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Synthesis of disubstituted 1,4-diazepines with affinity to $GABA_A$-receptor subtypes

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A series of tetrahydro-1*H*-1,4-diazepines **4a–c**, dihydro-1*H*-1,4-diazepine **5** and pyrido diazepines **8** and **10** was prepared. Originated form dehydroacetic acid (DHA) and aromatic aldehydes cinnamoyl compounds **3a–c** were obtained and converted with ethylenediamine to give tetrahydro-1*H*-1,4-diazepines **4a–c**. For the synthesis of pyrido[1,2-*d*][1,4]diazepines **8** and **10** a new snythetic approach is described. Compounds **4b** and **5** were investigated concerning their affinity to different benzodiazepine receptor subtypes. The determined IC_{50} values for **5** are 1.5 μM and 1.1 μM at 10 μM respectively.

1. Introduction

Benzodiazepine derivatives are well-known for their anxiolytic, anticonvulsant and sedative effects. They affect the γ-aminobutyric acid-A ($GABA_A$) receptor by an allosteric mechanism. The use of benzodiazepines is limited by the occurrence of side effects due to its unselective affinity to the α_1, α_2, α_3 and α_5 subunits of the $GABA_A$ receptor. There is a necessity for the development of subtype selective agents to improve pharmacological effects of benzodiazepines and simultaneously avoid undesirable side effects (Atack et al. 2006; Collins et al. 2002; Dias et al. 2005; Möhler and Rudolph 2004). The development of the sleep-inducing drugs zolpidem and zaleplon, which affect the α_1 subunit of the $GABA_A$ receptor, is only one example of these efforts. Compounds selective for the subunits α_2 and α_3 could lead to potent anxiolytic drugs with decreased hypnotic effects and less abuse potential (Atack 2005, 2008; Dawson et al. 2005; Dias et al. 2005; Dixon et al. 2008; Fradley et al. 2007; Rowlett et al. 2005).

In the course of our search for compounds with selective affinity to $GABA_A$ receptor subtypes (Grunwald et al. 2006) we were able to synthesize different partial hydrated 1,4-diazepine derivatives as potential agonists of the benzodiazepine receptor and to test them for their effect on benzodiazepine receptor.

