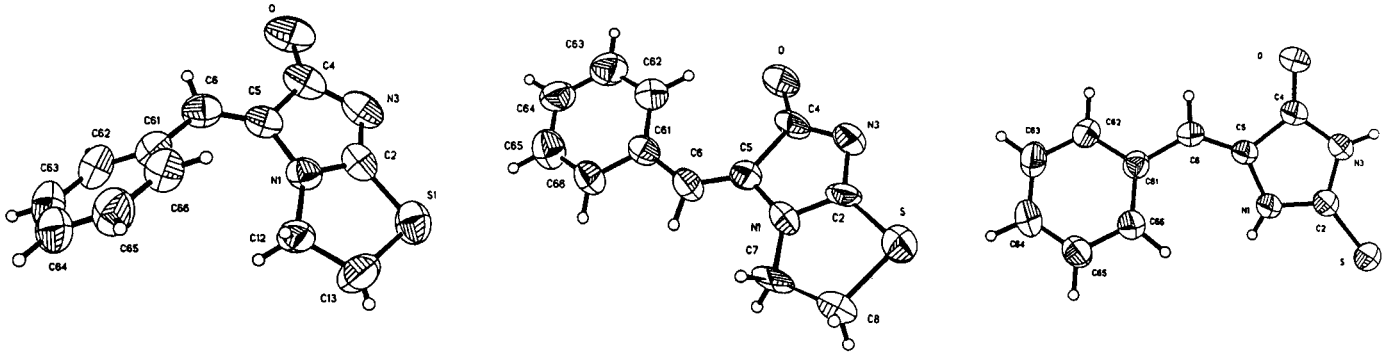


Figure 1. ORTEP view of **3a**, **4a** and **2a** molecules.

Table 1

X-ray Structure Analysis of **3a** and **4a**

A. Crystal Parameters

	data **3a**	data **4a**
formula	$C_{12}H_{10}N_2OS$;	$C_{12}H_{10}N_2OS$
molecular weight	230.28	230.28
crystallization medium	ethanol	ethanol
color	cream yellow	intensive yellow
crystal size, mm	0.4 x 0.2 x 0.2 mm	0.4 x 0.2 x 0.2 mm
cell dimensions	a = 6.8280(10) Å	a = 6.6390(1 0) Å
	b = 20.726(4) Å	b = 8.102(2) Å
	c = 7.671(2) Å	c = 20.047(4) Å
	α = 91.00°,	α = 90°
	β = 96.27(3)°,	β = 92.56(3)°
	γ = 91.00°;	γ = 90°;
	V = 1078.7(4) Å^3;	V = 1077.2(4) Å^3;
space group	$P2_1/n$;	$P2_1/n$;
molecules/unit cell	4	4
density calcd,	1.412 Mg•m^{-3}	1.420 Mg•m^{-3}

Table 1 (continued)

linear absorption factor, mm^{-1}	2.486	2.490
B. Refinement Parameters		
number of reflections	2842	3203
nonzero reflections [I>4σ(I)]	1313	674
R - index	0.060,	0.082
GOF	1.063	0.954
secondary extinction factor C,χ	NONE	NONE

the *Z* configuration as usually found for 5-arylidene-2-thiohydantoins and their bicyclic derivatives was established [19-21] (Figure 1c). The ORTEP drawings of structures with atom numbering are shown as Figure 1a and Figure 1b respectively for **3a** and **4a** while X-ray analysis details, non-hydrogen fractional atomic coordinates and

bond lengths with all important angle data are collected in Tables 1, 2 and 3 respectively.

To our knowledge, in contrast to 5-arylidene hydantoins [22,23], structures of *E* isomers of arylidene 2-thiohydantoin derivatives have not been described until the present. The X-ray analysis results also allowed us to describe the detailed conformation of both isomers present in the solid state. Two torsional angles: C(4)-C(5)-C(6)-C(61) and C(5)-C(6)-C(61)-C(62) are the fingerprint for both isomeric molecules (Table 3). The first gated in **4a**, which is reflected in the intense yellow color of the crystals (uv: λ max 370 nm). The crystals of **3a** are cream yellow (uv: λ max 347 nm).

