SYNTHESIS AND STRUCTURAL FEATURES OF 11*H*-TETRAZOLO[1,5-*c*][2,3]BENZODIAZEPINES

Alba Chimirri,* Maria Zappalà, Rosaria Gitto, Silvana Quartarone, and Francesca Bevacqua

Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, 98168 Messina, Italy

Abstract - A synthetic approach to new 11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepine derivatives starting from 3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones is described. The structural features of compounds obtained were ascertained by NMR spectroscopy. The proton and carbon assignments were made with the aid of two-dimensional heteronuclear chemical shift-correlation experiments.

INTRODUCTION

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). Excitatory synaptic transmission is mediated by different types of glutamate receptors (GluRs) that are commonly distinguished by their sensitivity to different GluRs agonists.[1,2]

2,3-Benzodiazepine derivatives are a new class of benzodiazepines which have recently acquired pharmacological importance owing to their anticonvulsant and neuroprotective properties acting as selective and noncompetitive blockers of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype of GluRs.[3,4]

Our previous publications reported chemical and structure-activity relationship (SAR) studies of 1-aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones (**1**),[5-9] which have shown significant anticonvulsant activity acting at the AMPA receptor complex in a non-competitive mode of action. In the course of our approach in synthesizing new 2,3-benzodiazepine derivatives as potential anticonvulsant agents, and in connection with our interest in the chemistry of annelated benzodiazepines[10-12] and benzothiazepines,[13,14] with particular reference to their stereochemical properties, in this paper we report the synthesis of the new 11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepine tricyclic system, which, to our knowledge, has never been

* Email: chimirri@pharma.unime.it; FAX: +39 090 355613

reported to date. Our interest is related to the investigation of the influence of the added tetrazole nucleus on the conformational preferences of the benzodiazepine heptatomic ring and on the anticonvulsant activity shown by their precursors (**1**). Continuous interest in structurally-modified benzodiazepine analogues lies in the potential changes in pharmacological activity caused by such modifications particularly in relation to CNS.

The structural features of the synthesized 11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepines (**4**) were assessed by two-dimensional NMR techniques.

RESULTS AND DISCUSSION

In the course of structural modifications in the benzodiazepine system, we report the synthetic pathway to 11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepines (**4a-d**) as described in Scheme 1. The 1-aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones (**1a-d**), obtained according to our previously reported procedure,[6] were activated by transformation into the corresponding thiocarbonyl analogs (**2a-d**) by treatment with Lawesson's reagent.[7]

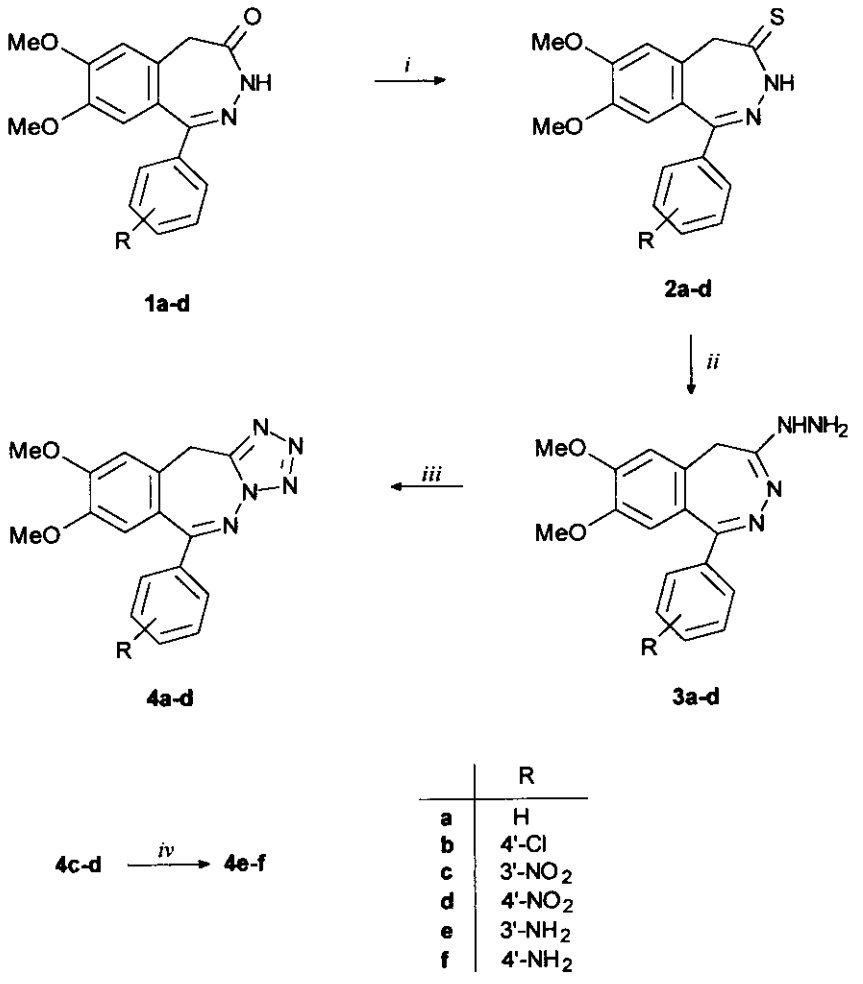
By reaction with hydrazine hydrate, compounds (**2a-d**) yielded 2,3-benzodiazepin-4-ylhydrazines intermediates (**3a-d**) which, as crude products, were treated with sodium nitrite in acidic medium to afford 11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepines (**4a-d**). Moreover, aminophenylsubstituted derivatives (**4e-f**) were prepared by reduction of the corresponding nitro analogues (**4c-d**) with Sn/HCl.

