ELSEVIER

Il Farmaco 53 (1998) 305–311

IL FARMACO

1,2,3-Triazolo[1,5-a][1,4]- and 1,2,3-triazolo[1,5-a]-[1,5]benzodiazepine derivatives: synthesis and benzodiazepine receptor binding

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Received 6 October 1997; accepted 4 March 1998

Abstract

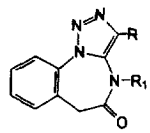
This paper reports the synthesis of new 1,2,3-triazolo[1,4]benzodiazepine and 1,2,3-triazolo[1,5]benzodiazepine derivatives and their evaluation toward benzodiazepine receptors. Receptor affinity gradually and remarkably increases by moving the nitrogen atom of the central ring from position 3 through 4 to position 5, to give the most effective compound **6a** ($K_i = 150$ nM). *N*-methylation of the diazepine ring (**7a**) lowers receptorial binding. Introduction of a chlorine atom on the benzene ring doubles the K_i value (**6b**) which remains unaltered by the *N*-methylation (**7b**).

Keywords: 1,2,3-Triazolo[1,5-a][1,4]benzodiazepine; 1,2,3-Triazolo[1,5-a][1,5]benzodiazepine; Benzodiazepine receptor binding

1. Introduction

The benzodiazepine receptor (BZR) is a cell-surface GABA receptor/chloride ion channel complex, which can bind either classical benzodiazepines or several other compounds with very different structures [1–7]. The interaction of these compounds can produce a continuum of intrinsic activity ranging from full agonists (sedative–hypnotic, anxiolytic, anticonvulsant and myorelaxant agents) to antagonists (devoid of pharmacological efficacy) or to inverse agonists (with proconvulsant, convulsant and anxiogenic properties). Considerable efforts have been directed toward the characterization of the essential features for ligand–BZR interaction, assuming that agonists, antagonists and inverse agonists bind to the same binding domain of the BZR. However, some pharmacophoric descriptors (hydrogen donors, hydrogen acceptors, and lipophilic groups) must differ to allow different efficacy [8].

In a previous paper [9] concerning a series of 1,2,3-triazolo[1,5-a][1,3]benzodiazepine derivatives (formula A)



A

which had shown a moderate affinity toward benzodiazepine receptors, theoretical calculations based upon the SAR suggested introduction of a substituent which generates a region of negative molecular electronic potential (MEP) on the benzene ring.

As a route to directly introduce substituents on the benzo-fused ring, we considered the nitration reaction of the triazolobenzodiazepine moiety; unfortunately, the nitration failed since the starting tricyclic system was recovered unreacted or it underwent oxidative demolition under stronger reaction conditions. Moreover, attempts of a preliminary substitution on the 2-aminophenylacetic acid, the precursor of the azide, which represents the key intermediate for the synthesis of the tricyclic system, failed because of facile intramolecular cyclization (under acid conditions) to 2-oxyindoline.

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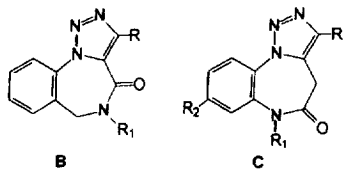
0014-827X/98/$19.00
PII S0014-827X(98)00025-1

Thus, we realized the synthesis of isomeric new 1,2,3-triazolo[1,5-a][1,4]benzodiazepines (formula B) and 1,2,3-triazolo[1,5-a][1,5]benzodiazepines (formula C).



B C

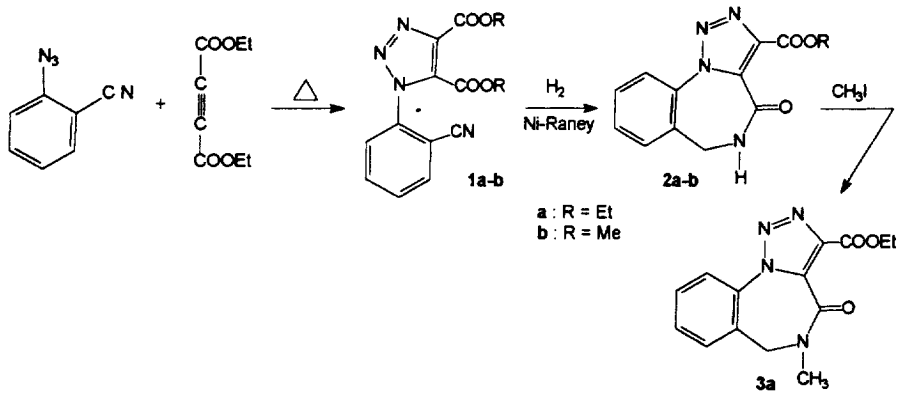
In the first case, the 2-cyanophenylazide [10] allowed the preparation of one triazolo[1,4]benzodiazepine derivative, unsubstituted on the benzene ring (Scheme 1). In fact, some attempts to introduce substituents on the benzene ring, starting from 2-cyanoaniline substituted with nitro or bromo groups to give the appropriate substituted 2-cyanophenylazides and/or the corresponding tricyclic derivatives, failed.

