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5-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-phenyl-2,3,6,7-tetrahydro-1,4-thiazepines as compounds with high affinity at the benzodiazepine binding site on $GABA_A$ receptors

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A series of thiazepines has been studied as new ligands for the benzodiazepine binding site of the $GABA_A$ receptor. Compounds with high affinity and weak selectivity regarding $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_5\beta_3\gamma_2$ subtypes were found. The pharmacophore is discussed based on experimental and theoretical results. The thiazepine sulfur atom was found to be able to act as hydrogen bond acceptor.

1. Introduction

$GABA_A$ receptors are the most important inhibitory transmitter receptors in the brain. They are chloride ion channels composed of five subunits. 21 different subunits types are known, and the majority of $GABA_A$ channels consists of two of the six different α subunits, two of the four β, and one of the four γ subunits (Bonnert et al. 1999; Korpi et al. 1997; Mehta and Ticku 1999). In our study, four $GABA_A$ subtypes consisting of $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_5\beta_3\gamma_2$ subunits were used. For simplicity, we will use here just the α subunit designation, e. g. α_1, instead of terms like "$GABA_A$ subtypes containing α_1 subunits".

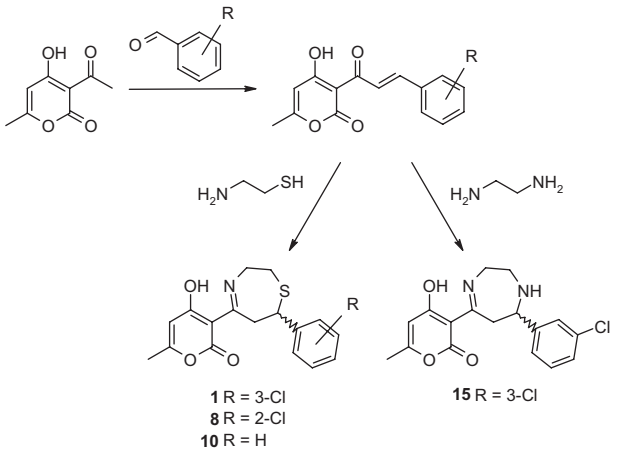
The benzodiazepine receptor (BzR) is one of several modulatory binding sites at the $GABA_A$ receptor. A wide variety of compounds are known to interact with the BzR site, modulating chloride ion conductivity of the $GABA_A$ channel and causing, for instance, anxiolytic and anticonvulsive effects but also unwanted side effects including sedation, ataxia, amnesia, and dependence (Albaugh et al. 2002; Basile et al. 2004). Since discovery of the 1,4-benzodiazepines, thousands of compounds of different compound classes have been synthesized with the aim to separate the desired medicinal properties from unwanted side effects. Studies using α type knockout mice suggest that anxiolytic properties of agonists are selectively mediated by activation of α_2 or α_3 subunits without inducing concomitant sedation and memory impairment. Inverse agonists acting at the α_5 subunit could play a role in certain aspects of cognition, whereas agonists at α_1 and probably also at α_5 subunits induce sedation (Atack 2005; Dawson et al. 2005; Möhler and Rudolph 2004; Rudolph et al. 1999; Savic et al. 2007; Sieghart 2006). These findings prompted the synthesis of many compounds with varying degrees of selectivity regarding affinity or efficacy for the different $GABA_A$ subtypes over the past decade (Da Settimo et al. 2007). However, to the best of our knowledge, no general strategy for the design of α_2 and α_3 selective agonists has been published to date. Size and topology of the included volumes of the α_1, α_2, and α_3 containing subtypes are apparently similar (Clayton et al. 2007).

Despite the difficulty in obtaining compounds with distinct affinity- or efficacy-selectivity for α_2 or α_3, some efficacy-selective compounds have been synthesized and investigated. For instance, 7-[1,1-dimethylethyl)-6-(2-ethyl-2*H*-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-triazolo[4,3-*b*]pyridazine (TPA023) displays selectivity regarding α_2/α_3 efficacy. TPA023 and the very similar compound 3-(2,5-difluorophenyl)-7-(1,1-dimethylethyl)-6-[(1-methyl-1 *H* -1,2,4,-triazol-5-yl)methoxy]-1,2,4-triazolo[4,3-*b*]pyridazine (L-838, 417) do not induce sedation or ataxia in rodents or primates. The intrinsic activities of these compounds are lower than that of 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one (diazepam) (Carling et al. 2005; Dawson et al. 2005; Scott-Stevens et al. 2005). Thus, the discovery of new $GABA_A$ modulators with selectivity towards α_2 or α_3 containing subtypes is still of interest.