The structural features of the compounds obtained were ascertained by analytical and spectroscopic measurements (^{1}H-NMR and ^{13}C-NMR).

The ^{1}H-NMR spectra of compounds (**4**), analogously to those of compounds (**1**), show an A_2 system for methylene protons at C-11. The observed magnetic equivalence of these diastereotopic hydrogens is attributable to a fast exchange between two limiting conformers and suggests that the presence of the tetrazolo heterocycle fused on the «c» edge of the benzodiazepine system in solution at room temperature do not affect the conformational mobility of the heptatomic ring. This hypothesis is supported by dynamic NMR experiments: in fact, at 209 K and below, the methylene protons appear as non equivalent and form an AB system.

In the ^{13}C-NMR spectra, the C-11 and C-11a resonances are mainly influenced by replacement of the lactam functionality with a tetrazole nucleus and are shifted upfield (about 12 and 20 ppm, respectively) with respect to the precursors.

Complete proton and carbon assignments were made by using direct and long-range heteronuclear chemical shift correlation experiments. The correlations observed in the HETCOR and LR-HETCOR spectra for compound (**4a**) are collected in Table 1. In particular, it has been possible to distinguish H-7 and H-10 resonances on the basis of the different long-range correlations to the carbon atoms of the



1a-d **2a-d** **3a-d** **4a-d**

	R
a	H
b	4'-Cl
c	3'-NO_2
d	4'-NO_2
e	3'-NH_2
f	4'-NH_2

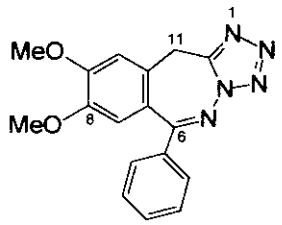
Reagents *i)* Lawesson's reagent, toluene, reflux, 1 h; *ii)* 85% $NH_2NH_2 \cdot H_2O$, THF, rt, 1 h; *iii)* $NaNO_2$/HCl, rt, 2 h; *iv*) Sn/HCl, Δ, 1 h.

Scheme 1

bicyclic skeleton; H-7 singlet resonating at 6.70 ppm is directly correlated with the carbon resonating 114.79 ppm.

Long-range couplings to H-7 are observed to three quaternary carbons resonating at 131.92, 153.25 and 168.07 ppm. The quaternary carbon resonating furthest downfield has, in turn, another long-range coupling with H-2'-6' and can be assigned to C-6 also on the basis of the high chemical shift value (168.07 ppm). Analogously H-10 singlet has been assigned on the basis of the long-range coupling with the methylene carbon at 28.57 ppm. Given these assignments, all the other protons and carbons can be immediately identified.

Table 1. Direct (●) and long-range (◆) heteronuclear correlations C-H for compound **(4a)**.