On the other hand, the 3-carbethoxy-1,2,3-triazolo[4,5-a]-[1,5]benzodiazepin-5-one had been described in the literature [11] starting from 2-nitrophenylazide, so that the same synthetic route (Scheme 2) could be employed from 2-nitrophenylazides previously substituted with a halogen atom (chlorine) or with an acetyl group, respectively, to give the corresponding substituted tricyclic analogues.

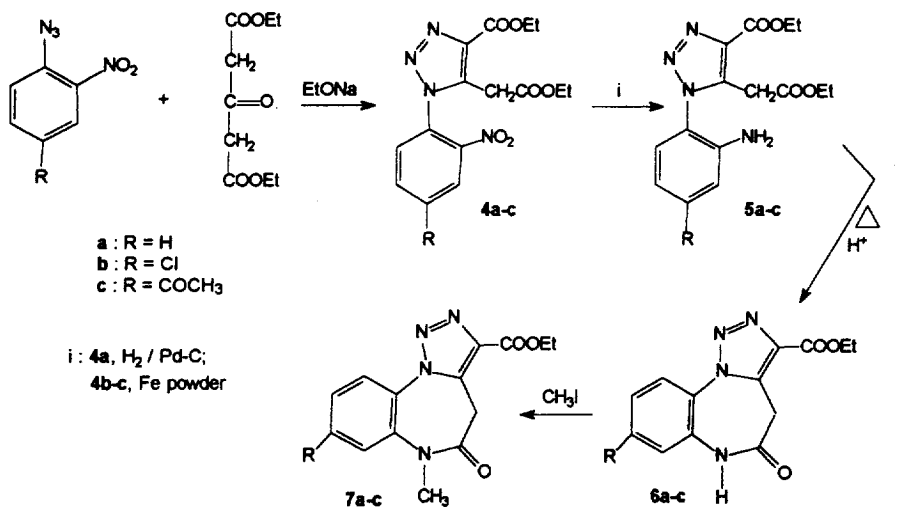
2. Chemistry

The 2-cyanophenylazide [10] reacted with diethyl or dimethyl acetylenedicarboxylate in refluxing benzene to give the expected triazole diesters **1a** and **1b** (Scheme 1). Reduction of the cyano group to amine directly provided the corresponding tricyclic derivatives 3-substituted-1,2,3-triazolo[1,5-a][1,4]benzodiazepin-4-ones **2a** and **2b** in good yield. Alkylation of **2a** with methyl iodide gave the corresponding *N*-methyl derivative **3a**.

The synthetic route which leads to the new 1,2,3-triazolo[1,5]benzodiazepines bearing a substituent on the benzene ring is described in Scheme 2. Thus, starting from 2-nitrophenylazide [12] and diethyl acetonedicarboxylate, according to the literature [11], the 1-(2-nitrophenyl)-4-carbethoxy-5-carbethoxymethyl-1*H*-1,2,3-triazole (**4a**), the corresponding 2-amino-phenyltriazole **5a** and the known 3-carbethoxy-1,2,3-triazolo[1,5-a][1,5]benzodiazepin-5-one (**6a**) were synthesized.



Scheme 1. Synthesis of 1,2,3-triazolo[1,5-a][1,4]benzodiazepine derivatives.



Scheme 2. Synthesis of 1,2,3-triazolo[1,5-a][1,5]benzodiazepine derivatives.

Similarly, by reacting 2-nitro-4-chlorophenylazide [13] or 2-nitro-4-acetylphenylazide [14] with diethyl acetonedicarboxylate, the expected triazole derivatives **4b** and **4c** were obtained in 25% and 18% yields, respectively. These target compounds were obtained in low yields because the main reaction product was the corresponding 4-substituted nitroaniline, probably resulting from the cleavage of the formed 1,2,3-triazole in an alkaline medium. Even using mild experimental conditions, the azide decomposed into benzofurazane *N*-oxide, and polymeric or tarry material was also recovered.

