Imidazo[2,1-*b*]thiazoles, Imidazo[2,1-*b*]imidazoles and Pyrrolo[1,2-*c*]imidazoles. Synthesis, Structure and Evaluation of Benzodiazepine Receptor Binding

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Received August 21, 2000

As a continuation of our studies on bicyclic heterocycles with benzodiazepine receptor affinity, derivatives with a 5:5 bicyclic skeleton, namely imidazo[2,1-*b*]thiazoles, imidazo[2,1-*b*]imidazoles and pyrrolo[1,2-*c*]imidazoles were prepared. The compounds possessed an aromatic substituent with different spatial arrangement and distance to the bicyclic skeleton. X-ray structure analysis was performed for *Z*-2-(4-chlorobenzylidene)-5,5-diphenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]imidazoline-3,6-dione (**6a**) and 5-amino-6-cyano-7-phenyl-1-oxo-3-thioxo-2,3-dihydro-1*H*-pyrrolo[1,2-*c*]imidazole (**20a**). In contrast to the previously described arylideneimidazo[2,1-*b*]thiazepinones the smaller heterocyclic ring systems investigated in this study were devoid of meaningful benzodiazepine receptor affinity as well as anticonvulsant activity.

J. Heterocyclic Chem., **39**, 243 (2002).

Introduction.

In our studies on bicyclic heterocycles with potential central nervous system activity containing an imidazole moiety, we focused on central benzodiazepine receptor (BZR) ligands [1–3]. Benzodiazepines [4], which mediate their actions at the BZR as full agonists, exhibit a wide variety of pharmacological actions, such as anxiolytic, sedative/hypnotic and anticonvulsant activities. They are the most widely studied and most commonly used drugs in the treatment of generalized anxiety disorders. Benzodiazepines are very potent, rapid-onset anxiolytics, however non-curative. In severe cases of anxiety, patients may need life-long therapy. Benzodiazepines exhibit numerous side effects such as physical dependence, amnesia, oversedation, muscle relaxation, or ethanol potentiation. For therapeutic purposes, it would be useful to find BZR partial agonists devoid of the above-mentioned side effects.

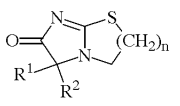
Ligands at BZR do not only belong to the structural class of benzodiazepines [5], but a large number of ligands from different chemical families including imidazopyridines [6], β-carbolines [7], imidazoquinoxalines [8], triazinobenzimidazolones [9] and pyrazoloquinolines [10] have been shown to bind with high affinity to BZR. All of them are condensed nitrogen-containing heterocyclic compounds. The importance of rotatable aromatic rings in BZR ligands for the recognition and activation of BZR has been underlined [11].

As part of our wider program on SAR studies evaluating the BZR binding of bicyclic heterocycles, we have previously synthesized and biologically examined diphenyl and arylidene imidazo[2,1-*b*]thiazines, imidazo[2,1-*b*]thiazepines [1] (Scheme 1a), imidazo-[2,1-*b*]pyrimidines, and imidazo[2,1-*b*]diazepines [3].

In the present work, the synthesis and BZR binding of a new series of imidazo[2,1-*b*]thiazoles, imidazo[2,1-*b*]-imidazoles and pyrrolo[1,2-*c*]imidazoles possessing aromatic groups, with different lipophilic, steric and electronic features are reported.

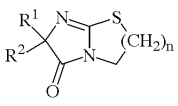
Various hetero-bicyclic structures with two five-membered rings (abbreviated as 5+5 bicyclic skeleton) were obtained. All of them contain at least two nitrogen atoms (Scheme 1b) and an –N=C– structure. For reasons of clarity in the future discussion, four quadrants in the 5+5 bicyclic skeleton were assigned as shown in Scheme 1b. According to this convention, the nitrogen that forms the double bond with a neighboring carbon atom is located