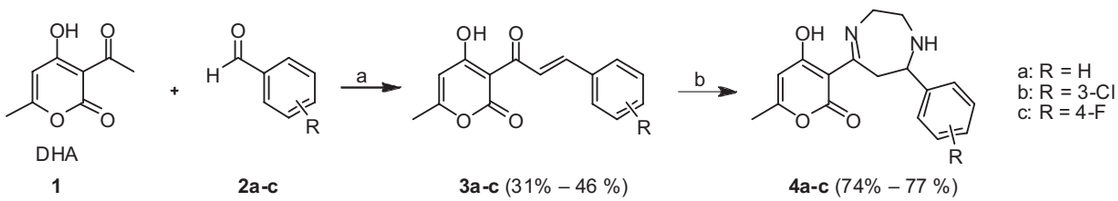
2. Investigations and results

2.1. Synthesis of compounds

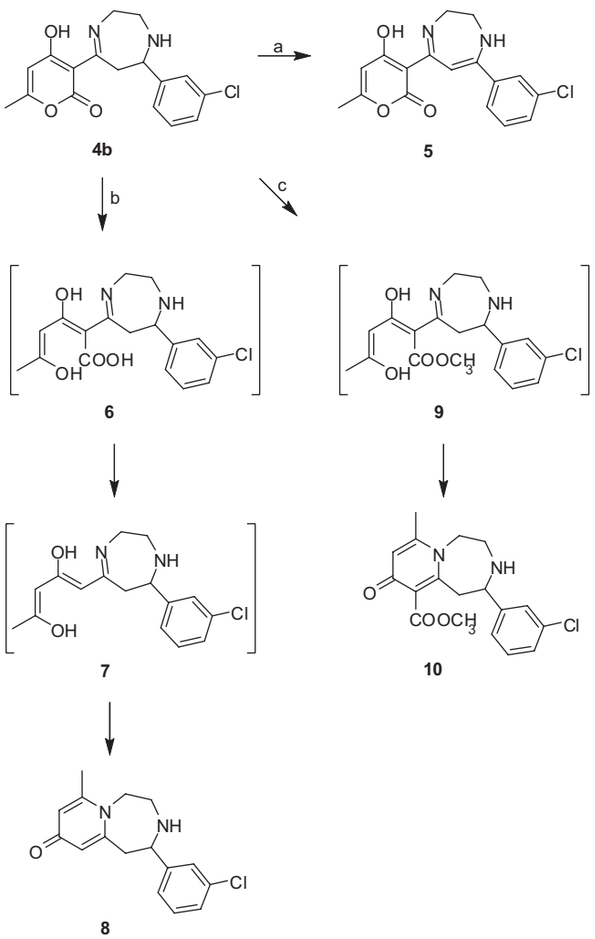
Originated from dehydroacetic acid (DHA, **1**) and aromatic aldehydes **2a–c** the 4-hydroxy-6-methyl-2*H*-pyran-2-ones **3a–c** were synthesized in a Claisen-Schmidt condensation according to the method of Hassan et al. (1985) with small modifications (Scheme 1). We observed the best yields (up to 46%) using toluene as solvent instead of chloroform. The conversion of these 4-hydroxy-6-methyl-2*H*-pyran-2-ones **3a–c** with ethylenediamine is known to be ambivalent. The formation of monocyclic diazepines is dependent on the reactivity and on the reaction conditions (Hideg and Lloyd 1971; Lloyd et al. 1981). In several cases only the olefinic carbon atom of the conjugated enone system reacts with the amino group of ethylenediamine to form an open-chain product and no cyclic diazepine (Hankovszky et al. 1979; Hideg and Lloyd 1971). The nucleophilic attack of the second amino group of ethylenediamine at the carbonyl carbon atom of the enone is dependent on the carbonyl activity and can be favoured be electron withdrawing groups (Hankovszky et al. 1979; Hideg and Lloyd 1971; Ried and Stahlhofen 1957). Conjugated enones with pyridyl-, (Samula and Jurkowska-Kowalczyk 1974) 4-hydroxy-coumarin-3-yl- (Tabakovic and Rapic 1987) and 3-hydroxyphenyl-substituents (Fenton et al. 1985; Srivastava et al. 1988) at the carbonyl group are known to give the corresponding diazepines (Lloyd et al. 1981). Furthermore the synthesis of 14-membered ring systems containing two molecules of enone and two molecules of ethylenediamine was reported.

Utilization of the electron withdrawing effect of the 4-hydroxy-6-methyl-2*H*-pyranon structure in the present enones **3a–c** and optimization of the reaction conditions gave the corresponding diazepines **4a–c** in good yields up to 77% (Scheme 1). We observed the best results using methylenechloride as solvent and two equivalents of ethylenediamine at room temperature. Changing the reaction settings according to already reported studies, e.g., solvents to stabilize the diazepines (Lloyd et al. 1981), using no solvent (Hankovszky et al. 1979; Hideg and Lloyd 1971) or various work-up techniques (Lloyd et al. 1981), led to lower yields or no formation of the corresponding diazepines. Although the formation of 14-membered ring systems is known (Curtis 2001; Hankovszky et al. 1979; Schönherr et al. 2006, 2004), we observed only the 7-membered diazepines (HPLC-purity > 95%). The structure was confirmed by the evaluation of NOESY, COSY and HSQC spectra and NMR-diffusion experiments in comparison to compound **2b**.