2. Investigations, results and discussion

2.1. Synthesis of compounds

We discovered 5-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-phenyl-2,3,6,7-tetrahydro-1,4-thiazepines as a new compound class with high affinity to the benzodiazepine site of $GABA_A$ receptors. First indications regarding the potential utility of these compounds were found during a random high-throughput screening of our compound library. Compounds **2**-**7**, **9**, **11**, and **12** are commercially available. Compounds **1**, **8**, **10** were prepared according to a modified reported procedure (Drewe et al. 2007; Sucheta et al. 1995), see Scheme 1. For example, condensation of dehydroacetic acid with 3-chlorobenzaldehyde in ethanol and piperidine gave the corresponding chalcone, which was reacted with 2-aminoethanethiol in ethanol to produce 5-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)- 7- (3 - chlorophenyl)-2, 3, 6, 7-tetrahydro-1, 4-thiazepine, compound **1**. Enantiomers **13** and **14** were obtained by separation of compound **1** by chiral preparative HPLC. 5-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(3-chlorophenyl)-2,3,



Scheme 1: Synthesis of compounds **1**, **8**, **10**, and **15**

6,7-tetrahydro-1,4-diazepine, compound **15**, was prepared according to Scheme 1. The cyclization with 1,2-diamino-ethane was carried out on silica gel.

2.2. Biology

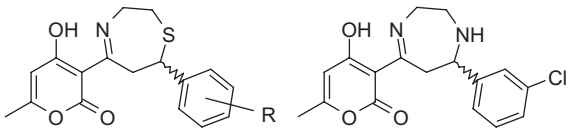
For our studies, human recombinant $GABA_A$ receptor subtypes $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_5\beta_3\gamma_2$ were used. The $\alpha_1\beta_2\gamma_2$ subtype was used instead of $\alpha_1\beta_3\gamma_2$ due to poor expression of $\alpha_1\beta_3\gamma_2$ receptors. This might reduce the comparability of our results. However, the benzodiazepine binding site is situated at the interface of α and γ subunits (Hanson et al. 2008) limiting effects of this substitution. Compounds were characterized regarding their affinity to the benzodiazepine binding site of the 4 $GABA_A$ subtypes applying radioligand binding studies. IC_{50} values of the chemical compounds were determined using filtration assays with 1 nM ^{3}H-Flumazenil at an optimized protein concentration. Diazepam was used as a reference compound. Results are listed in Table 1.

1,4-thiazepines have also been studied by other groups. Haggarty et al. (2000) have published that compounds of this type destabilize microtubules at 20 – 50 μM in a colchicine-like manner. Ohta et al. (2001) studied the inhibition of P-selectin-dependent cell adhesion detecting compound **11** (KF38789) as particular active with an IC_{50} value of 1.97 μM. For 7 of 10 compounds under study, no or only weak cytotoxicity was found at a concentration of 100 μM. Compound **11** was completely free of cytotoxicity. Contrary to this result, **11** was found to induce apoptosis by inhibiting tubulin polymerization in two human cancer cell assays with IC_{50} values of 0.41 and 0.56 μM, respectively (Drewe et al. 2007). Compounds **3** and **7** also displayed activity here whereas **5**, **6**, **9**, **10**, and **12** were inactive up to 10 μM in this investigation.

2.3. Structure-activity relationships

We have studied the effects of the different substituents at the 7-phenyl ring of 1,4-thiazepines on the affinity to $GABA_A$ receptors containing α_1, α_2, α_3, or α_5 subunits, respectively. Substituent effects apparently depend mainly on the position of substitution (ortho, meta, para) but not on whether substituents have polar or non-polar properties. Substitution in the meta position enhances affinity for all four subtypes. Compounds **1**, **2**, **3**, **4**, and **5** displays higher affinity than the unsubstituted compound **10**. Substitution in para position has only a marginal impact on affinity (**6**, **7**). The bulky isopropyl group decreases the affinity towards α_2 and α_3 (**12**). Substitution in ortho position (**8**, **9**) leaves affinities towards α_1, α_2, and α_3 virtually unchanged

Table 1: Influence of substituent R, ring configuration, and ring sulfur on BzR affinity