Moreover, based on the results of crystallographic studies from this paper and a previously published one [19], the thermodynamic stability of the *E*- and *Z*-isomers of bicyclic 5-arylidenehydantoins was examined. The heat of formation Hf of **3a** and **4a** calculated after geometry optimization (semiempirical quantum-chemistry approximation) is about 2kcal/mole lower for the *Z*-form **3a** than for

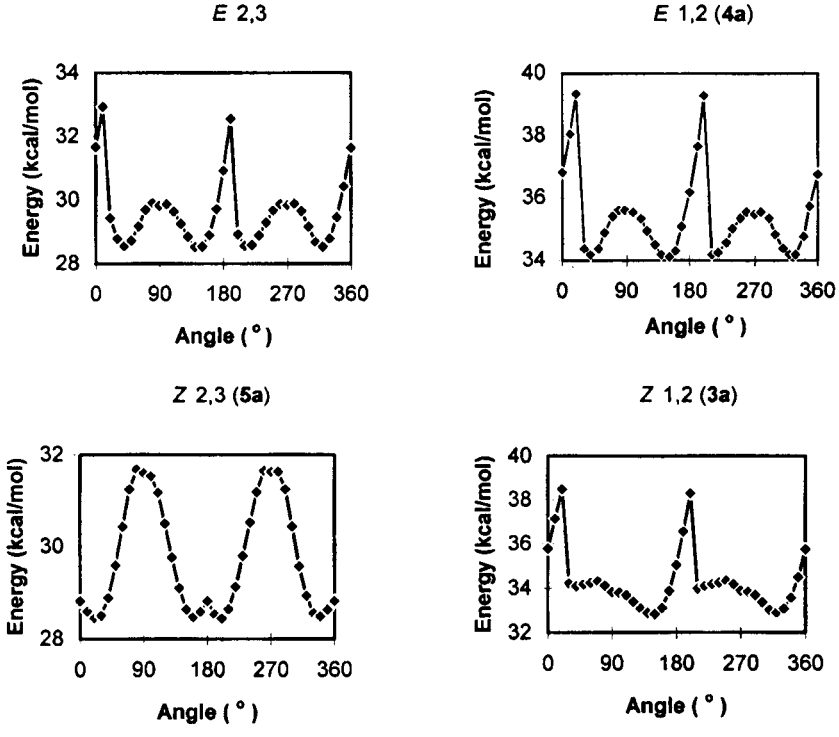


Figure 2. The conformation energy profiles upon rotation of the C6-C61 bond.

one is definitely close to 180° or to 0° respectively for the *Z*-**3a** or the *E*-form **4a**. The second one illustrates the planarity of the molecule on the whole: for the planar molecule of the *E*-isomer this angle is small equalling only 2.2(9)°. As a consequence of the space crowding in the *Z* isomer **3a** the second torsional angle from Table 3 is much higher, 128.8°. The dihedral angle formed by PL1 (phenyl ring) and PL2 (bicycle moiety) planes confirm reversibility in the planarity of both isomers (Table 3) as well.

The most important three dimensional differences in the molecules of both isomers are visible not only in their conformation. One can observe significant differences in the lengths of interatomic distances from the area of the conjugated endocyclic bonds especially the length of the C(6)-C(61) bond being 1.481 (5)Å and 1.424(6)Å for **3a** and **4a** respectively. Those bonds are much more conju-

the *E*-isomer **4a**. Almost identical ΔHf values were found for **5a** in *Z*- and *E*-configuration (rough models were built based on our previous studies [21]).

In order to evaluate the conformational freedom of the phenyl rings in the isomers investigated, the conformational analysis for one torsional angle rotation (C5-C6-C61-C62 in the range from 0° to 360°) was performed based on molecular mechanics calculations. It was found (Figure 2) that the energy differences between the rotamers ranged at 4-6 kcal/mole for all *Z* and *E* as well as for 1,2- and 2,3-substituted isomers. Therefore, independent of the profiles of the curves, conformational analysis of unsubstituted arylidene derivatives suggest almost free rotation for the phenyl ring.