According to the method of Larsen and Jørgensen (1992) we oxidized the 2,3,6,7-tetrahydro-1*H*-1,4-diazepine **4b** to 2,3-dihydro-1*H*-1,4-diazepine **5** using iodosobenzene in minor yields and several undefined oxidation products (Scheme 2). For



Scheme 1: Synthesis of cinnamoyl compounds **3a–c** and 2,3,6,7-tetrahydro-1*H*-1,4-diazepines **4a–c** a) toluene, piperidine, reflux 3 - 7 h b) CH_2Cl_2, H_2N-$(CH_2)_2$-NH_2, r.t., 2 h



Scheme 2: Synthesis of 2,3-dihydro-1,4-diazepine **5** and pyrido diazepines **8** and **10** a) CH_2Cl_2, iodosobenzene, 0 °C, 3 h, 6% b) MeOH, SiO_2, r.t., 7 d c) MeOH, NaOH, reflux, 2 h, 59%

the 2,3-dihydro-1*H*-1,4-diazepin **5** an equilibrium with the corresponding 3,6-dihydro-2*H*-1,4-diazepin is easily conceivable. In our NMR experiments we observed only one compound, the 2,3-dihydro-1*H*-1,4-diazepine **5**, distinctly marked by a signal at 5.85 ppm for the CH-proton in position 6 at the diazepin system. In the course of optimization of the synthesis of diazepam **4b** we also tried a procedure using ether, one equivalent of ethylenediamine and silica gel at 80 °C. After leaving the reaction mixture at room temperature for one week we observed only traces of the initial product **3b** and a new compound. NMR-studies indicated that the structure was a 2,3,4,5-tetrahydropyrido[1,2-*d*][1,4]diazepin-9(1*H*)-one **8** (Scheme 2). These observation can be explained by an rearrangement under DHA-ring cleavage to give the intermediate **6** followed by a decarboxlyation yielding **7** and an nucleophilic attack of the nitrogen atom at the 2-carbon atom of **7** to give compound **8**. Similar reactions were described by Arndt and Nachtwey (1924) and Wiley et al. (1955) originated from DHA (**1**) yielding 2,6-dimethyl-4*H*-pyran-4-one or from 3-cinnamoyl-4-hydroxy-6-methyl-2*H*-pyranones delivering 2-methyl-6-styryl-4*H*-pyran-4-ones. Different trials to synthesize **8** according to literature methods mentioned above under acidic conditions failed. A further approach to synthesize **8** from **4b** using a methanolic sodium hydroxide solution delivered another new 1,2,3,4,5,9-hexahydropyrido[1,2-*d*][1,4]diazepine **10**. The structure elucidation was performed by NMR. The formation of **10** can be explained by a mechanism similar to that described for **8**. Under basic conditions an esterification of the carboxylic acid takes place to give **9** and the decarboxylation step is no longer possible. We obtained **10** in 59% yield.

2.2. Biological studies

The compounds **4b** and **5** were tested for their affinity to different $GABA_A$ receptor subtypes in a radioligand binding assay.
With 2,3,6,7-tetrahydro-1*H*-1,4-diazepine **4b** (10 μM) we observed an inhibition of [^{3}H]-Ro15-1788 (flumazenil) binding to the subtypes $\alpha_2\beta_3\gamma_2$ and $\alpha_3\beta_3\gamma_2$ of 83% and 89% respectively. At a concentration of 1 μM **4b** we observed 34% and 45% inhibition.
For the 2,3-dihydro-1*H*-1,4-diazepine **5** we measured similar affinities to both receptor subtypes. The determined IC_{50} values are 1.5 μM to subtype $\alpha_2\beta_3\gamma_2$ and 1.1 μM to subtype $\alpha_3\beta_3\gamma_2$ respectively.
The results of the tests for 2,3-dihydro-1*H*-1,4-diazepine **5** and pyrido[1,2-*d*][1,4]diazepine **8** show little affinity to the subtypes $\alpha_2\beta_3\gamma_2$ and $\alpha_3\beta_3\gamma_2$ and no selectivity to the other $GABA_A$ subtypes $\alpha_1\beta_2\gamma_2$ and $\alpha_5\beta_3\gamma_2$ (data not shown).