Reduction of the nitro group of the triazole derivative **4a** was achieved by catalytic hydrogenation according to Smalley and Teguiche [11], while reduction of the analogues **4b** and **4c** was satisfactorily carried out with iron powder, in order to preserve the chloro and acetyl substituents. The aminophenyl 1,2,3-triazole esters **5a–c** obtained by intramolecular cyclization (in boiling xylene in the presence of a catalytic amount of *p*-toluenesulfonic acid (**5a**) [11] or boiling toluene in the presence of catalytic concentrated sulfuric acid for **5b** and **5c**) were converted into the corresponding tricyclic derivatives **6a–c**. Treatment of the latter compounds with methyl iodide provided the corresponding *N*-methyl derivatives **7a–c** in good yields.

The structures of all the newly prepared compounds were assigned according to the reaction mechanisms and were confirmed by analytical and spectroscopic data. ^{13}C NMR data of the tricyclic compounds are reported in Table 1.

3. Experimental

3.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra in nujol mulls were recorded on a Perkin-Elmer model 1310 spectrometer. ^1H NMR spectra (internal standard tetramethylsilane (TMS)) were recorded on a Varian CFT 20 or a Brüker AC 200 spectrometer operating at 80 MHz or at 200 MHz, respectively. ^{13}C NMR spectra were recorded with the Brüker AC 200 spectrometer at 50 MHz. Chemical shifts assignments were confirmed, when possible, with HETCOR experiments. Mass spectra were performed with a Hewlett Packard MS/System 5988. Thin-layer chromatography (TLC) data were obtained with Riedel de Haen, 37360 DC-Karten F_{254}, 0.2 mm, eluting with an AcOEt/petroleum ether 1:1 mixture. Elemental analyses (C, H, N) were within $\pm 0.4\%$ of the theoretical values and were performed on a Carlo Erba elemental analyzer model 1106 apparatus. Column chromatography was performed on silica gel 60 (230–400 mesh). Petroleum ether corresponds to the fraction boiling at 40–60°C.

3.1.1. General procedure for the synthesis of 1-(2-cyanophenyl)-1H-1,2,3-triazole-4,5-dicarboxylates 1a–b

A solution of 2-cyanophenylazide (3.00 g, 20.83 mmol) and 34.0 mmol of diethyl or dimethyl acetylenedicarboxylate in 90 ml of anhydrous benzene was heated under reflux for 20 h. The solvent was evaporated in vacuo and the oily residue underwent a short distillation at 150°C/5 mmHg to distil off the excess diethyl acetylenedicarboxylate. The distillation residue consisted of solidified **1a** or **1b**.

Compound **1a** (5.90 g, yield 90%) could be purified by distillation at 225°C/0.2 mmHg; m.p. 59–61°C; IR (nujol): ν (cm^{-1}) 2240 (C≡N), 1730 and 1230 (COOR). Anal. ($C_{15}H_{14}N_4O_4$): C, H, N.

Compound **1b** (4.91 g, yield 83%) could be purified by crystallization from MeOH; m.p. 104–106°C; IR (nujol): ν (cm^{-1}) 2240 (C≡N), 1730 and 1240 (COOR); MS (*m/z*): 286 (M^+), 227, 168, 155, 143, 102. Anal. ($C_{13}H_{10}N_4O_4$): C, H, N.

Table 1
^{13}C NMR data (δ, ppm) for compounds **2**, **3**, **6** and **7** in DMSO

	2a	3a	6a	6b	6c	7a	7b	7c
C-4	140.1	139.8	135.9	135.8	137.8	137.1	136.1	138.0
C-4a	134.1	134.9	134.4	134.5	134.7	134.4	134.6	134.5
C-5	157.4	155.8	30.7	30.7	30.7	31.0	31.0	30.8
C-6	41.2	49.1	167.6	167.5	167.5	166.3	166.8	166.5
C-7a	132.4	134.5	130.1	131.3	130.2	134.4	134.8	134.8
C-7	128.7	129.0	122.7	122.0	122.4	123.9	124.2	123.9
C-8	129.9	130.6	130.1	134.1	136.2	130.5	137.1	137.2
C-9	129.5	129.8	125.1	125.3	124.6	126.5	126.5	125.7
C-10	122.9	122.6	123.6	124.9	124.1	124.2	125.5	124.3
C-10a	135.1	134.8	126.3	125.3	129.2	127.5	126.7	130.5
COO	159.6	159.5	160.2	160.1	160.1	160.3	160.3	160.3
OCH_2	61.0	61.0	60.6	60.7	60.7	60.8	60.8	60.7
CH_3	13.7	13.6	13.9	13.8	13.7	14.0	14.0	13.8
NCH_3	–	33.9	–	–	–	36.5	36.5	36.5
CO-8	–	–	–	–	196.3	–	–	196.5
CH_3	–	–	–	–	26.5	–	–	26.7

*3.1.2. 5,6-Dihydro-4-oxo-4H-1,2,3-triazolo[1,5-a][1,4]-benzodiazepine-3-carboxylates **2a–b***

Aqueous Ni-Raney suspension (≅1 g) and ammonium hydroxide (32%, 2 ml) were added to a solution of **1a** or **1b** (7.0 mmol) in methanol (150 ml), and the mixture was hydrogenated at room temperature and pressure. The suspension was heated under reflux for 1 h, the catalyst was filtered off, washed with hot methanol and the combined filtrates evaporated in vacuo to give the title compounds.