1-14 **15**

Cpd	R	Cnf[b]	IC_{50} [nM] or inhibition[a]			
			$\alpha_1\beta_2\gamma_2$	$\alpha_2\beta_3\gamma_2$	$\alpha_3\beta_3\gamma_2$	$\alpha_5\beta_3\gamma_2$
1	3-Cl	r	7.1 (4.0)	12 (3)	7.8 (2.5)	3.9 (1.4)
2	3-I	r	3.6 (0.5)	10 (2)	9.5 (1.3)	4.6 (2.2)
3	3-MeO	r	13 (2)	52 (6)	38 (11)	15 (4)
4	3,4,5-tri-MeO	r	15 (1)	32 (2)	35 (13)	18 (2)
5	3-OH	r	17 (2)	52 (6)	51 (1)	16 (5)
6	4-OH	r	23 (2)	143 (4)	110 (32)	30 (5)
7	4-Cl	r	30 (8)	124 (27)	72 (17)	41 (13)
8	2-Cl	r	46 (5)	170 (15)	107 (14)	205 (24)
9	2-NO_2	r	55 (10)	147 (12)	108 (14)	471 (106)
10	H	r	56 (14)	129 (47)	83 (5)	60 (7)
11	2,4-di-MeO	r	59 (2)	373 (44)	189 (5)	180 (36)
12	4-Isopropyl	r	97 (22)	516 (13)	754 (226)	70 (6)
13	3-Cl	(-)	3.0 (0.4)	7.7 (2.2)	4.1 (0.6)	2.0 (0.6)
14	3-Cl	(+)	30 (10)	60 (19)	39 (19)	23 (9)
15		r	n.d.[c]	34 % (2 %)[d]	45 % (3 %)[d]	n.d.
dzp[e]		a	42 (13)	19 (5)	56 (22)	35 (5)

[a] Mean of IC_{50} or percentage inhibition and (standard deviation)
[b] Configuration: r racemate; (-) minus enantiomer; (+) plus enantiomer; a achiral.
[c] n.d.: not determined
[d] Percentual inhibition at a concentration of 1000 nM
[e] Reference diazepam

but reduces the α_5 affinity. Finally, compound **11** with methoxy substituents in ortho and para position displays a reduced affinity for α_2, α_3, and α_5, again compared with **10**. In summary, the investigated substituents at the 7-phenyl ring have only a weak influence on the selectivity.

The thiazapines studied are chiral compounds. To clarify if affinity depends on the enatiomeric form, the optically active enantiomers **13** and **14** of the racemic mixture **1** were examined. The minus enantiomer **13** displays approximately 10-fold greater affinity towards all receptor subtypes. As to be expected, the affinity of the racemate lies between the affinities of the enantiomers and is about half of that of the minus form. Compound **13** displays a 2-fold (α_2) up to 18-fold (α_5) higher affinity than the reference diazepam.

2.4. Pharmacophore

It is known from the pharmacophore model proposed by Cook et al. (Clayton et al. 2007; Zhang et al. 1995) that hydrogen bond acceptors play a significant role in binding of BzR ligands. We have speculated that the sulfur atom of the thiazepine ring might be involved in such an interaction. To check this hypothesis from a theoretical point of view, calculations based on the density functional theory (DFT) were used to characterize the hydrogen bond acceptor nature of the sulfur atom. In contrast to nitrogen and oxygen, sulfur is not generally recognized for its hydrogen bond acceptor properties in spite of theoretical (Sabin 1971) as well as experimental findings (Adman et al. 1975; Krepps et al. 2001; Wierzejewska 2000). Recently, Wierzejewska and Sałdyka (2004, 2006) have published results on analogous sulfur- and oxygen-containing systems regarding their hydrogen bonding features. Using *ab initio* and DFT methods, they found that complexes involving disulfide are only slightly weaker than the corresponding peroxide complexes. We used a modified version of the method described by Hao (2006) to calculate the strength of hydrogen bonds on the basis of DFT calculations. A system consisting of 5,7-dimethyl-2,3,6,7-tetrahydro-[1,4]thiazepine and a water molecule placed in the vicinity of the sulfur and in the direction of one of its lone electron pairs was used and a BSSE corrected B3LYP procedure on a 6-31++G(d,p) basis applied. An energy of 16.849 kJ/mol and a distance of 3.35 Å were calculated for the hydrogen bond between sulfur and water, indicating a weak hydrogen bond. Our result agrees well with experimental data of S···H-N bonds where distances in a range of 3.3-3.5 Å were found (Krepps et al. 2001). The calculated geometry of the thiazepine-water complex is depicted in Fig. 1.