Scheme 1a



1

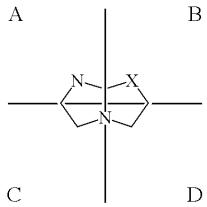
R^1, R^2 = Ar–(CH=CH)$_m$–CH=
m = 0, 1



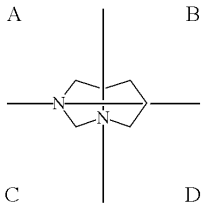
2

R^1, R^2 = Ph
n = 1, 2, 3

Scheme 1b

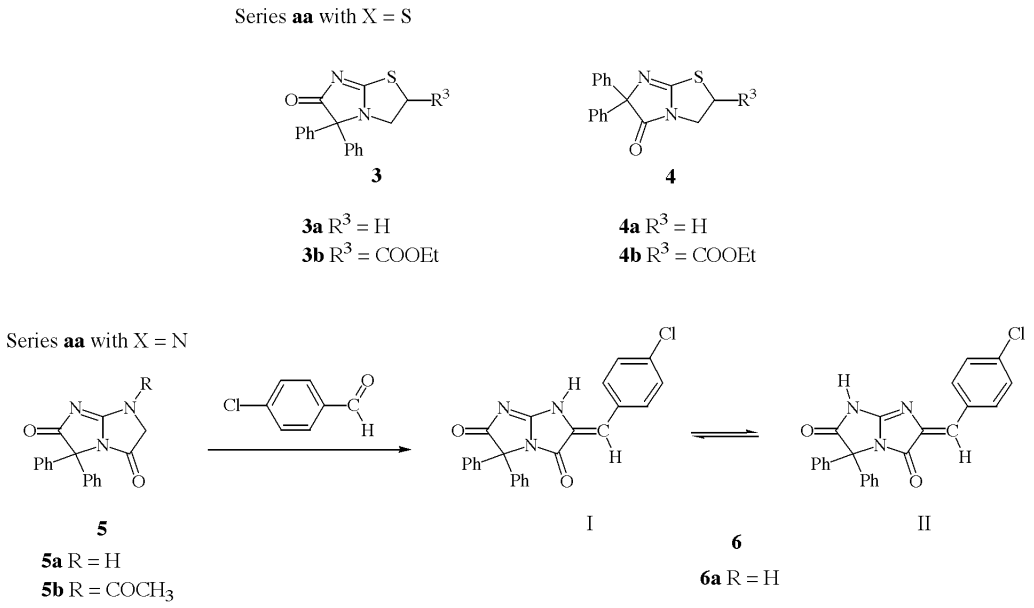


aa



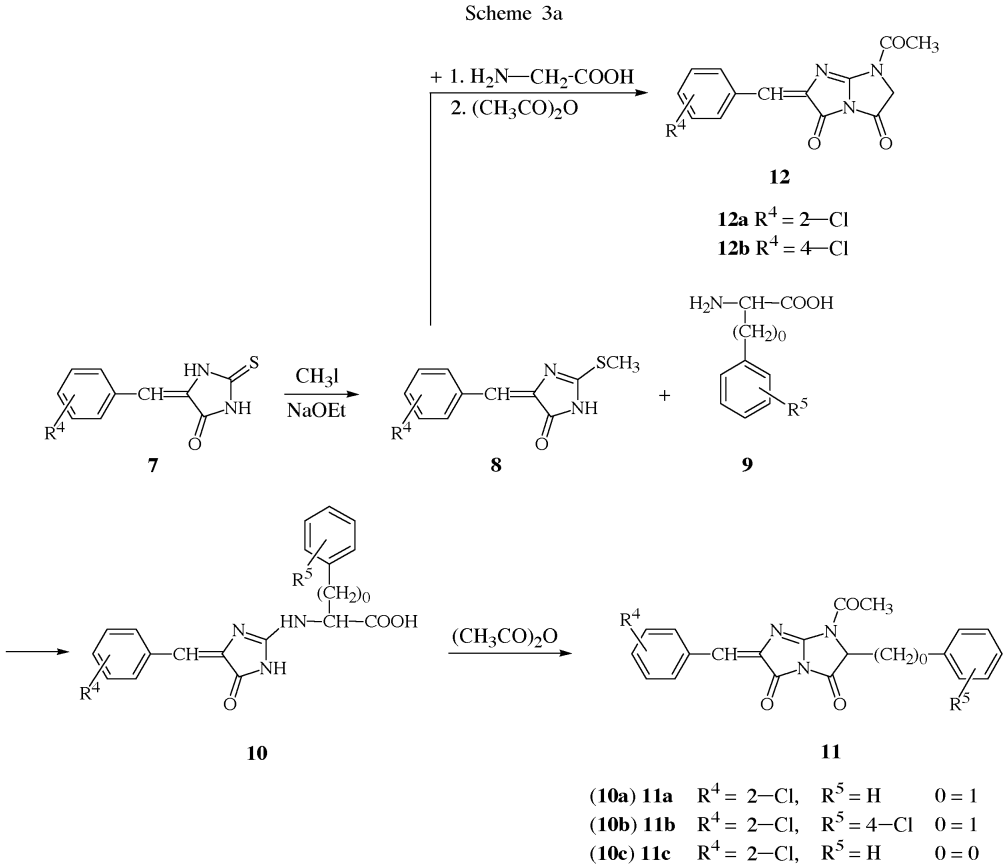
bb

Scheme 2



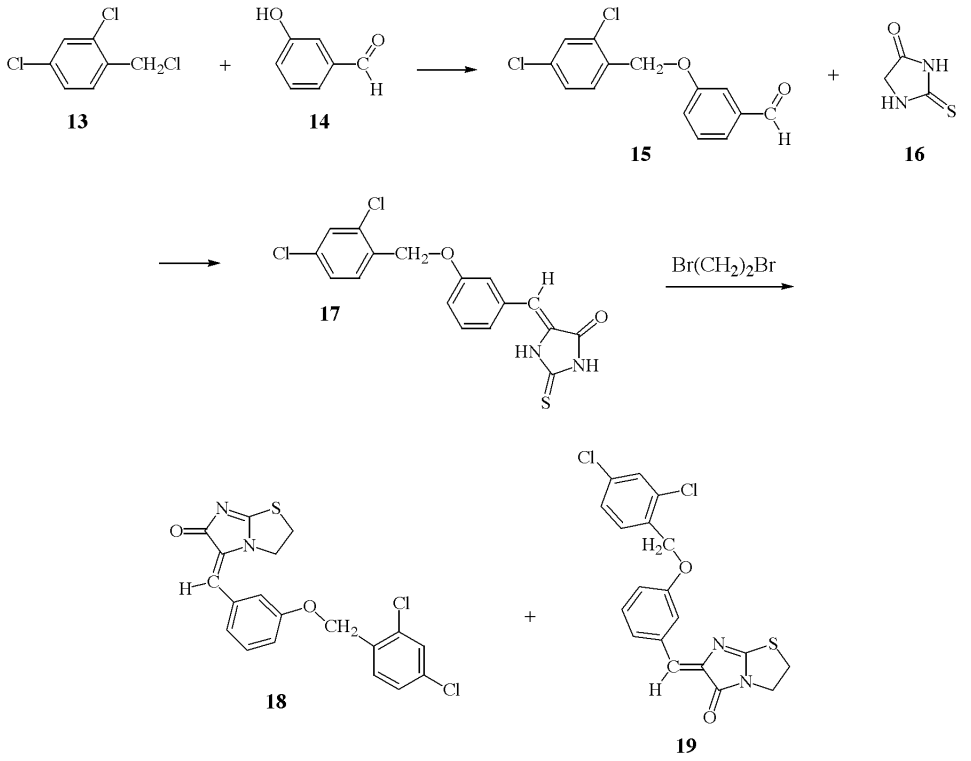
Diphenyl Derivatives from Series **aa**

Scheme 3a



Arylidene Derivatives from Series **aa** X = N

Scheme 3b



Arylidene Derivatives from Series **aa** X = S

on the axis separating quadrants C and D. Consequently, two possible positions for the second imidazole nitrogen atoms lead to two series of derivatives as shown in Scheme 1b: series **aa** with the second nitrogen atom in quadrant A (*e.g.*, imidazothiazoles **3**, **4** – Scheme 2, **18**, **19 –** Scheme 3b and imidazoimidazolones **5**, **6** – Scheme 2, **11**, **12 –** Scheme 3a) and series **bb** with a nitrogen atom on the line between quadrants A and C (Scheme 1b) (pyrroloimidazoles **20** – Scheme 4). It should be noted that the derivatives from the series **aa** contain an additional heteroatom in the quadrant B marked as X (sulfur or nitrogen). Moreover, some examples of that series can differ significantly in substitution pattern and hybridization of the four border carbon atoms. This results in different chemical and biological properties and causes a variety of geometrical phenomena. In the present paper, the chemical aspects of the 5+5 bicyclic compounds will be discussed. The description of structural variations will be the subject of a subsequent paper [12].

One important goal was to synthesize and investigate bicyclic compounds bearing aromatic substituents in different spatial positions. Up to now, the compounds that we had examined possessed phenyl substituents mainly in quadrants A and C [1–3]. In the present study, new compounds were to be synthesized in which the phenyl substituent (or substituents) should be placed in different spatial arrangements and different distances. So, diphenyl derivatives **3** and **4** (Scheme 2) as well as phenyl derivatives **20a** and **20b** (Scheme 4) were designed. In addition, the synthesis of compounds **6** (Scheme 2), **11a**–**11c** (Scheme 3a), **18** and **19** (Scheme 3b), possessing more than one aromatic substituent were projected. Among them, compounds **6**, **11a**–**11c** and **18** were aimed at probing the areas of quadrants B and D of the molecules with respect to aromatic substituents.

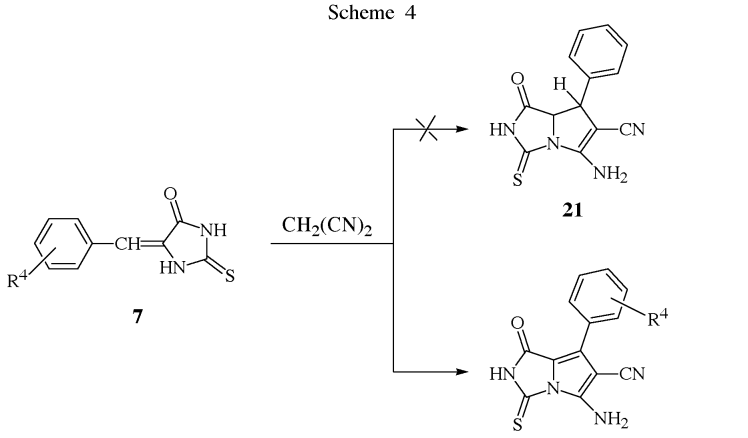
The second aim of this study was to examine the influence of the differently organized hetero-bicyclic skeletons (Scheme 1b) on BZR affinity of the compounds. The designed imidazothiazole derivatives **3**, **4**, **18,** and **19** were intended for comparison with the imidazoimidazole derivatives **6**, **11a**–**11c**, **12a**–**12b**, both from series **aa**, and with the pyrroloimidazoles **20a**, **20b** (Scheme 2–4) representing series **bb**.

Chemistry.

5+5 Bicyclic Derivatives from Series **aa**.

The synthesis of the diphenyl derivatives **3a**, **3b**, **4a**, **4b** with X=S and **5a**, **5b** with X=N (Scheme 1 and 2) was described previously [13–15]. Condensation of **5a** or **5b**

Scheme 4



Monophenyl Derivatives from Series **bb**

with 4-chlorobenzaldehyde, led to **6a** (X=N). Acetyl derivative **5b** was deacetylated during the reaction. The spectral (^{1}H, ^{13}C nmr, ms) and elemental analyses allowed to confirm structure **6a**. Such a compound may exist in *E* or *Z* form. Our experience with the condensation of benzaldehydes with compounds containing active methylene groups suggested that the *Z*-isomer would be preferred [2,15,16–20]. Moreover, **6a** may exist in the two tautomeric forms presented in Scheme 2. In order to confirm the structure, configuration and topological properties of **6a**, X-ray structure analysis was performed. The ORTEP drawing of structure **6a** with atom numbering is shown in Figure 1. All X-ray details, non-hydrogen atoms fractional coordinates and selected geometrical data are collected in Tables 1, 2 and 3.