2a: 1.81 g, yield 95%; m.p. 242–244°C (EtOH); IR (nujol): ν (cm^{-1}) 3230 (NH), 1660 (C=O), 1725 and 1220 (COOR); MS (*m/z*): 272 (M^+), 243, 229, 197, 171, 125, 116; ^{1}H NMR (DMSO, 200 MHz): δ (ppm) 9.09 (bt, 1H, NH), 8.00 (m, 1H, H-10), 7.68–7.58 (m, 3H, H-7, H-8 and H-9), 4.37 (q, 2H, J=7.1 Hz, OCH_2), 4.27 (d, 2H, CH_2-6), 1.32 (t, 3H, CH_3). ^{13}C NMR: see Table 1. Anal. ($C_{13}H_{12}N_4O_3$): C, H, N.

2b: 1.35 g, yield 75%; m.p. 270–272°C (MeOH); IR (nujol): ν (cm^{-1}) 3200 (NH), 1720 and 1220 (COOR), 1650 (C=O); MS (*m/z*): 258 (M^+), 229, 197, 129. Anal. ($C_{12}H_{10}N_4O_3$): C, H, N.

*3.1.3. Ethyl 5,6-dihydro-5-methyl-4-oxo-4H-1,2,3-triazolo[1,5-a][1,4]benzodiazepine-3-carboxylate **3a***

Methyl iodide (1.2 ml, 19.3 mmol) was added to a solution of **2a** (0.300 g, 1.10 mmol) in 70 ml of ethanolic 0.1% sodium hydroxide (1.75 mmol). The mixture was stirred at room temperature for 22 h. The mixture was neutralized with 10% hydrochloric acid, concentrated in vacuo, treated with H_2O and extracted with $CHCl_3$. The organic layer, dried ($MgSO_4$) and evaporated, provided **3a**: 0.280 g, yield 89%; m.p. 166–168°C (EtOH); IR (nujol): ν (cm^{-1}) 1720 and 1210 (COOR), 1650 (C=O); MS (*m/z*): 286 (M^+), 257, 185, 156, 128, 116; ^{1}H NMR (DMSO, 200 MHz): δ (ppm) 8.02 (m, 1H, H-10), 7.72 (m, 1H, H-8), 7.67 (m, 1H, H-7), 7.62 (m, 1H, H-9), 4.53 (s, 2H, CH_2-6), 4.37 (q, 2H, J=7.1 Hz, OCH_2), 3.11 (s, 3H, NCH_3), 1.33 (t, 3H, CH_3). ^{13}C NMR: see Table 1. Anal. ($C_{14}H_{14}N_4O_3$): C, H, N.

*3.1.4. General procedure for the synthesis of ethyl 4-carbethoxy-1-(2-nitroaryl)-1H-1,2,3-triazole-5-acetates **4a–c***

40 ml of ethanolic sodium ethoxide solution (0.350 g, 15.2 g atoms sodium) were slowly added dropwise (≅1 h) to a stirred and cooled (0–5°C) suspension of the suitable 2-nitrophenylazide (15.0 mmol) and diethyl acetonedicarboxylate (3.0 ml, 16.5 mmol) in 40 ml of absolute ethanol. The suspension was stirred at room temperature for one night (**4a**), for 5 h (**4b**) or for one night at 0–5°C (**4c**), then it was concentrated in vacuo (temperature ≤45°C), treated with H_2O and extracted with AcOEt. The organic layer, after washing with NaCl solution, was dried ($MgSO_4$) and evaporated to give a solid residue which underwent flash-chromatography through a silica gel column (≅14×4 cm). Elution with petroleum ether/AcOEt 3:1 and successively 2:1 provided the following results.

Isolation of **4a**: benzofurazane *N*-oxide [12]: 0.300 g, yield 15%, m.p. 69–71°C; 2-nitroaniline: 0.930 g, yield 45%, m.p. 71–72°C; **4a** [11]: 1.00 g, yield 19%; m.p. 78–79°C.