To further exclude that spatial differences between the thiazepine ring of **1** and its diazepine analog **15** lead to the large differences between affinities, geometries of the energetic minima were compared. They were found to be very similar with a root mean square deviation (RMSD) of the seven-membered rings of only 0.145 Å.

The IC_{50} values of compound **15** for α_2 and α_3 containing subtypes are greater then 1000 nM. Compared with the most potent enantiomer **13**, **15** shows more than 130 and 240 times lower affinity. It may therefore be assumed that the loss of affinity of **15** in comparison with the thiazepines is due to the qualitatively changed nature of the sp^3-nitrogen in place of the sulfur. Compound **15** contains a secondary amine nitrogen in place of the sulfur atom replacing the hypothetical hydrogen bond acceptor by a hydrogen bond donor.

Taken together, experimental and computational results strongly suggest that a H-bond acceptor is needed at the position of the sulfur and that the sulfur atom is able to accept H-bonds. This finding was used subsequently for an alignment of the compounds under study and known BzR ligands.

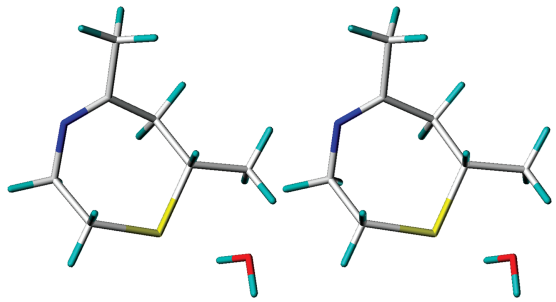


Fig. 1: Hydrogen bond between thiazepine sulfur and water, optimized geometry according to a DFT calculation. Stereo view

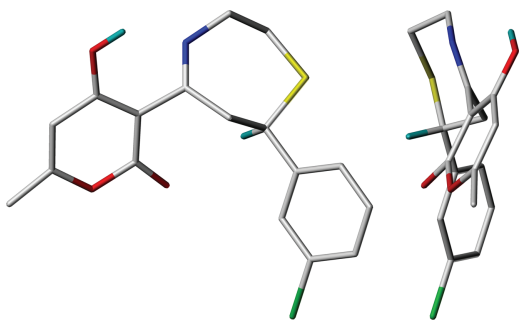


Fig. 2: Global energetic minimum of the R-enantiomer of compound **1**. Orthographic depiction. Non-polar and non-chiral hydrogens are not displayed

To be able to compare the geometry of our compounds with reference structures, the conformation of the seven-membered ring of **1** was extensively analyzed. A systematic approach yielded two chair-like, two twisted, and two boat-like conformers for each of the two enantiomers. These 12 ring geometries were minimized energetically using semiempirical AM1 calculations and analyzed regarding their heats of formation. For a depiction of the conformers and details of the calculations see the Experimental section below. A flat conformation of both the R- and the S-enantiomer was found to be energetically favored. The global minimum is a chair-like conformation of the thiazepine ring with the two substituents arranged in the plane of this ring as shown in Fig. 2. The essentially flat form is consistent with Cook's pharmacophore (Zhang et al. 1995). As to be expected for enantiomers, no differences were found for the two forms besides the chirality.

Based on the overlay of 2-(4-chlorophenyl)-1*H*-pyrazolo[4,5-*c*]quinolin-3-one (CGS-9896) and diazepam proposed by Cook et al. (Clayton et al. 2007; Zhang et al. 1995) and our findings, a superposition of CGS-9896, diazepam, and compound **1** was constructed. Due to its assumed function as H-bond acceptor, the sulfur was placed in the H_1 region to enable a rough fit of the shape of the three molecules. The chloro-phenyl ring was aligned with the fused chloro-phenyl of diazepam and 4-chloro-phenyl of CGS-9896 according to their lipophilic nature. The carbonyl oxygen of the pyranone ring is able to interact with the H-bond donor H_2. The acidic OH group of the pyranone ring corresponds to the NH group of CGS-9896 and interacts with A_2. It was not possible to favor one of the enantiomers for the alignment as the geometry of all three structures is essentially planar. The R-form was randomly selected for use here. The resulting alignment is depicted in Fig. 3, together with the arrangement of pharmacophoric receptor properties according to the Cook model (Clayton et al. 2007; Zhang et al. 1995).