		H-2',6' 7.85	H-4' 7.61	H-3',5' 7.52	H-10 6.93	H-7 6.70	H-11 4.25	MeO-9 4.00	MeO-8 3.69
C-11	28.57				◆		●		
MeO-8	56.12								●
MeO-9	56.27							●	
C-10	110.89				●		◆		
C-7	114.79					●			
C-6a	122.02				◆		◆		
C-3',5'	128.58			●					
C-2',6'	130.42	●	◆						
C-10a	131.92					◆	◆		
C-4'	131.96	◆	●						
C-1'	136.61			◆					
C-8	147.72				◆				◆
C-11a	149.52						◆		
C-9	153.25					◆		◆	
C-6	168.07	◆				◆			

The anticonvulsant properties of the new tricyclic compounds (**4a-f**) were evaluated in a genetic model of epilepsy, i.e. audiogenic seizures, to test the effect of the replacement of the amide group of compounds (**1**) with a tetrazole nucleus on anticonvulsant activity. None of the synthesized compounds appreciably prevented the clonic and tonic phases of sound-induced seizures, thus suggesting that the lactam moiety might have an important role in the anticonvulsant activity of 2,3-benzodiazepine derivatives.

In conclusion, a synthetic approach to a new cyclofunctionalized 2,3-benzodiazepine system has been carried out and the structures of 11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepines obtained were deduced. ^{1}H- and ^{13}C-NMR spectroscopy with the aid of two-dimensional heteronuclear chemical shift-correlation experiments was performed to unambiguously assign the chemical shift values of a new class of 2,3-benzodiazepines thus extending the existing data on benzodiazepine analogues.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out on a C. Erba Model 1106 Elemental Analyzer. All separations were done

under chromatography conditions on silica gel (Merck, 63-200 μm) and TLC was performed on silica gel plates (Merck, 60 F_{254}). ^1H and ^{13}C NMR spectra were measured with a Varian Gemini-300 instrument in $CDCl_3$ as solvent. Chemical shifts are expressed in δ (ppm) relative to TMS as internal standard and coupling constants (J) in Hz. All exchangeable protons were confirmed by addition of D_2O. HETCOR and LR-HETCOR experiments were carried out by using the standard software package. Compounds (**1a-d**) and (**2a-d**) were synthesized according to previously reported procedures.[6,7]

General procedure for the synthesis of 1-aryl-7,8-dimethoxy-5*H*-2,3-benzodiazepin-4-ylhydrazines (3a-d). To a solution of 85% hydrazine hydrate (0.5 mL, 8.0 mmol) in THF (5 mL) was added dropwise a solution of **2** (3.0 mmol) in 10 mL of the same solvent at rt. After stirring for 1 h, the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2, and the extract was washed with water, dried over Na_2SO_4, filtered and evaporated to give compound (**3**) as crude product.

General procedure for the synthesis of 6-aryl-8,9-dimethoxy-11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepines (4 a-d). A solution of sodium nitrite (300 mg, 4.2 mmol) in water (30 mL) was added dropwise to a suspension of **3** (2.8 mmol) in 0.5 N HCl (10 mL) at 5°C. After stirring for 2 h the reaction mixture was neutralized with a saturated solution of $NaHCO_3$ and the precipitate was filtered to give compounds (**4**), which were recrystallized from ethanol.

8,9-Dimethoxy-6-phenyl-11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepine (4a)

Yield 65%, mp 274-277°C. Anal. Calcd for $C_{17}H_{15}N_5O_2$: C, 63.54; H, 4.71; N, 21.79. Found: C,63.12; H, 4.48; N, 21.64. ^1H-NMR (298 K): 3.69 (s, 3H, MeO-8), 4.00 (s, 3H, MeO-9), 4.25 (s, 2H, CH_2), 6.70 (s, 1H, H-7), 6.93 (s, 1H, H-10), 7.52 (m, 2H, H-3',5'), 7.61 (m, 1H, H-4'), 7.85 (m, 2H, H-2',6'). ^1H-NMR (209 K): 3.71 (s, 3H, MeO-8), 4.02 (s, 3H, MeO-9), 4.10 and 4.48 (dd, 2H, J=13.4, CH_2), 6.66 (s, 1H, H-7), 6.93 (s, 1H, H-10), 7.53 (m, 2H, H-3',5'), 7.63 (m, 1H, H-4'), 7.84 (bs, 2H, H-2',6'). ^{13}C-NMR: 28.57 (C-11), 56.12 (Me-8), 56.27 (MeO-9), 110.89 (C-10), 114.79 (C-7), 122.02 (C-6a), 128.58 (C-3',5'), 130.42 (C-2',6'), 131.92 (C-10a), 131.96 (C-4'), 136.61 (C-1'), 147.72 (C-8), 149.52 (C-11a), 153.25 (C-9), 168.07 (C-6).