Isolation of **4b**: 6-chlorobenzofurazane *N*-oxide [13]: 0.700 g, yield 27%, m.p. 46–47°C; 2-nitro-4-chloroaniline: 0.920 g, yield 35%, m.p. 128–129°C; **4b**: 1.430 g; yield 25%; m.p. 121–122°C (EtOH); IR (nujol): ν (cm^{-1}) 1730 and 1220 (COOR); MS (*m/z*): 382 (M^+), 337, 280, 253, 207, 180, 127; ^{1}H NMR ($CDCl_3$, 80 MHz): δ (ppm) 8.11 (d, 1H, H-3′), 7.74 (dd, 1H, H-5′), 7.49 (d, 1H, H-6′), 4.38 and 4.04 (2q, 4H, $2OCH_2$), 3.91 (s, 2H, CH_2CO), 1.37 and 1.15 (2t, 6H, $2CH_3$). Anal. ($C_{15}H_{15}N_4O_6Cl$): C, H, N.

Isolation of **4c**: 6-acetylbenzofurazane *N*-oxide [14]: 0.147 g, yield 5.5%, m.p. 89–90°C; 2-nitro-4-acetylaniline: 1.447 g, yield 54%, m.p. 143–145°C; **4c**: 1.070 g, yield 18%; m.p. 134–135°C (EtOH); IR (nujol): ν (cm^{-1}) 1730 and 1230 (COOR), 1690 (C=O); MS (*m/z*): 390 (M^+), 261, 215, 187, 161, 127; ^{1}H NMR ($CDCl_3$, 80 MHz): δ (ppm) 8.68 (d, 1H, H-3′), 8.32 (dd, 1H, H-5′), 7.72 (d, 1H, H-6′), 4.45 and 4.11 (2q, 4H, $2OCH_2$), 4.00 (s, 2H, CH_2CO), 2.73 (s, 3H, CH_3CO), 1.44 and 1.21 (2t, 6H, $2CH_3$). Anal. ($C_{17}H_{18}N_4O_7$): C, H, N.

*3.1.5. Ethyl 1-(2-aminophenyl)-4-carbethoxy-1H-1,2,3-triazole-5-acetate **5a***

The compound was prepared as described in the literature [11] from **4a** (0.440 g, 1.26 mmol) by catalytic hydrogenation: 0.390 g, yield 97%; m.p. 100–102°C (EtOH).

*3.1.6. General procedure for the synthesis of ethyl 1-(2-aminoaryl)-4-carbethoxy-1H-1,2,3-triazole-5-acetates **5b–c***

5% $FeCl_3$ solution (0.3 ml) and iron powder (0.350 g) were added to a solution of **4b** or **4c** (1.70 mmol) in aqueous ethanol (60%, 60 ml). The mixture was heated under reflux for 3 h. The hot reaction mixture was filtered and the filtrate concentrated in vacuo. The residue was treated with H_2O and the suspension obtained was alkalinized with 32% ammonium hydroxide solution and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and evaporated to give the title compounds.

5b: 0.556 g, yield 93%; m.p. 105–107°C (EtOH); IR (nujol): ν (cm^{-1}) 3460 and 3340 (NH_2), 1720 and 1250 (COOR); MS (*m/z*): 352 (M^+), 265, 250, 222, 205, 177, 165, 142; ^{1}H NMR ($CDCl_3$, 80 MHz): δ (ppm) 7.03 (d, 1H, H-6′), 6.84 (d, 1H, H-3′), 6.79 (dd, 1H, H-5′), 4.43 and 4.11 (2q, 4H, $2OCH_2$), 3.91 (s, 2H, CH_2CO), 1.42 and 1.21 (2t, 6H, $2CH_3$). Anal. ($C_{15}H_{17}N_4O_4Cl$): C, H, N.

5c: 0.589 g, yield 95%; m.p. 105–107°C (EtOH). IR (nujol): ν (cm^{-1}) 3400 and 3330 (NH_2), 1730 and 1220 (COOR), 1690 (C=O); MS (*m/z*): 360 (M^+), 315, 273, 247, 230, 213, 185, 171, 143; ^{1}H NMR ($CDCl_3$, 80 MHz): δ (ppm) 7.43–7.20 (m, 4H, H-2′, H-3′, H-5′ and H-6′), 4.44 and 4.20 (2q, 4H, $2OCH_2$), 3.94 (s, 2H, CH_2CO), 2.59 (s, 3H, CH_3CO), 1.43 and 1.21 (2t, 6H, $2CH_3$). Anal. ($C_{17}H_{20}N_4O_5$): C, H, N.