2.5. Conclusion

In summary, thiazepines have been identified as a new compound class of ligands at the benzodiazepine binding site of $GABA_A$

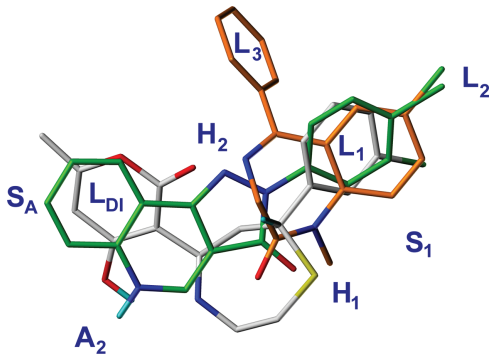


Fig. 3: Superposition of CGS-9896 (green), diazepam (orange), and compound **1** (white). Pharmacophoric features of the binding site according to Cook et al. (Zhang et al. 1995) are lipophilic pockets L, sterically restricted regions S, H-bond donors H, and an H-bond acceptor A_2. Non-polar and non-chiral hydrogens are not displayed

receptors. The affinity was studied with regard to substituents at the 7-phenyl ring and configuration of the 1,4-thiazepine ring. Potent compounds were identified. For instance, (-)-5-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(3-chlorophenyl)-2,3,6,7-tetrahydro-1,4-thiazepine **13** displays affinities between 2 and 8 nM at $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_5\beta_3\gamma_2$ $GABA_A$ subtypes. The investigated substituents at the 7-phenyl ring have only weak influence on the affinity-selectivity of α_2 and α_3 containing subtypes compared with α_1 and α_5, respectively. The overall structure of the thiazepine derivatives fits well with an established pharmacophore. Interestingly, it was shown by DFT calculations that the sulfur atom of the thiazepine ring is able to act as a hydrogen bond acceptor.

3. Experimental

3.1. Chemistry

3.1.1. Methods and instruments

Melting points were determined using a Boetius melting point apparatus PHMK 05 and are uncorrected. All substances were analyzed with an Agilent 1100 series HPLC/MSD system. The molecular mass was determined using a mass selective detector after ESI in positive scan mode. Purity was ascertained using the area percentage method on the UV trace recorded at a wavelength of 254 nm. Purities of purchased and synthesized compounds were found to be ≥ 95 %. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on a Bruker ARX 500 NMR spectrometer. Chemical shifts (δ) are represented in parts per million (ppm) relative to $Si(CH_3)_4$.

3.1.2. Preparation, yield, melting point, HPLC/MS, 1H- and 13C NMR of compounds **1**, **8**, **10**, **15**

3.1.2.1. 5-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-7-(3-chlorophenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**1**). Compound **1** was prepared based on a procedure published by Sucheta et al. (1995) and cited by Drewe et al. (2007). 0.58 g (2 mmol) of the appropriate chalcone (Rachedi et al. 1989) were dissolved in 20 ml of ether. 0.14 ml (2 mmol) of 2-aminoethanethiol were added dropwise. 20 g of silica gel were added. After stirring for 30 min the ether was evaporated and the residue was heated at 80 °C (bath temperature) for 90 min. The silica gel was then treated with 20 ml of hot ethyl acetate and separated by filtration. The filtrate was evaporated (residue 2 – 4 ml) to get yellow crystals. Yield: 43 %; mp: 147 °C. $C_{17}H_{16}ClNO_3S$ calculated m. w.: 349.84. ES-MS: *m/z* = 350 (M+1). ^{1}H NMR (DMSO-d_6) 2.1 (s, -C*H*$_3$), 2.9 (m, C*H*$_2$-S), 3.7 and 4.2 (dd, C*H*$_2$), 4.1 (m, C*H*$_2$-N), 4.2 (m, C*H*-S), 5.7 (s, Pyran-*H*), 7.4 (m, Ph-*H*), 13.8 (s, O*H*). ^{13}C NMR (DMSO-d_6) 19.6 (-*C*H$_3$), 29.4 (*C*H$_2$), 39.2 (*C*H$_2$-N), 40.7 (*C*H-S), 46.0 (*C*H$_2$-S), 96.0 *C*3 (pyran), 107.7 *C*5 (pyran), 126.4-130.9 (=*C*H-Ph), 133.5 (=*C*-Cl), 145.5 (=*C*-CH<), 163.2 (=*C*-CH$_3$), 163.5 (=*C*-OH), 177.6 (-*C*=N), 183.2 O-*C*=O).