Spectral analysis (^{1}H nmr) performed previously [16] for 1,2- and 2,3-substituted arylidenethiohydantoin

Table 2

Atomic Coordinates (x 10^4) and Equivalent Isotropic Displacement Parameters (Å^2 x 10^3)

Compound **3a**	x	y	z	U(eq)
S	2146(2)	6245(1)	1927(2)	75(1)
O	2377(4)	4671(2)	-2929(3)	89(1)
N(1)	2500(4)	5031(1)	1466(3)	53(1)
C(2)	2213(4)	5594(2)	577(5)	56(1)
N(3)	2080(4)	5557(2)	-1132(4)	66(1)
C(4)	2376(5)	4909(2)	-1477(5)	64(1)
C(5)	2673(4)	4552(2)	227(4)	52(1)
C(6)	3088(5)	3928(2)	409(5)	60(1)
C(7)	2783(6)	5060(2)	3363(5)	60(1)
C(8)	2651(9)	5752(2)	3874(6)	88(1)
C(61)	3373(5)	3537(2)	2007(5)	56(1)
C(62)	5042(6)	3160(2)	2348(6)	70(1)
C(63)	5357(7)	2804(2)	3857(7)	81(1)
C(64)	3996(7)	2813(2)	5073(6)	81(1)
C(65)	2317(7)	3175(2)	4723(6)	76(1)
C(66)	1995(6)	3537(2)	3222(5)	65(1)

Compound **4a**	x	y	z	U(eq)
S	531(2)	5703(2)	6136(1)	66(1)
0	-3292(4)	8430(6)	4250(2)	83(1)
N(1)	1157(5)	7517(5)	5116(2)	56(1)
C(2)	-300(6)	6766(6)	5458(2)	52(1)
N(3)	-2160(5)	7032(6)	5191(2)	63(1)
C(4)	-1940(6)	7981(6)	4628(2)	56(1)
C(5)	334(6)	8303(6)	4551(2)	52(1)
C(6)	1428(6)	9091(6)	4108(2)	56(1)
C(7)	3177(6)	7236(7)	5353(3)	72(2)
C(8)	3128(7)	6238(8)	5983(2)	73(2)
C(61)	971(6)	9909(6)	3491(2)	50(1)
C(62)	-960(7)	10032(7)	3171(2)	67(2)
C(63)	-1227(8)	10849(7)	2577(3)	76(2)
C(64)	381(8)	11622(7)	2290(3)	76(2)
C(65)	2266(9)	11512(7)	2600(3)	75(2)
C(66)	2577(7)	10688(6)	3186(2)	63(1)

Table 3

Bond Lengths (Å) and Bond Angles (°)

3a		**4a**	
S-C(2)	1.690(4)	S-C(2)	1.681(5)
S-C(8)	1.829(4)	S-C(8)	1.817(5)
O-C(4)	1.211(5)	O-C(4)	1.204(5)
N(1)-C(2)	1.369(4)	N(1)-C(2)	1.355(5)
N(1)-C(5)	1.378(4)	N(1)-C(5)	1.391(6)
N(1)-C(7)	1.446(4)	N(1)-C(7)	1.421(5)
N(2)-N(3)	1.305(5)	C(2)-N(3)	1.342(5)
N(3)-C(4)	1.386(5)	N(3)-C(4)	1.379(6)
C(4)-C(5)	1.510(5)	C(4)-C(5)	1.547(6)
C(5)-C(6)	1.334(5)	C(5)-C(6)	1.334(6)
C(6)-C(61)	1.479(5)	C(6)-C(61)	1.424(6)
C(7)-C(8)	1.489(5)	C(7)-C(8)	1.500(7)
C(61)-C(66)	1.395(5)	C(61)-C(66)	1.402(6)
C(61)-C(62)	1.396(5)	C(61)-C(62)	1.411(6)
C(62)-C(63)	1.383(6)	C(62)-C(63)	1.367(7)
C(63)-C(64)	1.387(6)	C(63)-C(64)	1.386(8)
C(64)-C(65)	1.385(6)	C(64)-C(65)	1.375(8)
C(65)-C(66)	1.385(5)	C(65)-C(66)	1.359(7)
C(2)-S-C(8)	91.79(18)	C(2)-S-C(8)	91.2(2)
C(2)-N(1)-C(5)	106.8(3)	C(2)-N(1)-C(5)	110.8(3)
C(2)-N(1)-C(7)	118.6(3)	C(2)-N(1)-C(7)	116.3(4)
C(5)-N(1)-C(7)	134.2(3)	C(5)-N(1)-C(7)	132.5(4)
N(3)-C(2)-N(1)	117.1(3)	N(3)-C(2)-N(1)	113.0(4)
N(3)-C(2)-S	130.1(3)	N(3)-C(2)-S	132.0(4)
N(1)-C(2)-S	112.8(3)	N(1)-C(2)-S	115.0(3)
C(2)-N(3)-C(4)	103.6(3)	C(2)-N(3)-C(4)	106.7(4)
O-C(4)-N(3)	124.7(4)	O-C(4)-N(3)	125.4(4)
O-C(4)-C(5)	125.7(4)	O-C(4)-C(5)	126.2(4)
N(3)-C(4)-C(5)	109.6(3)	N(3)-C(4)-C(5)	108.4(4)
C(6)-C(5)-N(1)	130.8(3)	C(6)-C(5)-N(1)	123.8(4)
C(6)-C(5)-C(4)	126.3(3)	C(6)-C(5)-C(4)	135.2(4)
N(1)-C(5)-C(4)	102.9(3)	N(l)-C(5)-C(4)	101.0(4)
C(5)-C(6)-C(61)	130.1(3)	C(5)-C(6)-C(61)	134.4(4)
N(1)-C(7)-C(8)	106.3(3)	N(1)-C(7)-C(8)	108.2(4)
C(7)-C(8)-S	110.5(3)	C(7)-C(8)-S	109.1(3)
C(66)-C(61)-C(62)	118.1(3)	C(66)-C(61)-C(6)	116.8(4)
C(66)-C(61)-C(6)	121.1(3)	C(66)-C(61)-C(62)	117.6(4)
C(62)-C(61)-C(6)	120.8(3)	C(6)-C(61)-C(62)	125.6(4)
C(63)-C(62)-C(61)	121.6(4)	C(63)-C(62)-C(61)	120.7(5)
C(62)-C(63)-C(64)	120.0(4)	C(62)-C(63)-C(64)	120.6(5)
C(65)-C(64)-C(63)	118.6(4)	C(65)-C(64)-C(63)	119.0(5)
C(64)-C(65)-C(66)	121.8(4)	C(66)-C(65)-C(64)	121.5(5)
C(65)-C(66)-C(61)	119.8(4)	C(65)-C(66)-C(61)	120.6(4)
C(4)-C(5)-C(6)-C(61)	-178.9(3)	C(4)-C(5)-C(6)-C(61)	-4.1(9)
C(5)-C(6)-C(6l)-C(62)	-128.8(4)	C(5)-C(6)-C(61)-C(6)	-2.2(9)
PLl/PL2)	55.2(2)	PL1/PL2	6.0(3)