6-(4'-Chlorophenyl)-8,9-dimethoxy-11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepine (4b)

Yield 48%, mp 245-248°C. Anal. Calcd for $C_{17}H_{14}N_5O_2Cl$: C,57.39; H, 3.97; N, 19.68. Found: C,57.12; H, 3.81; N, 19.97. ^1H-NMR: 3.70 (s, 3H, MeO-8), 3.99 (s, 3H, MeO-9), 4.23 (s, 2H, CH_2), 6.66 (s, 1H, H-7), 6.91 (s, 1H, H-10), 7.51 (d, J=8.7, H-3',5'), 7.82 (d, J=8.7, 2H, H-2',6'). ^{13}C-NMR: 28.69 (C-11), 56.28 (MeO-8) 56.33 (MeO-9), 111.10 (C-10), 114.64 (C-7), 121.71 (C-6a), 125.82 (C-4'), 129.01 (C-

3',5'), 131.75 (C-2',6'), 132.06 (C-10a), 138.45 (C-1'), 147.52 (C-8), 149.06 (C-11a), 153.64 (C-9), 166.62 (C-6).

8,9-Dimethoxy-6-(3'-nitrophenyl)-11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepine (4c)

Yield 78%, mp 248-250°C. Anal. Calcd for $C_{17}H_{14}N_6O_4$: C,55.74; H, 3.85; N, 22.98. Found: C,55.71; H, 3.68; N, 22.88. ^{1}H-NMR: 3.69 (s, 3H, MeO-8), 4.02 (s, 3H, MeO-9), 4.28(s, 2H, CH_2), 6.62 (s, 1H, H-7), 6.96 (s, 1H, H-10), 7.77 (m, 1H, H-5'), 8.31 (m. 1H, H-6'), 8.46 (m, 1H, 4'), 8.66 (m, 1H, H-2'). ^{13}C-NMR: 28.65 (C-11), 56.32 (MeO-8), 56.36 (MeO-9), 111.41 (C-10), 113.97 (C-7), 120.98 (C-6a), 125.38 (C-2'), 126.40 (C-4'), 129.93 (C-5'), 135.86 (C-6'), 132.28 (C-10a), 138.42 (C-1'), 148.20 (C-8), 148.20 (C-3'), 149.41 (C-11a), 154.00 (C-9), 165.48 (C-6).

8,9-Dimethoxy-6-(4'-nitrophenyl) -11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepine (4d)

Yield 66%, mp 249-251°C. Anal. Calcd for $C_{17}H_{14}N_6O_4$: C,55.74; H, 3.85; N, 22.94. Found: C,55.81; H, 3.68; N, 22.77. ^{1}H-NMR: 3.70 (s, 3H, MeO-8), 4.01 (s, 3H, MeO-9), 4.28(s, 2H, CH_2), 6.58 (s, 1H, H-7), 6.95 (s, 1H, H-10), 8.07 (d, J=8.7, 2H, H-2',6'), 8.38 (d, J=8.7, 2H, H-3',5'). ^{13}C-NMR: 29.15 (C-11), 56.23 (MeO-8) 56.31 (MeO-9), 111.28 (C-10), 113.68 (C-7), 121.45 (C-6a), 123.63 (C-3',5'), 130.93 (C-2',6'), 132.85 (C-10a), 143.41 (C-1'), 147.25 (C-8), 149.31 (C-4'), 149.65 (C-11a), 153.71 (C-9), 164.38 (C-6).

General procedure for the synthesis of 6-aminophenyl-7,8-dimethoxy-11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepines (4e-f). To a mixture of nitro derivative (**4c**) or (**4d**) (73.2 mg, 0.2 mmol) and granulated tin (47.5 mg, 0.4 mmol), 37% HCl (3 mL) was added dropwise. The reaction mixture was heated on a boiling water bath for 1 h. The mixture was cooled, treated with a solution of NaOH 2N and extracted with chloroform. The organic phase was dried over Na_2SO_4, the solvent was evaporated and the crude residue was purified by column chromatography using chloroform/methanol (95:5) as eluant.

6-(3'-Aminophenyl)-8,9-dimethoxy-11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepine (4e)

Yield 63%, mp 195-198°C. Anal. Calcd for $C_{17}H_{16}N_6O_2$: C,60.71; H, 4.79; N, 24.99. Found: C,60.81; H, 4.63; N, 24.94. ^{1}H-NMR: 3.70 (s, 3H, MeO-8), 3.98 (s, 3H, MeO-9), 4.21(s, 2H, CH_2), 6.74 (s, 1H, H-7), 6.85 (m, 1H, H-4'), 6.88 (s, 1H, H-10), 6.96 (m. 1H, H-6'), 7.26 (m, 1H, 5'), 7.32 (m, 1H, H-2'). ^{13}C-NMR: 28.68 (C-11), 56.26 (MeO-8), 56.29 (MeO-9), 110.77 (C-10), 115.11 (C-7), 115.93 (C-2'), 118.47 (C-4'), 121.28 (C-6'), 122.25 (C-6a), 129.37 (C-5'), 131.79 (C-10a), 137.72 (C-1'), 146.79 (C-4'), 147.75 (C-8), 149.51 (C-11a), 153.26 (C-9), 168.30 (C-6).