X-ray structure analysis (Figure 1) confirms the *Z*-configuration of **6a**. However, the unambiguous assignment to either tautomer **6a-I** or **6a-II** (Scheme 2) is not possible. This is due to the fact that in the independent unit cell two units of **6a** were found, marked as molecules a and b (Table 2). These molecules are not identical (Table 3); differences are found particularly in the bond lengths of C2-N (3 and/or 6). Molecule a, with double bond N3a-C2a = 1.293(4) Å corresponds to the tautomer **6a-I**, while the molecule b with shorter N6b-C2b = 1.266(4) Å suggests the existence of a **6a-II** tautomer. Unfortunately, the obtained difference electron density map did not allow for the assignment of the two hydrogen atoms attached to the heterocyclic nitrogen atoms (N3a or N6a in molecule a and N3b or N6b in molecule b) (Figure 2). Due to the monocrystal properties (crashing), low temperature diffraction measurement was impossible. The only rational interpretation of the H-bond pattern [with distances N3a (or b)···N6b (or a) equaling 2.995(3) Å] would be that in the crystal the dimeric associates shown in Figure 1 are statistically formed by two

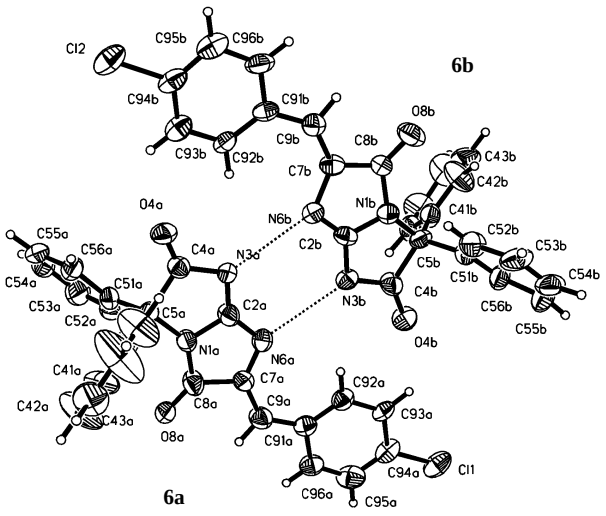


Figure 1. ORTEP view of two independent molecules of **6a**. Possibly H-bonds directions were marked by dotted lines.

molecules of **6a-I** or **6a-II**. Having this conclusion in mind, only H-bonds were marked in Figure 1 by dashed lines, but unquestionably both tautomers are present in the crystal. In addition, based on crystallographic data, it was established that elements of the bicyclic structure (5+5) are practically planar and incline to each other at 2.4(2)° and 3.9(2)° respectively in molecules a and b.

From theoretical calculations (MOPAC.6 in AM1 approximation [25]), tautomer **6a-II** is thermodynamically more stable by 11 kcal/mol. However, in solution (DMSO), we suppose that form **6a-I** dominates on the basis of ^{1}H nmr analysis, which is well in accordance with the structure of **5a**. An earlier performed X-ray analysis confirmed structure **5a** as presented in Scheme 2 [15]. The signal of the N–H

Table 1

X-ray Structure Analysis of **6a** and **20a**

	Data for **6a**	Data for **20a**
A. Crystals Parameters		
formula	$C_{24}H_{16}ClN_3O_2$	$C_{13}H_8N_4OS$, C_3H_7NO
molecular weight	413.86	341.39
crystallization medium	ethanol	DMF
colour	colourless	intensive yellow
crystal size, mm	0.1 x 0.15 x 0.3 mm	0.1 x 0.1 x 0.5 mm
cell dimensions	a = 9.232(2) Å	a = 3.9220(10) Å
	b = 14.366(3) Å	b = 39.026(8) Å
	c = 29.996(6) Å	c = 10.621(2) Å
	β = 96.98(3) °	β = 99.11(3) °
space group	$P2_1/n$	$P2_1/c$
molecules/unit cell	8	4
density calcd.	1.392 Mg•m^{-3}	1.413 Mg•m^{-3}
linear absorption factor, mm^{-1}	19.3	1.966
B. Refinement Parameters.		
number of reflections	4608	3361
nonzero reflection [I>4σ(I)]	1709	1319
R-index	0.0699	0.0482
GOF	0.73	0.677
secondary extinction factor C, χ	NONE	0.00000(17)

proton was observed at 10.30 ppm. The N–H signal of structure **6a** was found at 13.20 ppm. The significant downfield shift was presumably caused by the influence of the neighboring arylidene substituent. Such a deshielding effect was also observed when the signals of the unsubstituted 5,5-diphenyl hydantoin protons (N_1–H, N_3–H) observed at 9 and 11 ppm were compared with those of the 5-arylidene ones occurring both at ~12 ppm (*e.g.*, **17**).

Arylidene imidazo[2,1-*b*]imidazole-3,5-dione derivatives **11a–11c** and **12a**–**12b** were obtained by a method which had been developed in our group for the preparation of compound **12b** [15]. So, the appropriate 5-arylidene-2-thiohydantoin derivatives **7** were methylated using methyl iodide to yield the 2-methylthio-derivatives **8**, which were treated with glycine or glycine derivatives **9**. The resulting glycine-amidine derivatives were refluxed with acetic acid anhydride to yield the imidazo[2,1-*b*]imidazole-3,5-diones **11a–11c** and **12a–12b** (Scheme 3a). For the synthesis of compounds **18** and **19**, the starting benzaldehyde (**15**) was obtained by alkylation of 3-hydroxybenzaldehyde with 2,4-dichlorobenzyl chloride (Scheme 3b). Compound **15** was condensed with 2-thiohydantoin (**16**) to yield 5-arylidene-2-thiohydantoin derivative **17**. Cyclization of **17** with

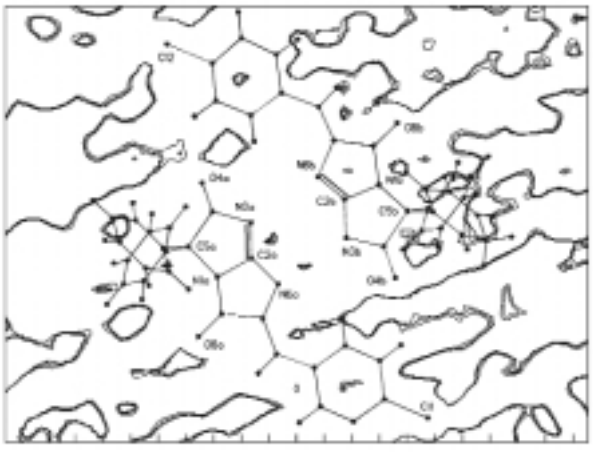


Figure 2. Different electron density map for **6a**. Projection in the plane passes by the atoms N3a, N6b and N6b.

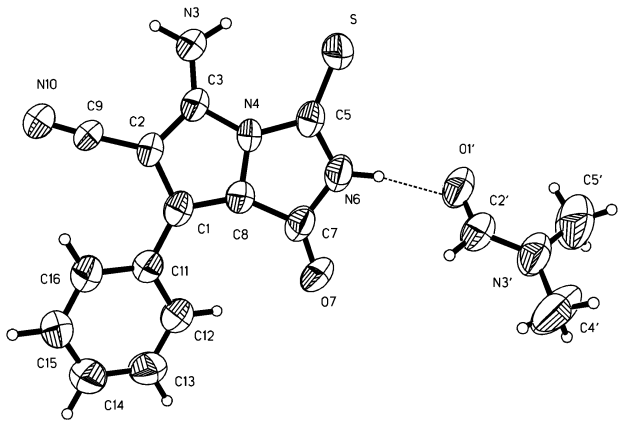


Figure 3. ORTEP view of **20a**, **20a** crystallized with dimethyl formamide molecule. Intramolecular H-bonds are marked by dotted lines.