derivatives allowed us to select the diagnostic properties which differentiate both types of isomers. The chemical shifts of the *ortho*-protons of the arylidene ring were shifted downfield ~8.00-9.00 ppm for the 2,3-substituted isomers which was caused by an anisotropic effect of the N-1 atom in comparison with the uninfluenced *ortho*-protons of the 1,2-substituted isomers at 7.06-7.62 ppm. The only exception was the benzylidene derivative which showed similar chemical shifts for both isomers. Thus, now, based on crystallographic confirmation of structures **3a** and **4a**, we can state that the *E* and *Z* isomers can be differentiated by proton nmr spectroscopy as follows: isomer *E* of the 1,2-substituted derivative **4a** shows chemical shifts for the *ortho*-protons at 8.18 ppm, in contrast to the *Z* isomer of 1,2-substituted derivative **3a** at 7.35-7.50 ppm. In this case the anisotropic effect of the carbonyl group causes the (Ho) peak to be shifted downfield.

Biological Evaluation of Compounds **3a-3d**, **3f**, **3k-3m**, **4a**, **5a-5m** as Ligands for Benzodiazepine Binding Sites.

The compounds were investigated in radioligand binding assays at rat brain cortical membranes. All compounds were screened for their potency to displace [^{3}H]

Table 4

Inhibition of [^{3}H]Diazepam Binding to Rat Brain by *un*- (**3a**, **4a**, **5a**) or *mono*- (**3b-d**, **3f** and **5b-i**) Substituted Compounds

Compound	R	% [a]	Ref	Log P [b]	Compound	R	% [a]	Ref	Log P [b]
3a	H	26 ±5	16	1.37	**5a**	H	12 ±10	16	1.61
4a	H	3 ±14		1.37					
3b	2-OCH_3	4 ±12	17	1.33	**5b**	2-OCH_3	0 ±11	17	1.57
3c	4-OCH_3	9 ± 1	16	1.32	**5c**	4-OCH_3	16 ±1	16	1.57
3d	4-Cl	7 ±1	16	2.09	**5d**	4-Cl	5.5 ±1.8	13	2.34
					5e	3-Cl	38.1 ±2.9	13	2.39
3f	2-Cl	14 ±5	16	2.08	**5f**	2-Cl	39.1 ±0.6	13	2.33
					5g	4-Br	0 ±8	16	2.50
					5h	4-NO_2	0 ±15	16	1.53
					5i	3-NO_2	44 ±8	16	1.59