*3.1.7. Ethyl 5,6-dihydro-5-oxo-4H-1,2,3-triazolo-[1,5-a][1,5]benzodiazepine-3-carboxylate **6a***

The compound was prepared as described in the literature [11] from **5a** (0.390 g, 1.22 mmol) by refluxing in 25 ml of xylene in the presence of a catalytic amount of *p*-toluenesulfonic acid: 0.265 g, yield 80%; m.p. 194–195°C (EtOH); ^{1}H NMR (DMSO, 200 MHz): δ (ppm) 10.49 (bs, 1H, NH), 7.97 (dd, 1H, $J_{9,10}$=8.02 Hz, $J_{8,10}$=1.50 Hz, H-10), 7.59 (ddd, 1H, $J_{8,9}$=7.56 Hz, H-8), 7.42 (ddd, 1H, $J_{7,9}$=1.44 Hz, H-9), 7.37 (dd, 1H, $J_{7,8}$=7.82 Hz, H-7), 4.39 (q, 2H, J=7.1 Hz, OCH_2), 4.14 (s, 2H, CH_2-5), 1.36 (t, 3H, CH_3). ^{13}C NMR see Table 1. Anal. ($C_{13}H_{12}N_4O_3$): C, H, N.

*3.1.8. General procedure for the synthesis of 8-substituted-5,6-dihydro-5-oxo-4H-1,2,3-triazolo[1,5-a][1,5]benzodiazepine-3-carboxylates **6b–c***

One drop of 98% sulfuric acid was added to a solution of **5b** or **5c** (1.50 mmol) in toluene (50 ml) and heated under reflux for 4 h. After cooling, the solution was washed with H_2O, dried ($MgSO_4$) and evaporated in vacuo to give the title compounds.

6b: 0.446 g, yield 97%; m.p. 268–270°C (AcOEt); IR (nujol): ν (cm^{-1}) 3180 (NH), 1710 and 1250 (COOR), 1670 (C=O); MS (*m/z*): 306 (M^+), 250, 204, 178, 165, 124; ^{1}H NMR (DMSO, 200 MHz): δ (ppm) 10.58 (bs, 1H, NH), 8.00 (d, 1H, $J_{9,10}$=8.65 Hz, H-10), 7.48 (dd, 1H, $J_{7,9}$=2.32 Hz, H-9), 7.42 (d, 1H, H-7), 4.39 (q, 2H, J=7.1 Hz, OCH_2), 4.17 (s, 2H, CH_2-5), 1.36 (t, 3H, CH_3). ^{13}C NMR see Table 1. Anal. ($C_{13}H_{11}N_4O_3Cl$): C, H, N.

6c: 0.461 g, yield 98%; m.p. 214–216°C (EtOH); IR (nujol): ν (cm^{-1}) 3160 (NH), 1710 and 1240 (COOR), 1660 (C=O); MS (*m/z*): 314 (M^+), 269, 258, 243, 185, 169, 141, 130; ^{1}H NMR (DMSO, 200 MHz): δ (ppm) 10.62 (bs, 1H, NH), 8.10 (d, 1H, $J_{9,10}$=8.24 Hz, H-10), 7.94 (dd, 1H, $J_{7,9}$=1.85 Hz, H-9), 7.92 (d, 1H, H-7), 4.40 (q, 2H, J=7.1 Hz, OCH_2), 4.18 (s, 2H, CH_2-5), 2.63 (s, 3H, $COCH_3$), 1.36 (t, 3H, CH_3). ^{13}C NMR see Table 1. Anal. ($C_{15}H_{14}N_4O_4$): C, H, N.

*3.1.9. Ethyl 5,6-dihydro-6-methyl-5-oxo-4H-1,2,3-triazolo-[1,5-a][1,5]benzodiazepine-3-carboxylate **7a**, ethyl 8-chloro-5,6-dihydro-6-methyl-5-oxo-4H-1,2,3-triazolo-[1,5-a][1,5]benzodiazepine-3-carboxylate **7b** and ethyl 8-acetyl-5,6-dihydro-6-methyl-5-oxo-4H-1,2,3-triazolo-[1,5-a][1,5]benzodiazepine-3-carboxylate **7c***

Methyl iodide (0.6 ml, 9.60 mmol) was added to a stirred solution of the triazolobenzodiazepine **6a**, **6b** or **6c** (0.80 mmol) in 0.5% NaOH ethanolic solution (10 ml), and stirring was continued at room temperature for 3 h. After dilution with H_2O the title compounds precipitated as a white solid which was collected by filtration and washed with H_2O.