2.3. Conclusions

The synthetic route to 4-hydroxy-6-methyl-2*H*-pyranon substituted 1,4-diazepines **4a–c** was optimized and shown to give good yields. According to a known procedure we were able to oxidize the 2,3,6,7-tetrahydro-1,4-diazepine **4b** to 2,3-dihydro-1,4-diazepine **5**. For the synthesis of pyrido[1,2-*d*][1,4]diazepines **8** and **10** a new synthetic method is described. The 1,4-diazepines **4b** and **5** and the pyrido[1,2-*d*][1,4]diazepine **8** were tested for their inhibitory effect on benzodiazepine receptor subtypes. For compound **5** we observed IC_{50} values of 1.5 μm for subtype $\alpha_2\beta_3\gamma_2$ and 1.1 μM for subtype $\alpha_3\beta_3\gamma_2$ respectively.

3. Experimental

3.1. Chemistry

3.1.1. General considerations

Chemicals were purchased from Merck, Sigma-Aldrich, Fluka or Lancaster. Melting points were determined on a Boetius melting point apparatus and are uncorrected. Proton (^{1}H), carbon (^{13}C) and fluor (^{19}F) NMR spectra were recorded on a VARIAN MERCURY-300 for solutions in deuterio chloroforme unless otherwise indicated. In some cases APT, HMBC, HMQC and COSY spectra were recorded. NOESY spectra were recorded on a BRUKER DRX-400 for solutions in deuterio chloroform. Residue of solvent was used as internal standard; chemical shifts (δ) are reported in parts per million

Table: HPLC settings

Column	Phenomenex Hyperclone 3u C8-BDS 130 Å 150 × 4.6 mm	
Detection	DAD UV detection at 300 nm with DIONEX	
Software	CHROMELEON	
Flow rate	1 ml/min	
Time (min)	*Eluent A (acetonitrile) (%)*	*Eluent B (phosphate buffer pH 2.5) (%)*
0–10	20	80
10–15	from 20 to 50	from 80 to 50
15–35	50	50
35–38	from 50 to 20	from 50 to 80
38–43	20	80

(ppm) and coupling constants (*J*) are given in Hertz (Hz). ESI mass spectra were recorded on a BRUKER DALTONICS ESQUIRE 3000plus. EI mass spectra were recorded on a VG ANALYTICS VG ZAB HSQ. High resolution ESI spectra were recorded on a BRUKER DALTONICS FTICR-APEX II. IR spectra were determined from KBr discs with a PERKIN-ELMER 16 PC FT-IR. Thin layer chromatography was performed on DC foils of Merck (DC-Alufolien Kieselgel F_{254}) and detected by UV. HPLC was performed on a DIONEX apparatus. Settings are listed in the Table.

For column chromatography silica gel 60 pure by Merck was used (0.063–0.200 mm). It was dried at 150 °C for 3 h, cooled and deactivated with 5% water (w/w). It was set aside in a closed container before use for three hours.

3.1.2. Synthetic procedures

3.1.2.1. General procedure for 4-hydroxy-6-methyl-2*H*-pyran-2-ones 3a–c (Birch et al. 1960; Hassan et al. 1985; Wiley et al. 1955). Dehydroacetic acid (DHA, **1**) (1 eq.) and aldehydes **2a–c** (1 eq.) were dissolved in toluene, piperidine was added and the mixture was heated for several hours. Water was removed by azeotropic distillation. Solvent was evaporated and the residue recrystallized from 2-propanol.

3-Cinnamoyl-4-hydroxy-6-methyl-2H-pyran-2-one (3a) Originated from dehydroacetic acid (**1**) (3.36 g, 20 mmol), benzaldehyde (**2a**) (2.05 ml, 20 mmol) and piperidine (0.5 ml) in toluene (100 ml). Orange crystals: Yield 46%; m.p. 129–133 °C (Lit.: 130–132 °C) (Ait-Baziz et al. 2008). ^{1}H NMR: δ 2.27 (d, J = 0.9 Hz, 3H, CH_3); 5.95 (d, J = 0.6 Hz, 1H, CH); 7.40–7.70 (m, 5H, aromat); 7.96 (d, J = 15.6 Hz, 1H, CH = CH); 8.31 (d, J = 15.9 Hz, 1H, CH = CH). ^{13}C NMR: δ 20.8 (CH_3); 99.7 (C-3); 102.6 (C-5); 123.2 (C-9); 129.1 (C-11, 15); 129.4 (C-12, 14); 131.3 (C-13); 134.9 (C-10); 146.5 (C-8); 161.4 (C-6); 168.9 (C-4); 183.4 (C-2); 193.0 (C-7). IR [KBr, cm^{-1}]: 3081, 1722, 1624, 1577. MS: (EI) (*m/z*) 256 (M^+). $C_{15}H_{12}O_4$.