[a] Percent specific inhibition of [^{3}H]diazepam binding to rat brain cortical membranes at a drug concentration of 25 μ*M*. [b] Log P values calculated by means of PALLAS program [24]

Table 5

Inhibition of [^{3}H]Diazepam Binding to Rat Brain by Disubstituted Compounds **3k-m** and **5k-m**

Compound	R^1	R^2	% [a]	Log P [b]	Compound	R^1	R^2 [a]	%	Log P [b]
3k	2-OCH_3	4-OCH_3	33 ±9	1.26	**5k**	2-OCH_3	4-OCH_3	0 ±24.0	1.51
3l	2-Cl	6-Cl	12 ±7	2.79	**5l** [c]	2-Cl	6-Cl	17.3 ±7.1	3.04
3m	2-Cl	6-F	14 ±7	2.24	**5m** [c]	2-Cl	6-F	21.5 ±4.4	2.48

[a] Percent specific inhibition of [^{3}H]diazepam binding to rat brain cortical membranes at a drug concentration of 25 μ*M*. [b] Log P values calculated by means of PALLAS program [24]. [c] Ref [13].

diazepam from its binding site in a single concentration (25 μ*M*).

Structure-Activity Relationships.

The results obtained for *mono*-substituted and *di*-substituted derivatives are presented in Tables 4 and 5. Generally the imidazothiazolones of type **3**, **4** and **5** were less potent compared to the imidazothiazepinones [13]. The spatial orientation of the phenyl ring has little influence on the benzodiazepine receptor affinity for compounds **3a**, **4a**, and **5a**. No meaningful difference was also observed for 1,2- and 2,3-substituted isomers. The calculated log P values [24] also could not be correlated with the affinity for benzodiazepine receptors (Table 4, 5). It appears that the greatest influence on biological activity has the nature and the number of substituents on the phenyl ring (Table 4). Among the *mono*-substituted derivatives the most active were *meta*-nitro and *ortho*-chloro substituted compounds **5i**, **5f** and **5e** respectively. The lack of substituents, or methoxy, bromo or nitro substituents placed at the 2 or 4 position reduced affinity *e.g.* **3a**, **4a**, **5a**, **3b**, **3c**, **5b**, **5c**, **5g** and **5h**. In the series of *di*-substituted compounds (Table 5) of type **3** and **5** the influence of the nature and the position of substituents on the affinity was less pronounced.

EXPERIMENTAL

Chemistry.

Melting points were not corrected. The tlc were performed on Merck Silica gel GF_{254} precoated tlc sheets; the used solvent system was chloroform:ethyl acetate (1:1). Electron impact and electron spray mass spectra were recorded on a Finningan MAT 95S spectrometer with a direct inlet. Infrared spectra were measured with a Specord M80 spectrometer (Carl Zeiss, Jena). The uv spectra were obtained with uv-vis-spectrophotometer (UV-VIS V-530 Jasco). The ^{1}H nmr spectra were performed on a Bruker AC-200F or a Bruker DPX 250 AVANCE spectrometer in deuteriodimethyl sulfoxide using tetramethyl silane as an internal standard. The elemental analyses were performed at the Department of Pharmaceutical Chemistry of Jagiellonian University.

Compounds **3a**, **3b-3d**, **3f**, **5a-5i**, **5l**, **5m** were obtained according to the references in Tables 4, 5.

Z-5-Benzylidene-2,3-dihydroimidazo[2,1-*b*]thiazol-6(5*H*)-one (**3a**) and *E*-5-Benzylidene-2,3-dihydroimidazo[2,1-*b*]thiazol-6(5*H*)-one (**4a**).

To the stirred suspension of 2.04 g (0.01 mole) of *Z*-5-benzylidene-2-thiohydantoin (**2a**), 4.0 g of potassium carbonate, and 0.3 g (0.001 mole) of benzyltriethylammonium chloride in 50 ml of acetone was added dropwise a solution of 2.06 g (0.011 mole) of 1,2-dibromoethane in 10 ml of acetone. The mixture was

stirred at room temperature for two days. The precipitate was removed, washed with water, and then with 1% aqueous sodium hydroxide and 1% aqueous hydrochloric acid solutions. The remaining solid and the residue after acetone filtrate evaporation were combined, suspended in chloroform and the suspension was filtered. The chloroform filtrate was washed with water, 1% aqueous sodium hydroxide and 1% aqueous hydrochloric acid solution, dried (sodium sulfate) and evaporated to dryness. The residue thus obtained was separated by column chromatography, silica gel 60 (70-230 mesh) using methylene chloride:ethyl acetate (1:1) as the eluent. Imidazothiazole **5a** was separated first, 0.93 g (41%), mp 197-198° (lit [16] 198-200°), tlc; R_f 0.41.