7a: 0.190 g, yield 83%; m.p. 183–185°C (EtOH); ^{1}H NMR (DMSO, 200 MHz): δ (ppm) 7.93 (m, 1H, H-10), 7.72 (m, 2H, H-7 and H-8), 7.53 (m, 1H, H-9), 4.39 (q, 2H, J=7.1 Hz, OCH_2), 3.29 (s, 2H, CH_2-5), 3.27 (s, 3H, NCH_3), 1.35 (t, 3H, CH_3). ^{13}C NMR see Table 1. Anal. ($C_{14}H_{14}N_4O_3$): C, H, N.

7b: 0.235 g, yield 92%; m.p. 150–152°C (EtOH); IR (nujol): ν (cm^{-1}) 1720 and 1210 (COOR), 1670 (C=O); MS (*m/z*): 320 (M^+), 235, 218, 191, 189, 155, 124; ^{1}H NMR (DMSO, 200 MHz): δ (ppm) 7.93 (d, 1H, $J_{9,10}$=8.68 Hz, H-10), 7.82 (d, 1H, $J_{7,9}$=2.23 Hz, H-7), 7.58 (dd, 1H, H-9), 4.39 (q, 2H, J=7.1 Hz, OCH_2), 3.30 (s, 2H, CH_2-5), 3.19 (s, 3H, NCH_3), 1.36 (t, 3H, CH_3). ^{13}C NMR see Table 1. Anal. ($C_{14}H_{13}N_4O_3Cl$): C, H, N.

7c: 0.210 g, yield 80%; m.p. 152–154°C (DMF–H_2O); IR (nujol): ν (cm^{-1}) 1710 and 1250 (COOR), 1680 (C=O); MS (*m/z*): 328 (M^+), 257, 243, 199, 187, 158, 130; ^{1}H NMR (DMSO, 200 MHz): δ (ppm) 8.15 (m, 1H, H-10), 8.06 (m, 2H, H-7 and H-9), 4.41 (q, 2H, J=7.1 Hz, OCH_2), 3.36 (s, 2H, CH_2-5), 3.20 (s, 3H, NCH_3), 1.36 (t, 3H, CH_3). ^{13}C NMR see Table 1. Anal. ($C_{16}H_{16}N_4O_4$): C, H, N.

4. Biological

The prepared tricyclic compounds **2a–b**, **3a**, **6a–c** and **7a–c** were tested for their ability to inhibit benzodiazepine receptor binding, by measuring the concentration able to displace the [^{3}H] Ro 15-1788 from bovine brain membranes.

The experimental details of the receptor binding assays were reported in a previous paper [15].

Furthermore, the GABA ratio values of active compounds were evaluated as an in vitro indicator of the agonist, inverse-agonist or antagonist properties [16].

5. Results and discussion

The results in Table 2 show that affinity toward the benzodiazepine receptors of the 1,2,3-triazolo[1,5-a]-benzodiazepine derivatives gradually and remarkably increases ($\cong$10-fold), by moving the nitrogen atom of the central ring from position 3 through 4 to position 5; in fact, the 3-carbethoxy-1,2,3-triazolo[1,5-a][1,3]benzodiazepin-5-one, the most effective derivative previously prepared [9], showed an inhibition constant K_i=1600 nM, while the [1,4]benzodiazepine isomer **2a** shows K_i=908 nM and the other [1,5]benzodiazepine isomer **6a** shows K_i=150 nM. In all cases the GABA ratio values ($\cong$1.2) indicate a antagonist action.

Moreover, comparison of these three isomers, which are members of three heterocyclic series, shows that the *N*-methylation of the diazepine ring lowers the receptor binding (see

Table 2
Inhibition of [^{3}H] Ro 15-1788 binding and GABA ratio

Comp.	K_i (nM) [a]	GABA ratio [b]
2a	908 ± 81	1.19
2b	1190 ± 97	0.60
3a	2600 ± 240	–
6a	150 ± 11	1.26
6b	348 ± 35	1.55
6c	7700 ± 755	–
7a	931 ± 91	1.12
7b	354 ± 31	1.93
7c	> 8000	–

The tests were carried out using dimethyl sulfoxide (2%) as solvent unless otherwise stated.
[a] K_i represents the mean ± SEM of three determinations.
[b] GABA ratio = K_i(compound)/K_i(compound + 50 μM GABA) performed in three experiments.

the *N*-methyl derivative of the previous [1,3] series [9] and compounds **3a** and **7a**).

It is worth noting for the [1,4]benzodiazepine series that the methyl ester **2b** is less effective than the ethyl ester **2a** and the GABA ratio value (0.60) suggests an inverse-agonist efficacy.

Regarding the derivatives of the [1,5]benzodiazepine series (compounds **6a–c** and **7a–c**), which interact better with the receptor site than the corresponding 1,3- and 1,4-derivatives, introduction of an 8-substituent on the benzo-fused ring decreases the receptor affinity.