6-(4'-Aminophenyl)-8,9-dimethoxy-11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepine (4f)

Yield 70 %, mp 259-261°C. Anal. Calcd for $C_{17}H_{16}N_6O_2$: C, 60.71; H, 4.79; N, 24.99. Found: C, 60.75; H, 4.58; N, 24.79. ^{1}H-NMR: 3.72 (s, 3H, MeO-8), 3.98 (s, 3H, MeO-9), 4.18(s, 2H, CH_2), 6.79 (s, 1H, H-7), 6.88 (s, 1H, H-10), 6.74 (d, J=8.7, 2H, H-3',5'), 7.69 (d, J=8.7, 2H, H-2',6'). ^{13}C-NMR: 28.67 (C-11), 56.23 (MeO-8 and MeO-9), 110.74 (C-10), 114.20 (C-3'), 115.18 (C-7), 122.24 (C-6a), 125.86 (C-1'), 131.93 (C-10a), 132.46 (C-2'), 147.71 (C-8), 149.72 (C-4'), 150.27 (C-11a), 152.93 (C-9), 167.80 (C-6).

ACKNOWLEDGEMENT

Financial support from CNR 98.01940.CT03 (Rome, Italy) is gratefully acknowledged

REFERENCES

1. D. T. Monaghan and R. J. Wenthold, 'The ionotropic glutamate receptors.' Humana Press, New Jersey, 1997.
2. P. Krogsgaard-Larsen, B. Ebert, T. M. Lund, H. Bräuner-Osborne, K. A. Slok, T. N. Johansen, L. Brehn, and U. Madsen, *Eur. J. Med. Chem.*, 1996, **31**, 515.
3. M. A. Rogawski, *Trends Pharmacol. Sci.*, 1993, **14**, 325.
4. S. D. Donevan, S. I. Yamaguchi, and M. A. Rogawski, *J. Pharmacol. Exp. Ther.* 1994, **271**, 25.
5. G. De Sarro, A: Chimirri, A. De Sarro, R. Gitto, S. Grasso, P. Giusti, and A. G. Chapman, *Eur. J. Pharmacol.*, 1995, **294**, 411.
6. A. Chimirri, G. De Sarro, A. De Sarro, R. Gitto, S. Grasso, S. Quartarone, M. Zappalà, P. Giusti, V. Libri, A. Constanti, and A. G. Chapman, *J. Med. Chem.*, 1997, **40**, 1258.
7. A. Chimirri, G. De Sarro, A. De Sarro, R. Gitto, S. Quartarone, M. Zappalà, V. Libri, and A. Constanti, *J. Med. Chem.*, 1998, **41**, 3409.
8. G. De Sarro, M. Rizzo, V. A. Sinopoli, R. Gitto, A. De Sarro, M. Zappalà, and A. Chimirri, *Pharmacol. Biochem. Behavior.*, 1998, **61**, 215.
9. A. De Sarro, G. De Sarro, R. Gitto, S. Grasso, N. Micale, S. Quartarone, and M. Zappalà, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 971.
10. G. Capozzi, A. Chimirri, S. Grasso, G. Romeo, and G. Zappia, *Heterocycles,* 1985, **23**, 2051.
11. M. Zappalà, A. Chimirri, S. Grasso, G. Romeo, and A. M. Monforte, *Il Farmaco, Ed. Sc.*, 1989, **44**, 185.
12. A. Chimirri, S. Grasso, A.M. Monforte, G. Romeo, and M. Zappalà, *Heterocycles*, 1993, **36**, 691.
13. A. Chimirri, R. Gitto, S. Grasso, P. Monforte, and M. Zappalà, *Heterocycles,* 1994, **38**, 2289.
14. G. Bruno, A. Chimirri, R. Gitto, S. Grasso, F. Nicolò, R. Scopelliti, and M. Zappalà, *J. Chem. Soc., Perkin Trans. 1*, 1997, **14**, 2211.

Received, 18th January, 1999