As a second fraction *Z*-imidazothiazole **3a** was separated as cream yellow crystals from chloroform:ethyl acetate 1:1 mixture, 230 mg (10%), mp 198-200°, tlc, R_f 0.13; ^{1}H nmr (250 MHz): δ 3.73 (def d, J = 3.2 Hz, 2H, SCH_2), 3.79 (def d, J = 3.2 Hz, 2H, NCH_2), 6.88 (s, 1H, ArCH=), 7.39-7.50 (m, 5H, Ar-H); ir (potassium bromide): ν 1700 (C=O), 1646 (ArCH=), 1488, 1472 (C=N), 1428, 1356, 1244, 760 cm^{-1}; uv (dioxane): λ max 347 nm (3.764), 283 nm (3.835), 245 nm (lg ε 3.662); ms: (electron spray) m/z 231 $(M+1)^{+\bullet}$.

Anal. Calcd. for $C_{12}H_{10}N_2OS$ (230.28): C, 62.59; H, 4.38; N, 12.16. Found: C, 62.37; H, 4.33; N, 11.97.

As a third fraction *E*-imidazothiazole **4a** was separated as intensely yellow crystals from chloroform:ethyl acetate (1:1 mixture), 200 mg (9%) mp 197-199°, tlc, R_f 0.08; ^{1}H nmr (250 MHz): δ 3.85 (def t, J = 7.5 Hz, 2H, SCH_2), 4.09 (def t, J = 7.5 Hz, 2H, NCH_2), 6.73 (s, 1H, ArCH=), 7.36-7.45 (m, 3H, 3'-H, 4'-H, 5'-H), 8.18 (dd, J = 8.0 Hz, J = 2.2 Hz, 2H, 2'-H, 6'-H); ir (potassium bromide): ν 1680 (C=O), 1608 (ArCH=), 1472 (C=N), 1412, 1386, 1250, 1108, 1092, 1072, 784; uv (dioxane): λ max 370 nm (log ε 3.722), 290 nm (log ε 3.803), 248 nm (log ε, 3.641); ms: (electron impact) m/z 230 $(M^{+\bullet})$, 202, 143, 129, 116, 102, 89, 86.

Anal. Calcd. for $C_{12}H_{10}N_2OS$ (230.28): C, 62.59; H, 4.38; N, 12.16. Found: C, 62.71; H, 4.30; N, 11.92.

Z-5-(2-Chloro-6-fluorobenzylidene)-2,3-dihydroimidazo[2,1-*b*]-thiazol-6(5H)-one (**3m**).

This compound was obtained as cream yellow crystals from ethanol, 250 mg (9%), mp 212-214°, tlc, R_f 0.27; ^{1}H nmr (250 MHz): δ 3.63 (def t, 2H, SCH_2), 3.76 (def t, 2H, NCH_2), 6.62 (s, 1H, ArCH=), 7.41-7.48 (m, 1H, 4'-H), 7.52-7.64 (m, 2H, 3'-H, 5'-H); ir (potassium bromide): ν 1712 (C=O), 1656, 1648 (ArCH=), 1596, 1552, 1516, 1484 (C=N), 1356, 1228, 888, 796, 668 cm^{-1}; ms: (electron impact) m/z 282 $(M^{+\bullet})$, 247, 168, 141, 133, 107, 86.

Anal. Calcd. for $C_{12}H_8N_2OSClF$ (282.71): C, 50.98; H, 2.85; N, 9.91. Found: C, 50.72; H, 2.94; N, 9.72.

Z-5-(2,6-Dichlorobenzylidene)-2,3-dihydroimidazo[2,1-*b*]thiazol-6(5*H*)-one (**3l**).