(E)-3-[3-(3-Chlorophenyl)acryloyl]-4-hydroxy-6-methyl-2H-pyran-2-one (3b) Originated from dehydroacetic acid (**1**) (16.8 g, 100 mmol), 3-chlorobenzaldeyde (**2b**) (12 ml, 100 mmol) and piperidine (2.0 ml) in toluene (250 ml). Yellow needles: Yield 39%; m.p. 147–150 °C (Lit.: 154–156 °C) (Ait-Baziz et al. 2008). ^{1}H NMR: δ 2.28 (s, 3H, CH_3); 5.96 (s, 1H, CH); 7.33–7.63 (m, 4H, aromat); 7.84 (d, J = 16.2 Hz, 1H, CH = CH); 8.28 (d, J = 15.9 Hz, 1H, CH=CH); 17.68 (s, 1H, OH). ^{13}C NMR: δ 20.9 (CH_3); 99.7 (C-3); 102.4 (C-5); 124.6 (C-9);127.3 (C-14); 128.9 (C-11); 130.3 (C-13); 131.0 (C-15); 135.2 (C-10); 136.7 (C-12); 144.5 (C-8); 161.3 (C-6); 169.2 (C-4); 183.2 (C-2); 192.8 (C-7). IR [KBr, cm^{-1}]: 3098, 1719, 1654, 1634, 1540. MS (EI) (*m/z*): 290 (M^+).
$C_{15}H_{11}ClO_4$.

(E)-3-[3-(4-Fluorophenyl)acryloyl]-4-hydroxy-6-methyl-2H-pyran-2-one (3c) Originated from dehydroacetic acid (**1**) (3.36 g, 20 mmol), 4-fluorobenzaldeyde (**2c**) (2.1 ml, 20 mmol) and piperidine (0.5 ml) in toluene (60 ml). Reddish crystalline powder: Yield 31%; m.p. 140–145 °C (Lit.: 147–149 °C) (Ait-Baziz et al. 2008). ^{1}H NMR: δ 2.27 (d, J = 0.6 Hz, 3H, CH_3); 5.95 (d, J = 0.9 Hz, 1H, CH); 7.09 (m, 2H, aromat); 7.67 (m, 2H, aromat); 7.89 (d, J = 15.9 Hz, 1H, CH=CH); 8.22 (d, J = 15.9 Hz, 1H, CH = CH). ^{13}C NMR: δ 20.8 (CH_3); 99.6 (C-3); 102.5 (C-5); 116.5 (d, J_{CF} = 21.9 Hz, C-12, 14); 122.9 (d, J_{CF} = 2.1 Hz, C-10); 131.2 (C-9); 131.4 (d, J_{CF} = 8.7 Hz, C-11, 15); 145.1 (C-8); 161.4 (C-6); 162.9, 166.3 (d, J_{CF} = 251.7 Hz, C-F); 168.9 (C-4); 183.3 (C-2); 192.8 (C-7). IR [KBr, cm^{-1}]: 3099, 1722, 1625, 1597, 1523. MS (ESI) (*m/z*): 275 ($M^+ + 1$).
$C_{15}H_{11}FO_4$.

3.1.2.2. General procedure for 2,3,6,7-tetrahydro-1*H*-1,4-diazepin-5-yl)-2*H*-pyran-2-ones **4a–c**. 3-Cinnamoyl-4-hydroxy-6-methyl-2*H*-pyran-2-ones **3a–c** were dissolved in dichlormethane and ethylene diamine (2 eq.) was added. The mixtures were stirred at room temperature. After reaction was finished, solvent was evaporated and the residue was recrystallized from ethanol.