Table 2

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

Compound **6a**

	x	y	z	U(eq)
Molecule a				
Cl1	8154(1)	6865(1)	1908(1)	79(5)
O4a	-2610(3)	9438(2)	3292(1)	68(1)
O8a	2825(3)	8159(2)	4074(1)	66(1)
N1a	0859(3)	8560(2)	3570(1)	50(1)
N3a	-0717(3)	8915(2)	2938(1)	52(1)
N6a	1761(3)	8324(2)	2929(1)	48(1)
C2a	0561(4)	8625(2)	3105(1)	54(2)
C4a	-1457(4)	9178(2)	3314(1)	49(2)
C5a	-0453(4)	8964(3)	3756(1)	50(2)
C7a	2820(4)	8064(3)	3268(1)	55(2)
C8a	2238(5)	8265(3)	3703(1)	63(2)
C9a	4235(4)	7721(3)	3285(1)	62(2)
C41a	0832(4)	9781(3)	4426(1)	109(2)
C42a	1200(7)	10533(4)	4679(2)	143(3)
C43a	0767(6)	11388(3)	4530(2)	107(3)
C44a	-0083(9)	11467(3)	4139(2)	178(4)
C45a	-0477(6)	10655(3)	3889(2)	120(3)
C46a	-0048(4)	9822(3)	4038(1)	60(2)
C51a	-1162(4)	8202(3)	4027(1)	52(2)
C52a	-0676(4)	7299(3)	4067(1)	65(2)
C53a	-1458(5)	6675(3)	4297(1)	82(9)
C54a	-2696(5)	6919(3)	4464(1)	75(9)
C55a	-3215(4)	7831(3)	4434(1)	70(2)
C56a	-2469(4)	8480(3)	4200(1)	60(2)
C91a	5084(4)	7497(2)	2930(1)	53(2)
C92a	4675(4)	7652(2)	2481(1)	55(2)
C93a	5592(4)	7450(3)	2170(1)	62(1)
C94a	7013(4)	7104(2)	2303(1)	51(2)
C95a	7431(4)	6960(3)	2752(1)	75(2)
C96a	6514(4)	7097(3)	3051(1)	58(2)
Molecule b				
Cl2	163(1)	10683(1)	3011(1)	79(5)
O4b	2818(3)	8103(2)	1596(1)	65(1)
O8b	-2728(3)	9295(2)	0823(1)	85(1)
N1b	-0664(3)	8931(2)	1327(1)	51(1)
N3b	0873(3)	8604(2)	1944(1)	45(1)
N6b	-1554(3)	9229(2)	1971(1)	50(1)
C2b	-0488(4)	8933(2)	1783(1)	49(2)
C4b	1563(4)	8379(3)	1574(1)	57(2)
C5b	0521(4)	8552(2)	1134(1)	48(1)
C7b	-2727(4)	9408(2)	1618(1)	46(1)
C8b	-2157(4)	9198(3)	1196(1)	54(2)
C9b	-4082(4)	9734(3)	1622(1)	55(2)
C41b	0128(4)	7634(3)	0878(1)	57(2)
C42b	-0733(5)	7717(3)	0456(1)	88(9)
C43b	-1071(4)	6888(3)	0236(1)	82(2)
C44b	-0801(6)	6053(3)	0408(1)	100(9)
C45b	0116(6)	5975(3)	0817(1)	99(9)
C46b	0460(4)	6782(3)	1053(1)	68(2)
C51b	1284(4)	9251(3)	864(1)	57(2)
C52b	0859(4)	10232(3)	847(1)	70(2)
C53b	1590(5)	10853(3)	605(1)	89(2)
C54b	2756(4)	10578(3)	407(1)	89(2)
C55b	3305(5)	9667(3)	423(1)	90(9)
C56b	2430(4)	9029(3)	656(1)	68(2)
C91b	-4969(4)	9958(2)	1969(1)	46(1)
C92b	-4531(4)	9825(3)	2420(1)	54(2)
C93b	-5403(4)	10054(3)	2739(1)	58(2)
C94b	-6746(4)	10436(3)	2601(1)	62(2)
C95b	-7275(4)	10642(3)	2156(1)	66(2)
C96b	-6339(4)	10382(3)	1842(1)	66(2)

Table 2 (continued)

Compound **20a**

	x	y	z	U(eq)
S	2214(2)	3659(1)	3834(1)	69(1)
C1	-2094(5)	4137(1)	7616(2)	56(1)
C2	-2282(5)	4395(1)	6638(2)	54(1)
C9	-3660(6)	4729(1)	6654(2)	59(1)
N10	-4826(5)	4998(1)	6587(2)	82(1)
C3	-1135(5)	4262(1)	5553(2)	53(1)
N3	-772(4)	4399(1)	4449(2)	66(1)
N4	-234(4)	3927(1)	5854(2)	53(1)
C5	1121(5)	3652(1)	5234(2)	53(1)
N6	1185(4)	3395(1)	6093(2)	62(1)
C7	34(5)	3483(1)	7250(2)	59(1)
O7	-79(4)	3289(1)	8134(1)	77(1)
C8	-895(5)	3845(1)	7061(2)	54(1)
C11	-3008(5)	4176(1)	8877(2)	55(1)
C12	-3926(6)	3892(1)	9569(2)	68(1)
C13	-4795(6)	3938(1)	10760(2)	78(1)
C14	-4698(6)	4258(1)	11307(2)	79(1)
C15	-3706(6)	4537(1)	10658(2)	75(1)
C16	-2890(5)	4497(1)	9454(2)	71(1)
O1'	3398(4)	2748(1)	5728(2)	89(1)
C2'	4941(7)	2629(1)	6694(3)	81(1)
N3'	6195(5)	2312(1)	6864(2)	83(1)
C4'	7974(8)	2188(1)	8019(3)	136(1)
C5'	5587(9)	2079(1)	5777(3)	138(1)

dibromoethane yielded a mixture of imidazo[2,1-*b*]thiazole derivatives **18** and **19**. In accordance with previously described results [19], compound **19**, the product of the 2,3-cyclization of 2-thiohydantoin **17**, was obtained as the major product and **18**, **19** are in *Z*-configuration.

5+5 Bicyclic Derivatives from Series **bb**.

The synthesis of the monophenyl derivatives **20a** and **20b** from series **bb** of the 5+5 bicyclic derivatives under discussion is presented in Scheme 4. The compounds were obtained according to a method described in the literature [21] with the addition of malononitrile to the 5-arylidene-2-thiohydantoin. However, in contrast to the described product (**21**) and the claimed yields, we were only able to isolate starting material **7** in addition to small amounts of pyrrolo[1,2-*c*]imidazoles **20a** and **20b** as products. The structures of the obtained compounds **20** were confirmed by elemental and spectral analyses. In the mass spectra, the molecular ions of the compounds were observed. In the ^{1}H nmr spectra, the absence of signals characteristic of alkane protons eliminated structure **21**. Due to unclear structural identification, the structure of **20a** was determined by means of X-ray structure analysis. The ORTEP drawing of structure **20a** with atom numbering is shown in Figure 3. All X-ray details, non-hydrogen atoms fractional coordinates and selected geometrical data are collected in Tables 1, 2 and 3, respectively.

The structure of **20a** (Figure 3) was unambiguously confirmed by the values of the bond lengths observed in the 5+5 skeleton (Table 3). All bonds in the

Table 3

Selected Geometrical Details in the Structures of **6a** and **20a**

Compound **6a**: Selected bond distances (Å)

Molecule A			Molecule B		
N1a	-C2a	1.393(3)	N1b	-C2b	1.356(4)
N3a	-C2a	1.293(4)	N3b	-C2b	1.374(4)
N3a	-C4a	1.437(4)	N3b	-C4b	1.385(4)
N6a	-C2a	1.354(4)	N6b	-C2b	1.266(4)
N6a	-C7a	1.374(4)	N6b	-C7b	1.442(4)
C4a	-C5a	1.554(4)	C4b	-C5b	1.555(5)
C7a	-C8a	1.498(5)	C7b	-C8b	1.461(5)
N3b……..N6a	2.995(3)		N6a……..N3b	2.995(3)	

Compound **20a**

a. Selected bond distances (Å)

S-C5	1.611(2)	C3-N4	1.379(2)
C1-C8	1.399(2)	N4-C8	1.386(2)
C1-C2	1.442(2)	N4-C5	1.407(2)
C2-C3	1.402(2)	C5-N6	1.355(2)
C2-C9	1.412(2)	N6-C7	1.418(2)
C9-C10	1.144(2)	C7-O7	1.213(2)
C3-N3	1.316(2)	C7-C8	1.463(2)

b. H-bonds geometry in the crystal of **20a**

D-H….a	d(D-H)	d(H….a)	d(D….a)	<(DHa)[°]	symm. cod.
N3 --H3a…S	0.9000	2.5340	3.2241(18)	133.91	
N3 --H3b…N10	0.8985	2.2095	3.019(3)	149.65	-1-x, 1-y, 1-z
N6 --H6a…O1'	0.8989	1.8182	2.716(2)	175.94	

five-membered ring defined by the atoms C1, C2, C3, N4 and C8 are conjugated bonds. Therefore, all carbon atoms are in sp^2 hybridization including C1 holding the aromatic substituent. Moreover, the C1–C8 bond of 1.399(2) Å showed double bond character with the shortest C-C bond in the 5+5 skeleton. The second C-C double bond corresponding to C2–C3 shows a distance of 1.402(2) Å. In the structure of **20a**, both five-membered rings from 5+5 moiety are planar and only slightly inclined against each other at 1.9(1)°. The planarity of the skeleton is also stabilized by an intramolecular N3–H···S bond (Table 3 and Figure 3). In the crystal, two additional intermolecular H-bonds are stabilizing the structure (Table 3).

Biological Evaluation of Selected Compounds.

Biological evaluation was performed for selected compounds, namely **3a**, **3b**, **4a**, **4b**, **6a**, **12a**, **12b**, **18**, **19**, **20a**, and **20b** (Table 4). These compounds were investigated in radioligand binding assays at rat brain cortical membranes [1]. All compounds were screened for their potency to displace [^{3}H]diazepam from its binding site in a single concentration (25 μ*M*). Compounds **11a**–**11c** were excluded from the biological evaluation since they were insoluble in the test concentration of 25 μ*M*. In addition, compounds **6a**, **12a** and **12b** were evaluated by the Antiepileptic Drug Development (ADD) Program with Phase I testing procedures [22,23]. Phase I of the evaluation involved three tests: maximal electroshock (MES), subcutaneous pentylenetetrazole (scMet), and neurologic toxicity.

Results and Discussion.