This compound was obtained as cream yellow crystals from ethanol, 300 mg (10%), mp 241-243°, tlc; R_f 0.23; ^{1}H nmr (250 MHz): δ 3.44 (def t, J = 7.5 Hz, 2H, SCH_2), 3.68 (def t, J = 7.5 Hz, 2H, NCH_2), 6.66 (s, 1H, ArCH=), 7.48 (dd, J = 6.5 Hz, J = 0.5 Hz, 1H, 4'-H), 7.62 (s, 1H, 3'-H), 7.63 (d, 1H, 5'-H); ir (potassium bromide): ν 1712 (C=O), 1656, 1648 (ArCH=), 1552, 1472 (C=N), 1350, 1228, 1100, 782, 668 cm^{-1}; ms: (electron impact) m/z 299 $(M^{+\bullet})$, 263, 184, 157, 114, 86.

Anal. Calcd. for $C_{12}H_8N_2OSCl_2$ (299.17): C, 48.18; H, 2.69; N, 9.36. Found: C, 48.01; H, 2.55; N, 9.24.

Z-5-(2,4-Dimethoxybenzylidene)-2,3-dihydroimidazo[2,1-*b*]thiazol-6(5*H*)-one (**3k**) and *Z*-6-(2,4-Dimethoxybenzylidene)-2,3-dihydroimidazo[2,1-*b*]thiazol-5(6*H*)-one (**5k**).

Compound **5k** was separated first as yellow crystals from dimethylformamide 0.63 g (22%), mp 212-215°, tlc, R_f 0.40; ^{1}H nmr (200 MHz): δ 3.35 (s, 2H, SCH_2), 3.83 (s, 3H, 2'-OCH_3), 3.86 (s, 2H, NCH_2), 3.88 (s, 3H, 4'-OCH_3), 6.61 (s, 1H, 3'-H), 6.65 (dd, J = 9.7 Hz, J = 2.4 Hz, 1H, 5'-H), 7.06 (s, 1H, ArCH=), 8.59 (d, J = 8.5 Hz, 1H, 6'-H); ir (potassium bromide): ν 1704 (C=O), 1648 (ArCH=), 1590 (C=N), 1270, 1204, 1088, 1022, 668 cm^{-1}; ms: (electron impact) m/z 290 $(M^{+\bullet})$, 261, 259, 176, 167, 160, 146, 139, 121.

Anal. Calcd. for $C_{14}H_{14}N_2O_3S$ (290.34): C, 57.92; H, 4.86; N, 9.65. Found: C, 57.77; H, 4.81; N, 9.53.

Compound **3k** was separated as a second fraction as light yellow, cotton-like crystals from ethanol, 120 mg (4%), mp 201-203°, tlc; R_f 0.12; ^{1}H nmr (200 MHz): δ 3.35 (s, 2H, SCH_2), 3.76 (dd, J = 6.7 Hz, J = 2.1 Hz, 2H, NCH_2), 3.82 (s, 3H, 2'-OCH_3), 3.84 (s, 3H, 4'-OCH_3), 6.61 (dd, J = 10.9 Hz, J = 2.3 Hz, 1H, 5'-H), 6.64 (d, J = 2.3 Hz, 1H, 3'-H), 6.80 (s, 1H, ArCH=), 7.33 (d, J = 8.2 Hz, 1H, 6'-H); ir (potassium bromide): ν 1692 (C=O), 1648 (ArCH=), 1600, 1502, 1478 (C=N), 1296, 1236, 1162, 1132, 1024, 668 cm^{-1}; ms: (electron impact) m/z 290 $(M^{+\bullet})$, 259, 176, 146.

Anal. Calcd. for $C_{14}H_{14}N_2O_3S$ (290.34): C, 57.92; H, 4.86; N, 9.65. Found: C, 57.65; H, 4.93; N, 9.44.

Benzodiazepine Binding Assays

Frozen rat brains were obtained from Pel-Freez, Rogers, Arkansas, USA. The cortex was dissected and inhibition of binding of [^{3}H]diazepam to rat brain cortical mernbranes was determined as previously described [13]. The compounds were dissolved in dimethyl sulfoxide and further diluted with tris(hydroxymethyl)aminomethane-hydrochloride buffer (50 m*M*, *p*H 7.4), (final dimethyl sulfoxide concentration 1%). In a final volume of 1 ml, each test tube contained 790 µl of tris(hydroxymethyl)-aminomethane-hydrochloride buffer (50 m*M*, *p*H 7.4), 10 µl of investigated compound solution, 100 µl of rat cerebral cortical membrane preparation with a protein concentration of *ca.* 100 µg per tube, and 100 µl of [^{3}H]diazepam solution, to give a final concentration of 1 n*M*. Dimethyl sulfoxide (final concentration: 1%) was necessary since the compounds possessed low water-solubility. Higher concentrations of dimethyl sulfoxide, however, were not tolerated by the receptors. Dimethyl sulfoxide without test compound served as the control.