(E)-4-Hydroxy-6-methyl-3-(7-phenyl-2,3,6,7-tetrahydro-1H-1,4-diazepin-5-yl)-2H-pyran-2-one (4a) Originated from **3a** (0.80 g, 3.12 mmol). Yellow crystals: Yield 77%; m.p. 174–177 °C. ^{1}H NMR: δ 2.10 (s, 3H, CH_3); 2.85–2.96 (m, 2H); 3.26 (dd, J = 5.1, 13.2 Hz, 1H); 3.62–3.70 (m, 1H); 3.90–3.96 (m, 2H); 4.76 (d, J = 14.4 Hz, 1H); 5.96 (s, 1H, CH); 7.24–7.55 (m, 5H, aromat); 14.24 (s, 1H, OH). ^{13}C NMR: δ 19.9 (CH_3); 40.3 (CH_2); 46.6 (CH_2); 48.0 (CH_2); 59.0 (CH); 96.5 (C-3); 107.5 (C-5); 126.6 (C-15, 19); 127.9 (C-17); 128.8 (C-16, 18); 143.5 (C-14); 163.0 (C-6); 163.9 (C=N); 179.6 (C-4); 184.9 (C-2). IR [KBr, cm^{-1}]: 3313, 3034, 2997, 2887, 2831, 1694, 1661, 1591, 1574. MS (ESI) (*m/z*): 299 ($M^+ + 1$, 100% rel.Int.), 597 ($2\,M^+ + 1$, 7.5% rel.int.).
$C_{17}H_{18}N_2O_3$.

(E)-3-(7-(3-Chlorophenyl)-2,3,6,7-tetrahydro-1H-1,4-diazepin-5-yl)-4-hydroxy-6-methyl-2H-pyran-2-one (4b) Originated from **3b** (4.35 g, 15.0 mmol). Yellow crystals: Yield 75%; m.p. 133–135 °C. ^{1}H NMR: δ 2.11 (s, 3H, CH_3); 2.90–2.98 (m, 2H); 3.31 (dd, J = 5.1, 13.2 Hz, 1H); 3.64–3.74 (m, 1H); 3.90–4.01 (m, 2H); 4.74 (d, J = 13.8 Hz, 1H); 5.96 (s, 1H, CH); 7.24–7.56 (m, 4H, aromat); 14.23 (s, 1H, OH). ^{13}C NMR: δ 19.8 (CH_3); 40.4 (CH_2); 46.7 (CH_2); 48.0 (CH_2); 58.4 (CH); 96.5 (C-3); 107.5 (C-5); 125.1 (C-18); 126.5 (C-15); 128.0 (C-19); 130.1 (C-17); 134.5 (C-16); 145.7 (C-14); 163.0 (C-6); 163.9 (C=N); 179.2 (C-4); 184.9 (C-2). IR [KBr, cm^{-1}]: 3495, 3286, 3051, 2947, 2826, 1683, 1659, 1598, 1574. MS (ESI) (*m/z*): 333 ($M^+ + 1$, 100% rel.int.), 665 ($2\,M^+ + 1$, 62.5% rel.int.).
$C_{17}H_{17}ClN_2O_3$.