3.1.2.2. 5-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-7-(2-chlorophenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**8**). Compound **8** was prepared in the same manner as compound **1** using the appropriate chalcone (Qamar and Siddiq 1988). Yield: 36%; mp: 200 °C. $C_{17}H_{16}ClNO_3S$ calculated m. w.: 349.84. ES-MS: *m/z* = 350 (M+1). ^{1}H NMR (DMSO-d_6) 2.1 (s, -C*H*$_3$), 2.9 (m, C*H*$_2$-S), 3.8 and 4.2 (dd, C*H*$_2$), 4.1 (m, C*H*$_2$-N), 4.5 (m, C*H*-S), 5.7 (s, Pyran-*H*), 7.4 (m, Ph-*H*), 13.7 (s, O*H*). ^{13}C NMR (DMSO-d_6) 19.6 (-*C*H$_3$), 29.4 (*C*H$_2$), 39.9 (*C*H$_2$-N), 41.2 (*C*H-S), 46.2 (*C*H$_2$-S), 96.0 *C*3 (pyran), 107.7 *C*5 (pyran), 127.6-130.0 (=*C*H-Ph), 133.3 (=*C*-Cl), 140.0 (=*C*-CH<), 162.9 (=*C*-CH$_3$), 163.5 (=*C*-OH), 177.6 (-*C*=N), 183.7 O-*C*=O).

3.1.2.3. 5-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-7-phenyl-2,3,6,7-tetrahydro-1,4-thiazepine (**10**). Compound **10** was prepared in the same manner as compound **1** using the appropriate chalcone (Takeuchi et al. 1980). Yield: 89%; mp: 148 °C. $C_{17}H_{17}NO_3S$ calculated m. w.: 315.39. ES-MS: *m/z* = 316 (M+1). ^{1}H NMR (DMSO-d_6) 2.1 (s, -C*H*$_3$), 2.9 (m, C*H*$_2$-S), 3.7 and 4.2 (dd, C*H*$_2$), 4.2 (m, C*H*$_2$-N), 4.5 (m, C*H*-S), 5.8 (s, Pyran-*H*), 7.5 (m, Ph-*H*), 13.7 (s, O*H*).
^{13}C NMR (DMSO-d_6) 19.6 (-*C*H$_3$), 29.4 (*C*H$_2$), 39.9 (*C*H$_2$-N), 41.2 (*C*H-S), 46.2 (*C*H$_2$-S), 96.0 *C*3 (pyran), 107.7 *C*5 (pyran), 127.9-129.3 (=*C*H-Ph), 143.1 (=*C*-CH<), 162.9 (=*C*-CH$_3$), 163.4 (=*C*-OH), 178.0 (-*C*=N), 183.7 O-*C*=O).

3.1.2.4. 5-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(3-chlorophenyl)-1H-2,3,6,7-tetrahydro-1,4-diazepine (**15**). 0.58 g (2 mmol) of 3-(3-chloro-cinnamoyl)-4-hydroxy-6-methyl-2H-pyran-2-one (Rachedi et al. 1989), dissolved in 20 ml of ether, and 0.14 ml (2 mmol) of 1,2-diaminoethane, dissolved in 20 ml of ether, were mixed. 2 g of silica gel were added. After stirring for 30 min the ether was evaporated and the residue was heated at 80 °C (bath temperature) for 90 min. The silica gel was then treated with 20 ml of hot ethyl acetate and separated by filtration. The filtrate was evaporated to get yellow crystals. Yield: 59 %; mp: 132-134 °C. $C_{17}H_{17}ClN_2O_3$ calculated m. w.: 332.78. ES-MS: m/z = 333 (M+1). ^{1}H NMR (DMSO-d_6) 2.1 (s, -C*H*$_3$), 3.1 (m, C*H*$_2$-NH), 2.7 and 3.1 (dd, C*H*$_2$), 3.8 (m, C*H*$_2$-N, N*H*), 4.5 (d, C*H*-N), 5.7 (s, Pyran-*H*), 7.4 (m, Ph-*H*), 13.7 (s, O*H*).
^{13}C NMR (DMSO-d_6) 19.1 (-*C*H$_3$), 40.0 (*C*H$_2$), 46.8, 46.9 (*C*H$_2$-N), 57.0 (*C*H-N), 95.3 *C*3 (pyran), 107.3 *C*5 (pyran), 125.3-130.1 (=*C*H-Ph), 132.8 (=*C*-Cl), 147.5 (=*C*-CH<), 162.0 (=*C*-CH$_3$), 163.3 (=*C*-OH), 178.7 (-*C*=N), 183.1 (O-*C*=O).