The physicochemical properties of the obtained compounds were preliminarily evaluated by calculation (PALLAS program [24]) of their log P and log D values. For **6a**, log P and log D values were calculated for both tautomers I and II. Calculated physicochemical properties of the obtained compounds (Table 4) have shown that only compounds **20a** and **20b** are very sensitive to the pH of the milieu showing very different values of log P and log D at pH 7 equal to pH 7.4, respectively, the remaining compounds showed equal values. Calculated values of log P and log D for both tautomers (I and II) of **6a** were slightly different. Although the log P/log D values of **18** and **19** are very high, higher than those of **11a** – **11c**, contrary to **11a** – **11c** (which were insoluble at test concentration of 25 μ*M*) it was possible to examine the *in vitro* **18** and **19** biological properties.

The results of the biological *in vitro* evaluation obtained for the 5+5 bicyclic diphenyl, arylidene and phenyl derivatives are presented in Table 4. The 5+5 bicyclic derivatives

Table 4

Inhibition of [^{3}H]Diazepam Binding to Rat Brain Cortical Membranes by Bicyclic Diphenyl (**3a**, **3b**, **4a**, **4b**, **6a**), Arylidene (**12a**, **12b**, **11a-11c**, **18**, **19**), and Phenyl Derivatives (**20a**, **20b**)

Compound	% [a]	Ref.	Log P [b]	log D [b]	
				pH=7.0	pH=7.4
3a	3 ± 10	[13]	2.06	2.06	2.06
4a	8 ± 6	[13]	2.23	2.23	2.23
3b	0 ± 11	[14]	2.64	2.64	2.64
4b	0 ± 22	[14]	2.81	2.81	2.81
6a	8 ± 2		3.49 [c]	3.49 [c]	3.49 [c]
			3.79 [c]	3.79 [c]	3.79 [d]
11a	[e]		2.60	2.60	2.60
11b	[e]		3.33	3.33	3.33
11c	[e]		2.15	2.15	2.15
12a	38 ± 1		0.39	0.39	0.39
12b	10 ± 11	[15]	0.39	0.39	0.39
18	28 ± 3		4.54	4.54	4.54
19	1		4.79	4.79	4.79
20a	0 ± 10		0.33	-2.50	-2.50
20b	14 ± 5		0.97	-2.06	-2.06

[a] Percent specific inhibition of [^{3}H]diazepam binding to rat brain cortical membranes at a drug concentration of 25 μ*M*; results from at least 3 separate experiments each in triplicate; [b] Log P, log D values calculated by means of the program PALLAS [24]; [c], [d] log P, log D values calculated for **6a** tautomer I [c] and tautomer II [d]; [e] Insoluble at the test concentration of 25 μ*M*.

were generally less potent as compared to the previously described imidazothiazinones and imidazothiazepinones [1] with bicyclic 5+6 and 5+7 skeletons. For the 5+5 bicyclic derivatives, the character of the nature of the annelated ring (imidazothiazoles and imidazoimidazoles from series **aa** or pyrroloimidazoles belonging to series **bb**) as well as the nature of the attached aromatic ring(s) (diphenyl, monophenyl, and arylidene) as well as their distance or localization in the quadrants were without influence on the activity. Similarly, neither the spatial localization of the aromatic rings nor the physicochemical properties of the compounds had any influence on the BZR affinity. It seems that the small size and the coplanarity of the 5+5 bicyclic skeleton are the factors that eliminate BZR binding in this group of compounds. In the *in vivo* tests, compounds **6a**, **12a** and **12b** were devoid of anticonvulsant activity (in doses up to 300 mg/kg), they did not show neurological toxicity (in doses up to 300 mg/kg). Thus, pharmacological *in vitro* and *in vivo* test results were in accordance.

EXPERIMENTAL

Chemistry.

Melting points were measured on a Mel-Temp II apparatus (LD Inc, USA) and were not corrected. The tlc was performed on Merck Silica gel GF_{254} precoated tlc Al sheets; the solvent systems used were: A, methylene chloride; B, chloroform:ethyl acetate (1:1); C, toluene:acetone (20:1.5); D, chloroform: isopropanol:ammonia aq 25% (9:11:2). Column chromatography was performed on Merck silica gel 60 (70–230 mesh). Electron impact mass spectra were recorded on a Finningan MAT CH 7A (70eV, 170 °C) spectrometer with direct inlet. Infrared spectra were measured with an FT IR 410 spectrometer (Jasco). The ^{1}H nmr spectra were performed on a Bruker DPX 400, DPX 250 AVANCE, or a Bruker AC-200F in [d_6]dimethylsulfoxide using tetramethylsilane as internal standard; D_2O exchangable signals were marked (*), splitting patterns are designated as s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; def, deformed. The ^{13}C nmr spectra were measured with a Varian-Mercury 300 MHz spectrometer. The uv spectra were obtained for solutions of $5 \cdot 10^{-5}$ mol/L concentration with a uv-vis-spectrometer (UV-VIS V-530 Jasco). The elemental analyses were performed at the Department of Pharmaceutical Chemistry of the Jagiellonian University, Medical College.

The starting 2-thiohydantoins (**7** and **17**) were obtained as previously described or in analogy to the described procedure [26]. The new derivative was prepared described below.

3-(2,4-Dichlorobenzyloxy)-benzaldehyde (**15**).

a: A suspension of 3-hydroxybenzaldehyde (**14**) (6.1 g, 0.05 mole), 2,4-dichlorobenzyl chloride (9.8 g, 0.05 mole) and K_2CO_3 (3.45 g, 0.025 mole) in 50 ml of ethanol was refluxed for 4.5 hours. After cooling the solid was filtered off and washed several times with water to give **15** (13.5 g), mp. 78–80 °C, tlc R_f (A) – 0.61.

Z-5-[3-(2,4-Dichlorobenzyloxy)benzylidene]-2-thiohydantoin (**17**).

b: A mixture of 3-(2,4-dichlorobenzyloxy)benzaldehyde (**15**) (15.46 g, 0.055 mole), anhydrous sodium acetate (16.5 g, 0.20 mole) and 2-thiohydantoin (5.8 g, 0.05 mole) in 65 ml of glacial acetic acid was refluxed for 0.5 hour. The obtained solution became dark. After cooling the precipitate that formed was isolated by filtration, washed with water and recrystallized from acetic acid to give **17** (15.6 g; 82%), mp 209–210 °C; tlc, R_f

(A) –0.85; ^{1}H nmr (250 MHz): δ 5.22 (s, 2H, CH_2O), 6.46 (s, 1H, ArCH=), 7.03–7.07 (m, 1H, 5'-H), 7.34–7.39 (m, 3H, 3"-H, 5"-H, 6"-H), 7.47–7.51 (m, 1H, 4'-H), 7.63–7.70 (m, 2H, 2'-H, 6'-H), 12.20 (s, 1H, N_1–H), 12.39 (s, 1H, N_3–H); ir (KBr): ν 3172, 3052 (NH), 1727 (C=O), 1654 (ArCH=), 1484, 1369, 1241, 1031, 788, 689, 676.

Anal. Calcd. for $C_{17}H_{12}N_2O_2SCl_2$ (379.27): C, 53.83; H, 3.19; N, 7.39. Found: C, 53.64; H, 3.13; N, 7.04.

Compounds **3a**, **4a** [13], **3b**, **4b** [14], **5a**, **5b** [27], **8**, [28], **12b** [15] were prepared as previously described.

Z-2-(4-Chlorobenzylidene)-5,5-diphenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]imidazoline-3,6-dione (**6a**).

a: A mixture of **5a** (2.91 g, 0.01 mole), anhydrous sodium acetate (5.0 g, 0.06 mole) and 4-chlorobenzaldehyde (1.54 g, 0.011 mole) in 100 ml of acetic acid was refluxed for 4 hours. After cooling, the formed precipitate was isolated by filtration and recrystallized from ethanol to give **6** (1.9 g; 46%), mp 266–268 °C; tlc, R_f (B) – 0.72; ^{1}H nmr (300 MHz): δ 6.92 (s, 1H, ArCH=), 7.35–7.41 (m, 10H, ArH), 7.53 (d, J = 8.0 Hz, 2H, 3'-H, 5'-H), 8.08 (d, J = 8.0 Hz, 2H, 2'-H, 6'-H), 13.20 (br s, 1H, N_7-H); ^{13}C nmr: δ 73.50 (Ph_2C), 127.39, 128.73, 128.87 (aromatic carbons), 128.73 (ArCH=), 132.48 (3'-C, 5'-C), 134.08 (2'-C, 6'-C), 136.21 (2'-C); ir (KBr): ν 3438, 3058 (NH), 1760, 1743 (C=O), 1673 (ArCH=), 1608 (C=N), 1488, 1120, 943, 790, 692; uv (MeOH) λ_{max} 339 nm (log ε 4.55), λ_{max} 262 nm (log ε 3.96), λ_{max} 214 nm (log ε 4.22); ms: m/z 413 ($M^{+\bullet}$, 79), 385 (14), 357 (53), 308 (9), 274 (14), 246 (14), 208 (19), 205 (25), 194 (12), 180 (13), 165 (100), 150 (65), 129(20), 104 (57), 89(13).