(E)-3-(7-(4-Fluorophenyl)-2,3,6,7-tetrahydro-1H-1,4-diazepin-5-yl)-4-hydroxy-6-methyl-2H-pyran-2-one (4c) Originated from **3c** (0.56 g, 2.04 mmol). Orange crystals: Yield 74%; m.p. 182–186 °C. ^{1}H NMR: δ 2.10 (s, 3H, CH_3); 2.83–2.97 (m, 2H); 3.27 (dd, J = 4.8, 13.2 Hz, 1H); 3.63–3.71 (m, 1H); 3.88–3.96 (m, 2H); 4.71 (d, J = 13.8 Hz, 1H); 5.69 (s, 1H, CH); 7.03 (m, 2H, aromat); 7.49 (m, 2H, aromat); 14.22 (s, 1H; OH). ^{13}C NMR: δ 19.8 (CH_3); 40.5 (CH_2); 46.5 (CH_2); 48.1 (CH_2); 58.3 (CH); 96.5 (C-3); 107.5 (C-5); 115.4 (d, J_{CF} = 21.4 Hz, C-16, 18); 128.2 (d, J_{CF} = 8.2 Hz, C-15, 19); 139.2 (d, J_{CF} = 2.8 Hz, C-14); 163.2 (C-6); 160.8; 164.1 (d, J_{CF} = 244.6 Hz, C-F); 164.0 (C = N); 179.4 (C-4); 184.9 (C-2). ^{19}F NMR: δ -114.8. IR [KBr, cm^{-1}]: 3295, 2977, 2813, 2788, 1688, 1652, 1588, 1570. MS (ESI) (*m/z*): 317 ($M^+ + 1$, 100% rel.Int.), 633 ($2\,M^+ + 1.2$% rel.int.).
$C_{17}H_{17}FN_2O_3$.

3.1.2.3. **3-((4*E*,6*Z*)-7-(3-Chlorophenyl)-2,3-dihydro-1*H*-1,4-diazepin-5-yl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (5)**. Compound **4b** (1.50 g, 4.50 mmol) was dissolved in dichlormethane (100 ml), freshly prepared iodosobenzene (Saltzman and Sharefkin 1973) (0.30 g, 1.50 mmol) was added and the suspension was stirred at 0 − 5 °C for 3 h. The addition of iodosobenzene was repeated. Solvent was evaporated, keeping the bath temperatur below 25 °C. The residue was purified by column chromatography (eluent chloroform:methanol 100:3) and slowly recrystallized from *n*-hexane. Slight brown powder: Yield 6%; m.p. 105–108 °C. ^{1}H NMR: δ 2.10 (s, 3H, CH_3); 3.67–3.71 (m, 4H, 2 CH_2); 5.84 (s, 1H, CH); 6.83 (s, 1H, CH); 6.93 (s, 1H, NH); 7.29–7.56 (m, 4H, aromat); 12.80 (s, 1H; OH). ^{13}C NMR: δ 18.6 (CH_3); 46.8 (CH_2); 48.1 (CH_2); 91.4 (C-12); 94.1 (C-3); 106.0 (C-5); 124.7 (C-18); 126.3 (C-15); 129.2 (C-19); 129.6 (C-17); 133.7 (C-16); 139.2 (C-14); 157.4 (C-13); 160.5 (C-6); 164.0 (C=N); 166.0 (C-4); 180.5 (C-2). IR [KBr, cm^{-1}]: 3295, 2977, 2813, 2788, 2717, 1688, 1652, 1588, 1570. MS (ESI) (*m/z*): 331 ($M^+ + 1$, 100% rel.int.).
$C_{17}H_{15}ClN_2O_3$.

3.1.2.4. **2-(3-Chlorophenyl)-7-methyl-2,3,4,5-tetrahydropyrido[1,2-*d*][1,4]diazepin-9(1*H*)-one (8)**. Compound **3b** (1.16 g, 4.00 mmol) was grinded with silica gel (4 g) for 30 min while ethylene diamine (2.8 ml, 40 mmol) was added slowly. The mixture was kept at RT. After 12 h methanol (70 ml) was added and the suspension was stirred at RT for 3 h. Silica gel was removed by filtration. The filtrate was kept in a closed container at RT for 14 d. Methanol was evaporated and the oily residue was purified by column chromatography (eluent chloroform:methanol 100:8) and recrystallized from acetone. Slight brown powder: Yield 30%; m.p. 168–172 °C. ^{1}H NMR: δ 2.31 (s, 3H, CH_3); 2.76 (d, J = 15.3 Hz, 1H of CH_2); 2.91 (dd, J = 8.7, 13.5 Hz, 1H of CH_2); 3.27 (dd, J = 9.6, 15.0 Hz, 1H of CH_2); 3.44 (dd, J = 6.3, 13.5 Hz, 1H of CH_2); 3.83 (d, J = 9.0 Hz, 1H, CH); 4.10 (m, 1H of CH_2); 4.34 (m, 1H of CH_2); 6.16 (m, 2H, CH); 7.23–7.40 (m, 4H, aromat). ^{13}C NMR: δ 21.3 (CH_3); 44.7 (CH_2); 48.4 (CH_2); 50.6 (CH_2); 62.2 (CH); 118.6, 118.8 (C-8, 10); 125.0 (C-15); 126.9 (C-12); 128.5 (C-14); 130.5 (C-16); 134,9 (C-13); 145.4; 148.6 (C-7); 152.1 (=C-N); 179.2 (C-9). IR [KBr, cm^{-1}]): 3426, 1632, 1548. MS (ESI) (*m/z*): 289 ($M^+ + 1$, 29% rel.Int.), 577 ($2\,M^+ + 1$, 100% rel.int.).
$C_{16}H_{17}ClN_2O$.