However, introduction of a chlorine atom maintains good activity (compounds **6b** and **7b**), whilst the acetyl group strongly decreases the activity (compounds **6c** and **7c**). The chloro-substituted compounds **6b** (K_i = 348 nM) and the corresponding *N*-methyl derivative **7b** (K_i = 354 nM) show equivalent affinities and the GABA ratio values (1.55 and 1.93, respectively) suggest a partial-agonist/agonist activity.

These considerations indicate that the presence of a substituent such as Cl or $COCH_3$ influences receptor binding probably by electronic and steric effects.

Moreover, the recently proposed inclusive pharmacophore model of agonist/inverse-agonist binding sites [8] allows us to evaluate the interactions of the functional groups present on these structures. The possible and indicative interactions of the more active ligands **6a**, **6b** and **7b** are illustrated in Figs. 1 and 2. Fig. 1 shows the most active 8-unsubstituted compound **6a**: the NH function may be able to form an H-bond with the A_2 site; the CO amide function may be able to accept an H-bond from the H_1 site; likewise, a nitrogen atom of the triazole ring may accept an H-bond from the H_2 site; the ethyl group may be arranged in the lipophilic region L_1. All these interactions can agree with an antagonist/inverse-agonist activity. Similarly, the receptor binding hypothesized for 8-chloro-substituted **6b** and **7b**, which possess similar affinity, is reported in Fig. 2. This result indicates that in this case *N*-methylation did not influence the affinity, therefore the NH function is not involved in the receptor binding in the

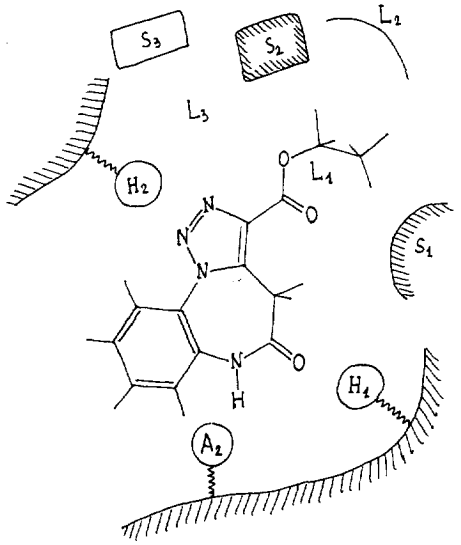


Fig. 1. Interactions of **6a** with A_2 (H-bond acceptor site), H_1 and H_2 (H-bond donor sites) and L_1 (lipophilic region).

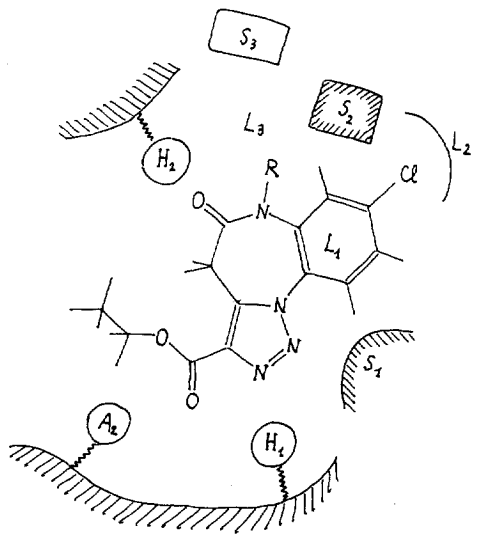


Fig. 2. Interactions of **6b** or **7b** with L_1 and L_2 (lipophilic regions) and H_1 and H_2 (H-bond donor sites); NH or NCH_3 functions are not involved in the A_2 but in the L_3 region.

A_2 area. Thus, according to this hypothesis, **6b** and **7b** can occupy the lipophilic areas L_1 and L_2 with the 8-chlorobenzo-fused moiety and interact with the H_1 and H_2 donor sites through the triazole nitrogen atoms and the carbonyl functions, respectively. This arrangement can agree with the agonist activity of **6b** and **7b**; in fact, the A_2 interaction site, which is characteristic of inverse-agonist/antagonist efficacy, is not involved. The NH or NCH_3 groups fall in the L_3 lipophilic area, which together with the L_2 one, is essential for agonist biological function. Occupation of the L_3 region justifies the higher GABA ratio of the NCH_3 derivative **7b** with respect to the *N*-unsubstituted **6b**.

Acknowledgements

Thanks are due to A. Frangioni for technical assistance. This work was supported by a grant from MURST.

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