3.1.3. Separation, yield, melting point, HPLC/MS, 1H- and 13C NMR of enantiomers **13** *and* **14**

3.1.3.1. Separation of **13** and **14**. 1 g of compound **1** was stirred in 300 ml of propan-2-ol and refluxed for 5 min under an argon atmosphere until completely dissolved. After cooling to room temperature separation of enantiomers was achieved by chiral preparative HPLC (Chiralpak AD, 50 mm x 250 mm, n-hexane/propan-2-ol = 3/2).

3.1.3.2. (-)-5-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-7-(3-chlorophenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**13**). The second fraction eluted contained compound **13**. Yield: 350 mg; mp: 147 °C; $[\alpha]_D^{20}$ – 185.8° (EtOH). $C_{17}H_{16}ClNO_3S$ calculated m. w.: 349.84. ES-MS: *m/z* = 350 (M+1). ^{1}H NMR (DMSO-d_6) 2.1 (s, -C*H*$_3$), 2.9 (m, C*H*$_2$-S), 3.7 and 4.2 (dd, C*H*$_2$), 4.1 (m, C*H*$_2$-N), 4.5 (m, C*H*-S), 5.7 (s, Pyran-*H*), 7.4 (m, Ph-*H*), 13.7 (s, O*H*).
^{13}C NMR (DMSO-d_6) 19.6 (-*C*H$_3$), 29.4 (*C*H$_2$), 39.5 (*C*H$_2$-N), 40.6 (*C*H-S), 46.0 (*C*H$_2$-S), 96.0 *C*3 (pyran), 107.7 *C*5 (pyran), 126.4-130.7 (=*C*H-Ph), 133.5 (=*C*-Cl), 145.5 (=*C*-CH<), 162.8 (=*C*-CH$_3$), 163.5 (=*C*-OH), 177.6 (-*C*=N), 183.7 (O-*C*=O).

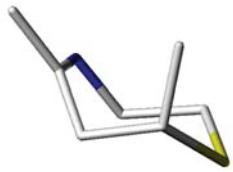
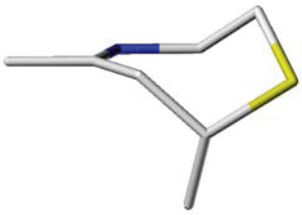
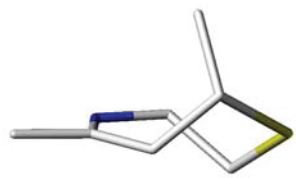
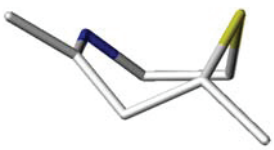
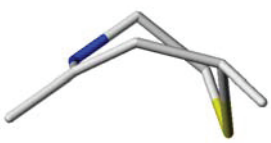
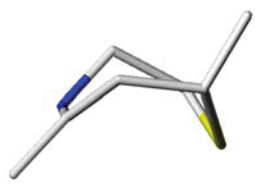
3.1.3.3. (+)-5-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-7-(3-chlorophenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**14**). The first fraction eluted contained compound **14**. Yield: 250 mg; mp: 147 °C, $[\alpha]_D^{20}$ + 226.8° (EtOH). $C_{17}H_{16}ClNO_3S$ calculated m. w.: 349.84. ES-MS: *m/z* = 350 (M+1). ^{1}H NMR (DMSO-d_6) 2.1 (s, -C*H*$_3$), 2.9 (m, C*H*$_2$-S), 3.7 and 4.2 (dd, C*H*$_2$),
4.1 (m, C*H*$_2$-N), 4.5 (m, C*H*-S), 5.7 (s, Pyran-*H*), 7.4 (m, Ph-*H*), 13.7 (s, O*H*).
^{13}C NMR (DMSO-d_6) 19.6 (-*C*H$_3$), 29.4 (*C*H$_2$), 39.5 (*C*H$_2$-N), 40.6 (*C*H-S), 46.0 (*C*H$_2$-S), 96.0 *C*3 (pyran), 107.7 *C*5 (pyran), 126.4-130.7 (=*C*H-Ph), 133.5 (=*C*-Cl), 145.5 (=*C*-CH<), 162.8 (=*C*-CH$_3$), 163.5 (=*C*-OH), 177.6 (-*C*=N), 183.7 (O-*C*=O).

3.1.4. HPLC/MS of commercially obtained compounds **2**-**7**, **9**, **11**, **12**

3.1.4.1. 5-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-7-(3-iodophenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**2**). $C_{17}H_{16}INO_3S$ calculated m. w.: 441.28. ES-MS: *m/z* = 442 (M+1).