3.1.2.5. **Methyl 2-(3-chlorophenyl)-7-methyl-9-oxo-1,2,3,4,5,9-hexahydropyrido[1,2-*d*][1,4]diazepine-10-carboxylate (10)**. Compound

4b (1.08 g, 3.25 mmol) was dissolved in methanol (50 ml) and aq. 0.1 M NaOH solution (25 ml) was added. The mixture was refluxed for 2 h, the solvent was evaporated and the residue was recrystallized from acetone (5 ml). Slightly yellow crystalline powder: Yield 59%; m.p. 222–227 °C. ^{1}H NMR: δ 2.30 (s, 3H, CH_3); 2.83 (dd, J = 1.5, 15.0 Hz, 1H of CH_2); 2.89 (dd, J = 8.4, 13.2 Hz, 1H of CH_2); 3.04 (dd, J = 9.0, 15.9 Hz, 1H of CH_2); 3.40 (dd, J = 6.0, 13.2 Hz, 1H of CH_2); 3.76 (s, 3H, OCH_3); 3.89 (d, J = 8.1 Hz, 1H, CH); 4.09 (dd, J = 8.7, 15.6 Hz, 1H of CH_2); 4.34 (dd, J = 6.0, 15.6 Hz, 1H of CH_2); 6.02 (s, 1H, CH); 7.23–7.41 (m, 4H, aromat). ^{13}C NMR: δ 21.7 (CH_3); 42.5 (CH_2); 48.3 (CH_2); 50.9 (CH_2); 52.6 (OCH_3); 62.2 (CH); 119.3 (C-8); 124.7 (C-15); 125.1 (C-11); 126.5 (C-12); 128.2 (C-14); 130.3 (C-16); 134.8 (C-13); 146.2; 148.2; 150.0; 167.8; 175.2. IR [KBr, cm^{-1}]: 3307, 2950, 2805, 1726, 1634, 1572. MS (HR-ESI) (*m/z*): 369 (M^+ + Na, 100% rel.int.), 715 (2 M^+ + Na, 74.7% rel.int.).
$C_{18}H_{19}ClN_2O_3$.

3.2. Biology

The radioligand binding assay was performed on membranes of transiently transfected HEK293 tsa cells. These cells are transfected with $GABA_A$ subunits $\alpha_2\beta_3\gamma_2$ and $\alpha_3\beta_3\gamma_2$ respectively by calcium phosphate DNA precipitation. 24 h after transfection the cells were scraped from the plates in PBS and the membrane fractions prepared after different steps of separation. The radioactive binding assay with [^{3}H]-Ro15–1788 was performed in MultiScreen glass fiber filterplates. 10–100 µg protein per well was incubated with 1 nM [^{3}H]-Ro15–1788 in 50 mM Tris, 200 mM NaCl, pH = 7.1 for 60 min at 4 °C. Nonspecific binding was estimated in the presence of 10 µM diazepam. The assay was terminated by aspiration using the MultiScreen vacuum manifold and washing with 200 µl assay buffer. After removing residues of buffer from the plate, the plate was 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 in order to determine the specific binding of [^{3}H]-Ro15–1788.

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