Anal. Calcd. for $C_{24}H_{16}N_3O_2Cl$ (413.87): C, 69.65; H, 3.90; N, 10.15; Found: C, 69.53; H, 3.92; N, 9.84.

b: Compound **6a** was obtained with 64% yield under similar conditions as described above, starting from **5b**.

N-[*Z*-5-(2-Chlorobenzylidene)-4-oxo-2-imidazolidinyl]phenylalanine (**10a**).

A mixture of *Z*-5-(2-chlorobenzylidene)-2-methylthioimidazoline-4-one (**8**) (2.53 g, 0.01 mole) and phenylalanine (1.7 g, 0.011 mole) in 50 ml of acetic acid was refluxed for 6 hours. After cooling the obtained precipitate was recrystallized from DMF/H_2O to give **10a** (2.5 g; 68%), mp 224–226 °C; tlc, R_f (D) 0.32; ^{1}H nmr (400 MHz): δ 3.09 (def t, 1H, CH_2Ph), 3.24 (def t, 1H, CH_2Ph), 4.71 (br s, 1H, C*H*CH_2), 6.64 (s, 1H, ArCH=), 7.29–7.37 (m, 8H, 3'-H, 4'-H, 5'-H, 2"-H, 3"-H, 4"-H, 5"-H, 6"-H), 7.92 (br s, 1H, N*H*CH)*, 8.72 (br s, 1H, 6'-H), 10.63 (br s, 1H, NH)*, 12.83 (br s, 1H, COOH)*; ir (KBr): ν 3421 (NH, COOH), 3064, 3031, (C–H), 1700 (C=O), 1602 (C=N), 1357, 1290, 1033, 761, 700.

Anal. Calcd. for $C_{19}H_{16}N_3O_3Cl$ (369.80): C, 61.71; H, 4.36; N, 11.36; Found: C, 61.46; H, 4.31; N, 11.22.

1-Acetyl-2-benzyl-*Z*-6-(2-chlorobenzylidene)-2,3,5,6-tetrahydroimidazo[2,1-*b*]imidazole-3,5-dione (**11a**).

A suspension of **10a** (1 g) in 10 ml of acetic acid anhydride was refluxed for 4 hours. The obtained precipitate was recrystallized from acetic acid (yield 35%), mp 218–220 °C; tlc, R_f (D) 0.77; ^{1}H nmr (200 MHz): δ 2.68 (s, 3H, CH_3CO), 3.31–3.41 (m, 2H, CH_2Ph), 5.24–5.29 (m, 1H, C*H*CH_2), 7.11 (dd, J = 7.6 Hz, J = 1.5 Hz, 2H, 2"-H, 6"-H), 7.12 (s, 1H, ArCH=), 7.23–7.31 (m, 3H, 3"-H, 4"-H, 5"-H), 7.41-7.46 (m, 2H, 3'-H, 5'-H), 7.53–7.58 (m, 1H, 4'-H), 8.56–8.61 (m, 1H, 6'-H); ir (KBr): ν 3085, 3066, 3027, (C–H), 1808 (CH_3CO), 1722 (C_2=O, C_4=O), 1664 (ArCH=), 1600 (C=N), 1419, 1257, 894, 773, 715.

Anal. Calcd. for $C_{21}H_{16}N_3O_3Cl$ (393.82): C, 64.04; H, 4.09; N, 10.67; Found: C, 64.29; H, 3.85; N, 10.46.

N-[*Z*-5-(2-Chlorobenzylidene)-4-oxo-2-imidazolidinyl]-(4-chlorophenyl)alanine (**10b**).

Compound **10b** was obtained by reaction of **8** and 4-chlorophenylalanine according to the method described for the preparation of **10a**; yield 62%, mp 248–250 °C (from DMF/H_2O); tlc, R_f (D) 0.39; ^{1}H nmr (400 MHz): δ 3.09 (br s, 1H, CH_2Ph), 3.23 (br s, 1H, CH_2Ph), 4.70 (br s, 1H, C*H*CH_2), 6.64 (br s, 1H, ArCH=), 7.33 (m, 7H, 3'-H, 4'-H, 5'-H, 2"-H, 3"-H, 5"-H, 6"-H), 7.94 (br s, 1H, N*H*CH)*, 8.71 (br s, 1H, 6'-H), 10.66 (br s, 1H, NH)*, 13.13 (br s, 1H, COOH)*; ir (KBr): ν 3400 (COOH), 3164 (NH), 3083 (C–H), 1704 (C=O), 1650 (ArCH=), 1616 (C=N), 1536, 1357, 1292, 1186, 1035, 767.

Anal. Calcd. for $C_{19}H_{15}N_3O_3Cl_2$ (404.24): C, 56.45; H, 3.74; N, 10.39; Found: C, 56.22; H, 3.65; N, 10.01.

1-Acetyl-2-(4-chlorobenzyl)-*Z*-6-(2-chlorobenzylidene)-2,3,5,6-tetrahydroimidazo[2,1-*b*]imidazole-3,5-dione (**11b**).

Compound **11b** was obtained as described for **11a**; yield 25%, mp 199–201 °C (from acetic acid); tlc, R_f (D) 0.77; ^{1}H nmr (200 MHz): δ 2.69 (s, 3H, CH_3CO), 3.33–3.39 (m, 2H, CH_2Ph), 5.21–5.26 (m, 1H, C*H*CH_2), 7.14 (s, 1H, ArCH=), 7.15 (d, J = 8.3 Hz, 2H, 3"-H, 5"-H), 7.37 (d, J = 8.3 Hz, 2H, 2"-H, 6"-H), 7.42–7.46 (m, 2H, 3'-H, 5'-H), 7.52–7.58 (m, 1H, 4'-H), 8.58–8.63 (m, 1H, 6'-H); ir (KBr): ν 3085, 3060, 3019, 2931 (C–H), 1803 (CH_3CO), 1737 (C_2=O), 1725 (C_4=O), 1664 (ArCH=), 1600 (C=N), 1492, 1411, 1369, 1290, 1276, 1257, 1238, 1120, 894, 763.

Anal. Calcd. for $C_{21}H_{15}N_3O_3Cl_2$ (428.26): C, 58.89; H, 3.53; N, 9.81; Found: C, 58.65; H, 3.37; N, 9.59.

N-[*Z*-5-(2-Chlorobenzylidene)-4-oxo-2-imidazolidinyl]-phenylglycine (**10c**).

Compound **10c** was obtained as described for **10a**; yield 35%, mp 268–270 °C (from DMF/H_2O); tlc, R_f (D) 0.36; ^{1}H nmr (400 MHz): δ 3.82 (s, 1H, CHPh), 6.63 (s, 1H, ArCH=), 7.35–7.68 (m, 8H, 3'-H, 4'-H, 5'-H, 2"-H, 3"-H, 4"-H, 5"-H, 6"-H), 8.46 (br s, 1H, N*H*CH)*, 8.79 (s, 1H, 6'-H), 10.41 (br s, 1H, NH)*, 13.22 (br s, 1H, COOH)*; ir (KBr): ν 3261 (NH, COOH), 3060 (C–H), 1700 (C=O), 1650 (ArCH=), 1614 (C=N), 1531, 1369, 1288, 1024, 750, 705.

Anal. Calcd. for $C_{18}H_{14}N_3O_3Cl$ (355.77): C, 60.76; H, 3.97; N, 11.81; Found: C, 60.48; H, 3.91; N, 11.58.

1-Acetyl-2-phenyl-*Z*-6-(2-chlorobenzylidene)-2,3,5,6-terahydroimidazo[2,1-*b*]imidazole-3,5-dione (**11c**).

Compound **11c** was obtained as described for **11a**; yield 35%, mp 175–176 °C (from acetic acid); tlc, R_f (D) 0.69; ^{1}H nmr (200 MHz): δ 2.61 (s, 3H, CH_3CO), 5.93 (s, 1H, CHPh), 7.26 (s, 1H, ArCH=), 7.37–7.61 (m, 8H, 3'-H, 4'-H, 5'-H, 2"-H, 3"-H, 4"-H, 5"-H, 6"-H), 8.24–8.28 (m, 1H, 6'-H); ir (KBr): ν 3099, 3062, 3033, 2948 (C–H), 1805 (CH_3CO), 1766 (C_2=O), 1735 (C_4=O), 1660 (ArCH=), 1598 (C=N), 1432, 1236, 1122, 906, 763.