Incubations were performed at 2° for 1 hour and were terminated by rapid filtration through glass fiber filters (Schleicher & Schüll GF 51) using a Brandel cell harvester M-24 (Brandel, Gaithersburg, Maryland, USA). Three 5 ml washes with ice-cold tris(hydroxymethyl)aminomethane-hydrochloride buffer were performed. Unlabelled diazepam (5 µ*M*) was used to define non specific binding. All compounds were tested in a single concentration of 25 µ*M* in at least three independent experiments each in triplicate.

Crystal Structure Determination.

The crystals of **3a** and **4a** were obtained by slow evaporation of an ethanol solution. All crystallographic data are in Table 1. Preliminary crystallographic data were obtained from KM4 four-cycle diffractometer; the accurate cell dimensions were determined by the least-squares refinement from the angular settings of 25 reflections located within $10<\theta<40°$ for compound **3a** and for com-

pound **4a** of 25 reflections located within $10<\theta<40°$; crystal of 0.4 x 0.2 x 0.2 mm for compound **3a** and respectively 0.4 x 0.2 x 0.2 mm for compound **4a** were applied to collect diffraction data on KM4 diffractometer by the $\omega/2\theta$ scan technique and using graphite monochromated CuKα radiation at room temperature for $\theta<84°$ for **3a** and $\theta<80°$ for **4a** [h:-8 → 8, k: -1 → 26, l: -1 → 9 for compound **3a** and h: -8 → 1, k: -1 → 10, l: -25 → 25 for compound **4a**]; an absorption correction was not applied; the intensity of three standard reflections monitored every 100 reflections showed no significant fluctuations for both compounds; 2842 for **3a** and 3203 for **4a** reflections were measured, 1313 for **3a** and 674 for **4a** reflections were considered observed using the criterion $F_0>4\sigma(F_0)$. The structures were solved by a direct method (SHELXTL-PC) [25]. The E-map provided positions for all non-H-atoms; full-matrix least-squares refinement was carried out on F^2-s using anisotropic temperature factors for all non-H-atoms; the positions of all H-atoms were from $\Delta\rho$-maps; isotropic thermal parameters of H-atoms were taken as 1.5 times of the temperature factors for their parent-atoms than the positions of H-atoms were refined in the riding model, being finished at R1 = 0.060, wR2 = 0.149 for **3a** and R1 = 0.082, wR2 = 0.221 for **4a** (with $w = 1/[\rho^2(Fo^2)+(0.1089)^2+0.4P]$ for **3a** and $w = 1/[\rho^2(Fo^2)+(0.1461)^2+2.3P]$ for **4a** where $P = Fo^2 + 2Fc^2)/3$) and empirical extinction correction coefficient g = 0.0112(17) for **3a** and g = 0.0023(6) for **4a**, S = 1.063 (167 parameter) for **3a** and S = 0.954 (146 parameter) for **4a**; final changes $\Delta/\rho < 0.01$; $\Delta\rho$ min = -0.402 e A^{-3}, $\Delta\rho$ max = 0.357 e A^{-3} for **3a** and $\Delta/\rho < 0.01$; $\Delta\rho$ min = -0.50 e A^{-3}, $\Delta\rho$ max = 0.552 e A^{-3} for compound **4a**. The atomic scattering factors were taken from SHELXL-97 [26]. The X-ray structure analysis results are presented in the form of non-H-atoms coordinates in Table 2 as well as in Figure 1a-b [29].

Computational Procedures.

The geometry of all the isomers were optimized with MOPAC 6.0 using AM1 Hamiltonians in an aqueous environment (dielectric constants equals 78.4) [27]. The values of log P for the compounds investigated were calculated by means of PALLAS (version 1.2) program [24]. The conformation analyses were performed by molecular mechanics methods using the PCMOD.6 program [28]. The energy was minimized after each 10° clockwise rotation of two torsional angles in the 360° range.

Acknowledgements.

The authors wish to thank Mrs. Maria Kaleta for excellent technical assistance. The financial support for the synthetic, crystallographic and computational parts of this research carried out under the Polish State Committee for Scientific Research, grant No 4 P05F 007 13 is gratefully acknowledged.

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