3.1.4.2. 5-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(3-methoxyphenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**3**). $C_{18}H_{19}NO_4S$ calculated m. w.: 345.42. ES-MS: *m/z* = 346 (M+1).

Table 2: Starting thiazepine ring conformations Cnf and calculated differences of heats of formation ΔE. Phenyl and pyrane rings are not displayed for clarity of depiction

Cnf. [a]	S enantiomer	E [b] [kJ/mol]	R enantiomer	E [b] [kJ/mol]
C1		0.00		13.03
C2		13.12		0.00
T1		11.93		2.42
T2		2.41		11.93
B1		8.17		9.77
B2		9.80		2.41[c]

[a] Conformation: C chair-like, T twisted, B boat-like. [b] Relative heat of formation related to the minimum found for C2-R: -245.104 kJ/mol. [c] Converted to T1-R during the AM1 calculation

3.1.4.3. 5-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(3,4,5-trimethoxyphenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**4**). $C_{20}H_{23}NO_6S$ calculated m. w.: 405.47. ES-MS: *m/z* = 406 (M+1).

3.1.4.4. 5-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(3-hydroxyphenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**5**). $C_{17}H_{17}NO_4S$ calculated m. w.: 331.39. ES-MS: *m/z* = 332 (M+1).

3.1.4.5. 5-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(4-hydroxyphenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**6**). $C_{17}H_{17}NO_4S$ calculated m. w.: 331.39. ES-MS: *m/z* = 332 (M+1).

3.1.4.6. 5-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(4-chlorophenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**7**). $C_{17}H_{16}ClNO_3S$ calculated m. w.: 349.84. ES-MS: *m/z* = 350 (M+1).

3.1.4.7. 5-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(2-nitrophenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**9**). $C_{17}H_{16}N_2O_5S$ calculated m. w.: 360.39. ES-MS: *m/z* = 361 (M+1).

3.1.4.8. 5-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(2, 4-dimethoxyphenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**11**). $C_{19}H_{21}NO_5S$ calculated m. w.: 375.44. ES-MS: *m/z* = 376 (M+1).

3.1.4.9. 5-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-7-(4-isopropylphenyl)-2,3,6,7-tetrahydro-1,4-thiazepine (**12**). $C_{20}H_{23}NO_3S$ calculated m. w.: 357.47. ES-MS: *m/z* = 358 (M+1).

3.2. Biology

For binding studies, membranes of transiently transfected HEK293 tsa cells were used. Cells were transfected with $GABA_A$ subunits $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_5\beta_3\gamma_2$ respectively by calcium phosphate DNA precipitation. 24 h after transfection cells were scraped from the plates in PBS and the membrane fractions prepared after different steps of separation.
Radioactive binding assays with ^{3}H-flumazenil were performed using MultiScreen glass fiber filterplates. 10-100 μg protein per well was incubated with 1 nM ^{3}H-flumazenil in 50 mM Tris, 200 mM NaCl, pH 7.1 for 60 min at 4 °C. Nonspecific binding was determined in the presence of 10 μM diazepam. Compounds were solubilized in 100 % DMSO and tested in 1 % DMSO at a range of 6 to 10 different concentrations. Assays were terminated by aspiration using the MultiScreen vacuum manifold and washing with 200 μl assay buffer. After removing the underdrain from the plates, plates were dryed at 50 °C for 2 h. 50 μl liquid scintillator was applied to each well and radioactivity was counted in a Wallac Microplate Beta counter.

3.3. Computational methods

3.3.1. Molecular modelling and conformational analysis

Program Sybyl (SYBYL 2006) was used to sketch molecular structures. For the conformational analysis of compound **1**, 12 starting conformations were built including two chair-like, twisted, and boat-like conformers for each of the two enantiomers. Initial models were mechanically minimized applying the Tripos Force Field. Subsequently, geometries were optimized by means of the semiempirical procedure AM1 (Clark et al. 1999). Calculated heats of formation are listed in Table 2. They were directly used to compare the conformers. Resulting geometries were used for alignments and as input for DFT calculations.

3.3.2. DFT calculations

The method described by Hao (2006) was applied with the exception that none of the atoms were fixed. The calculation was performed with the PC GAMESS program (Granovsky 2007) using the B3LYP functional density approach with a 6-31++G(d,p) basis set. To correct results for the basis set superposition error (BSSE), the counterpoise procedure was used, i.e., the basis sets of both molecules were included into the calculation of the energetic contributions of the single molecules. Strength of the hydrogen bond was calculated as difference between the energy of the energetically optimized two-molecule system and the (single point) energies of the two single molecules.

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