Anal. Calcd. for $C_{20}H_{14}N_3O_3Cl$ (379.79): C, 63.25; H, 3.71; N, 11.06; Found: C, 63.01; H, 3.58; N, 10.80.

1-Acetyl-*Z*-6-(2-chlorbenzylidene)-2,3,5,6-tetrahydroimidazo[2,1-*b*]imidazole-3,5-dione (**12a**).

Compound **12a** was prepared analogous to **12b** [3]; yield 49%, mp 271–273 °C (from acetic acid); tlc R_f (D) 0.20; ^{1}H nmr (400 MHz): δ 2.66 (s, 3H, CH_3CO), 4.63 (s, 2H, CH_2CO), 7.21 (s, 1H, ArCH=), 7.42–7.51 (m, 2H, 3'-H, 5'-H), 7.59–7.61 (m, 1H, 4'-H), 8.70 (dd, J = 7.7 Hz, J = 1.8 Hz, 1H, 6'-H); ir (KBr): ν 3093, 2973, 2942 (C–H), 1805 (CH_3CO), 1741 (C_2=O), 1716 (C_4=O), 1671 (ArCH=), 1600 (C=N), 1446, 1405, 1270, 1240, 904, 781.

Anal. Calcd. for $C_{14}H_{10}N_3O_3Cl$ (303.70): C, 55.36; H, 3.32; N, 13.84; Found: C, 55.28; H, 3.17; N, 13.68.

Z-5-[3-(2,4-Dichlorobenzyloxy)benzylidene]-2,3-dihydroimidazo[2,1-*b*]thiazol-6(5*H*)-one (**18**); *Z*-6-[3-(2,4-dichlorobenzyloxy)benzylidene]-2,3-dihydroimidazo[2,1-*b*]thiazol-5(6*H*)-one (**19**).

To a stirred suspension of thiohydantoin derivative **17** (1.9 g, 5 mmole), potassium carbonate (2.0 g), and benzyltriethylammonium chloride (0.15 g, 0.5 mmole) in 20 ml of acetone, a solution of 1,2-dibromoethane (1.03 g, 5.5 mmole) in 5 ml of acetone was added dropwise. The mixture was stirred at room temperature for two days. Then, the solvent was removed *in vacuo*. The residue was washed with water and subsequently with 1% aqueous sodium hydroxide and 1% aqueous hydrochloric acid solutions, and separated by means of column chromatography using methylene chloride:ethyl acetate (1:1) as the eluent. Product **19** was eluted first: yellow crystals 1.30 g (64%). **19:** mp 142–145 °C (from dioxane); tlc, R_f (B) 0.66; ^{1}H nmr (250 MHz): δ 3.87 (s, 4H, CH_2CH_2), 5.16 (s, 2H, CH_2O), 6.73 (s, 1H, ArCH=), 7.05 (d, J = 8.0 Hz, 1H, 5''-H), 7.33 (t, J = 7.8 Hz, 1H, 5'-H), 7.45 (d, J = 6.3 Hz, 1H, 4'-H), 7.48–7.68 (m, 3H, 3''-H, 6''-H, 6'-H), 7.87 (s, 1H, 2'-H); ir (KBr): ν 1720 (C=O), 1637 (ArCH=), 1571, 1508 (C=N), 1251, 1116, 1045, 817, 690.

Anal. Calcd. for $C_{19}H_{14}N_2O_2SCl_2$ (405.32): C, 56.30; H, 3.48; N, 6.91; Found: C, 56.53; H, 3.31; N, 6.76.

As a second fraction, **18** was obtained as yellow crystals, 120 mg (6%). **18**: mp 187–190 °C (from methylene chloride:ethyl acetate, 1:1); tlc, R_f (B) 0.26; ^{1}H nmr (250 MHz): δ 3.68 (def t, 2H, SCH_2), 3.81 (def t, 2H, NCH_2), 5.17 (s, 2H, CH_2O), 6.84 (s, 1H, ArCH=), 7.07 (t, J = 8.5 Hz, 2H, 5''-H, 6''-H), 7.17 (s, 1H, 3''-H), 7.37 (def t, 1H, 5'-H), 7.47 (d, J = 8.2 Hz, 4'-H), 7.61–7.69 (m, 1H, 6'-H), 7.69 (d, J = 2.0 Hz, 2'-H); ir (KBr): ν 1706 (C=O), 1650 (ArCH=), 1596 (C=N), 1471, 1353, 1236, 1041, 794, 698.

Anal. Calcd. for $C_{19}H_{14}N_2O_2SCl_2$ (405.32): C, 56.30; H, 3.48; N, 6.91; Found: C, 56.21; H, 3.35; N, 6.78.

5-Amino-6-cyano-7-phenyl-1-oxo-3-thioxo-2,3-dihydro-1*H*-pyrrolo[1,2-*c*]imidazole (**20a**).

A suspension of *Z*-5-benzylidene-2-thiohydantoin (4.08 g, 0.02 mole), triethylamine (2 ml) and malononitrile (1.32 g, 0.02 mole) in 40 ml of absolute ethanol was refluxed for 6 hours and then allowed to cool. The solid isolated by filtration (2.0 g) was identified as starting 2-thiohydantoin (**7**). The filtrate was evaporated *in vacuo*, washed with water and crystallized from dimethylformamide to give lemon yellow crystals of **20a** (500 mg; 9%), mp 288–290 °C (from dimethylformamide); tlc, R_f (B) 0.77; R_f (C) 0.10; ^{1}H nmr (400 MHz): δ 7.79 (s, 3H, 3'-H, 4'-H, 5'H), 8.11 (s, 2H, 2'-H, 6'-H), 8.60* (s, 2H, NH_2), 13.01* (s, 1H, NH); ^{13}C nmr: δ 75.57 (7a-C), 110.76 (6-C), 115.18 (CN), 127.95 (3'-C, 5'-C), 128.81 (2'-C, 6'-C), 128.84 (4'-C), 130.39 (1'-C), 130.70 (7-C), 149.09 (5-C), 157.29 (1-C), 172.00 (3-C); ir (KBr): ν 3380, 3289, 3221, 3163 (NH, NH_2), 2359, 2213 (CN) 1705 (C=O), 1631 (CN), 1555, 1447, 1347, 1170, 1017, 828, 742; ms: (electron impact) m/z 268($M^{+\bullet}$, 94), 209 (100), 181 (46), 153 (31), 133 (15), 127 (20), 100 (4), 77 (9), 60 (3).

Anal. Calcd. for $C_{13}H_8N_4OS$ (268.29): C, 58.19; H, 3.00; N, 20.88; Found: C, 58.02; H, 3.09; N, 21.03.

5-Amino-6-cyano-7-(4-chlorophenyl)-1-oxo-3-thioxo-2,3-dihydro-1*H*-pyrrolo[1,2-*c*]imidazole (**20b**).

Compound **20b** was obtained according to the procedure described for **20a** as dark yellow crystals (7.2%), mp 302–304 °C (from dimethylformamide); tlc, R_f (B) 0.76, R_f (C) 0.35; ^{1}H nmr (300 MHz): δ 7.65 (d, J = 8.7 Hz, 2H, 3'-H, 5'-H); 8.07 (d, J = 8.7 Hz, 2H, 2'-H, 6'-H), 8.57* (s, 2H, NH_2), 13.01* (s, 1H, NH); ^{13}C nmr: δ 75.40 (7a-C), 111.00 (6-C), 115.06 (CN), 127.75 (1'-C), 128.99 (3'-C, 5'-C), 129.05 (7-C), 129.59 (2'-C, 6'-C), 134.96 (4'-C), 149.04 (5-C), 157.38 (1-C), 172.04 (3-C); ir (KBr): ν 3320, 3164, (NH_2, NH), 2360, 2200 (CN), 1728, 1712 (C=O), 1666, 1620 (CN), 1564, 1492, 1440, 1164, 744, 668; ms: (electron impact) m/z 302 ($M^{+\bullet}$, 4), 301 (25), 242 (38), 208 (8), 180 (100), 161 (30), 153 (16), 112 (17), 85 (16), 73 (8).

Anal. Calcd for $C_{13}H_7N_4OSCl \bullet C_3H_7NO$ (302.75•73.09 = 375.84): C, 51.13; H, 3.75; N, 18.64; Found: C, 50.74; H, 3.59; N, 18.35.

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 membranes was determined as previously described [1]. The compounds were dissolved in dimethyl sulfoxide and further diluted with tris(hydroxymethyl)-aminomethane hydrochloride buffer (50 m*M*, pH 7.4); the final dimethyl sulfoxide concentration was 1%. In a final volume of 1 mL, each test tube contained 790 μL of 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 was necessary since the compounds possessed low water solubility.

Incubations were performed at 2 °C 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.

Anticonvulsant Assays.

Evaluation for anticonvulsant activity of **6a**, **12a** and **12b** was done by the Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA. These tests were performed in male Carworth Farms No. 1 (CF 1) mice. Phase I of the evaluation was a qualitative assay, which used small groups of animals (1-8) and included three tests: maximal electroshock (MES), subcutaneous pentylenetetrazole (scMet), and rotorod test for neurological toxicity (Tox).

Compounds were suspended in 30% polyethylene glycol 400 and were administered by intraperitoneal injection at three dosage levels (30, 100 and 300 mg/kg) with anticonvulsant activity and neurotoxicity noted 30 minutes and 4 hours after administration. Details of these procedures have been published [22,23].

Crystal Structure Determination.

The crystals of **6a** were obtained by slow evaporation of an ethanol solution, while crystals of **20a** were selected from dimethylformamide solution. All crystallographic data are gathered in Table 1. Preliminary crystallographic data were obtained from a 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 both compounds; crystal of 0.1 x 0.15 x 0.3 mm for **6a**, and 0.1 x 0.1 x 0.5 mm for **20a** were applied to collect diffraction data on a KM4 diffractometer by the $\omega/2\Theta$ scan technique and using graphite monochromated CuKα radiation at room temperature for $\Theta<60°$ [h:-9/9, k: 0/16, l: 0/33] for **6a**, and $\Theta<80°$ [h:-4/4, k: 0/49, l: 0/13] for **20a**; an absorption correction was not applied; the intensity of three standard reflections monitored every 100 reflections showed no significant fluctuations for both compounds; 4608 reflections for **6a** and 3361 reflections for **20a** were measured, 1709 for **6a** and 1319 for **20a** reflections were considered observed using the criterion $F_0>4\sigma(F_0)$. The structures were solved by a direct method (SHELXTL-PC) [29]. E-map provided positions for all non-H-atoms; full-matrix least-squares refinement was carried out on F's using anisotropic temperature factors for all non-H-atoms; the positions of all H-atoms were from $\Delta\rho$-maps except two amide hydrogens in **6a**; such atoms were localised geometrically at four nitrogens and subsequently refined with occupation factor of 0.5; isotropic thermal parameters of all 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 = 0.0699, wR2 = 0.0794 (with $w=1/[\rho^2(Fo^2)]$) for **6a** and R1 = 0.0482, wR2 = 0.0526 (with $w=1/[\rho^2(Fo^2)$ 0.1128 $P)^2+0.2078$ P] where $P=(Fo^2+ 2Fc^2)/3$) for **20a**; S = 0.73(543 parameters) for **6a** and S=0.66(218 parameters) for **20a**; final changes $\Delta / \rho < 0.01$; $\Delta\rho_{min}$ = -0.33 e Å^{-3}, $\Delta\rho_{max}$ = 0.35 e Å^{-3} for **6a** and $\Delta\rho_{min}$ = -0.213 e Å^{-3}, $\Delta\rho_{max}$ = 0.251 e Å^{-3} for **20a**. The atomic scattering factors were taken from SHELXL-93 [30]. The X-ray structure analysis results are presented in the form of non-H-atoms coordinates in Table 2 as well as in Figures 1 and 3 [31].

Acknowledgements.

The authors wish to thank Professor James Stables for providing pharmacological data through the Antiepileptic Drug Development Program National Institutes of Health in Bethesda. We 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.

REFERENCES AND NOTES

[1] U. Geis, K. Kieć-Kononowicz and C.E. Müller, *Sci. Pharm.*, **64**, 383 (1996).

[2] K. Kieć-Kononowicz, J. Karolak-Wojciechowska, C. E. Müller, U. Geis, W. Książek and E. Szymańska, *J. Heterocyclic Chem.*, **36,** 257 (1999).

[3] K. Kieć-Kononowicz J. Karolak-Wojciechowska, C. E. Müller, E. Pękala and E. Szymańska, *Eur. J. Med. Chem.*, **36**, 407 (2001).

[4] M. Chebib and G. A. R. Johnston, *J. Med. Chem.*, **43**, 1427 (2000).

[5] Q. Huang, X. He, C. Ma, R. Liu, S. Yu, C. A. Dayer, G. R. Wenger, R. McKernan and J. M. Cook, *J. Med. Chem.*, **43**, 71 (2000).

[6] S. Z. Langer and S. Arbilla, *Pharmacol. Biochem. Behav.*, **29**, 763 (1988).

[7] G. Dorey, L. Dubois, L. Prado de Carvalho, P. Potier and R. H. Dodd, *J. Med. Chem.*, **38**, 189 (1995).

[8] E. J. Jacobsen, R. E. TenBrink, L. S. Stelzer, K. L. Belonga, D. B. Carter, H. K. Im, W. B. Im, V. H. Sethy, A. H. Tang, P. F. Von Voigtlander and J. D. Petke, *J. Med. Chem.*, **39**, 158 (1996).

[9] G. Primofiore, F. Da Settimo, S. Taliani, A. M. Marini, C. La Motta, E. Novellino, G. Greco, M. Gesi, L. Trincavelli and C. Martini, *J. Med. Chem.*, **43**, 96 (2000).

[10] C.-G. Wang, T. Langer, P. G. Kamath, Z.-Q. Gu, P. Skolnick and R. I. Fryer, *J. Med. Chem.*, **38**, 950 (1995).

[11] W. Zhang, K. F. Koehler, P. Zhang, and J. M. Cook, *Drug. Des. Discov.*, **12**, 193 (1995).

[12] A. Mrozek, J. Karolak-Wojciechowska, K. Kieć-Kononowicz, *J. Mol. Str*. in preparation.

[13] J. Karolak-Wojciechowska, M. Mikołajczyk, A. Zatorski, A. Zejc and K. Kieć-Kononowicz, *Tetrahedron*, **41**, 4593 (1985).

[14] J. Karolak-Wojciechowska and K. Kieć-Kononowicz, *J. Crystallogr. Spectrosc. Res.*, **17**, 485 (1987).

[15] K. Kieć-Kononowicz and J. Karolak-Wojciechowska, *Arch. Pharm. (Weinheim)*, **328**, 119 (1995).

[16] J. Karolak-Wojciechowska, W. Kwiatkowski and K. Kieć-Kononowicz, *Acta Cryst.*, **C45**, 1467 (1989).

[17] W. Kwiatkowski, J. Karolak-Wojciechowska and K. Kieć-Kononowicz, *Acta Cryst.*, **C47**, 1256 (1991).

[18] J. Karolak-Wojciechowska and K. Kieć-Kononowicz, *Acta Cryst.*, **C47**, 2371 (1991).

[19] K. Kieć-Kononowicz and J. Karolak-Wojciechowska, *Phosphorus, Sulfur and Silicon*, **73**, 235 (1992).

[20] J. Karolak-Wojciechowska, A. Mrozek and K. Kieć-Kononowicz, *J. Mol. Str.*, **516**, 113 (2000).

[21] H. A. F. Daboun, S. E. Abdou, M. M. Hussein and M. H. Elnagdi, *Synthesis*, **502** (1982).

[22] R. L. Krall, J. K. Penry, B. G. White, H. J. Kupferberg, E. A. Swinyard, *Epilepsia*, **19**, 400 (1978).

[23] R. J. Porter, J. J. Cereghino, G. D. Gladding, B. J. Hessie, H. J. Kupferberg, B. Scoville, B. G. White, *Cleveland Clin. Q.*, **51**, 293 (1984).

[24] PALLAS for Windows 1.2, CompuDrug Chemistry Ltd., 1995.

[25] MOPAC 6.0, QCPE No. 455; Department of Chemistry, Indiana University, Bloomington, IN 47405, 1990.

[26] A. F. A. Shalaby, H. A. Daboun and S. S. M. Z. Boghdadi, *Z. Naturforsch*., **29B**, 99 (1974).

[27] K. Kieć-Kononowicz, J. Karolak-Wojciechowska, A. Mrozek and M. Poseł, *Arch. Pharm. (Weinheim)*, **328,** 517 (1995).

[28] K. Kieć-Kononowicz, J. Karolak-Wojciechowska and J. Handzlik, *Acta Polon.-Drug Res.*, **55**, 381 (1998).

[29] G. M. Sheldrick, *Acta Cryst*., **A 46**, 467 (1990).

[30] G. M. Sheldrick, SHELXL-93. Program for the Refinement of the Crystal Structures. University of Göttingen, Germany (1993).

[31] Further details of the crystal structure **6a** and **20a** including structure factors, anisotropic displacement parameters and H-atom coordinatea are available on request from one of the authors [J. K.-W.].