Specific Inhibition of Binding to Benzodiazepine Receptors by 1,2,3-Triazole Derivatives

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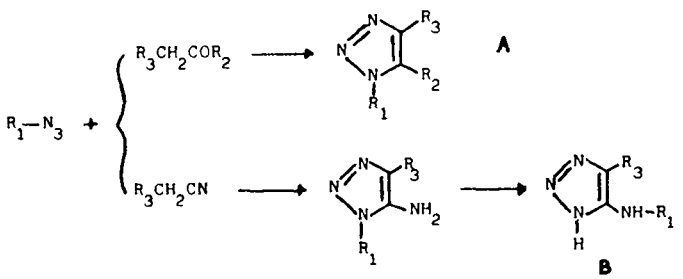
Abstract □ Certain 1,2,3-triazole derivatives were prepared and tested for their ability to displace [^{3}H]diazepam that was bound to bovine brain membrane protein. All the tested compounds are essentially lacking in this ability, except for **B.1**, which inhibited binding of [^{3}H]diazepam in 50% of the trials at 2.5 μM. The structure of **B.1**, with a 1,2,3-triazole ring with acidic properties, supports the hypothesis proposed for binding to the benzodiazepine receptor site. Comparison of **B.1** with 1,2,3-triazole derivatives bearing a bicyclic substituent in position 1 of the heterocyclic ring suggests that a high steric hindrance increases the affinity of a compound for the benzodiazepine receptor.

High-affinity, specific binding sites or receptors for benzodiazepines exist in the central nervous system. Compounds of different structures bind to these binding sites. Purines that have been suggested as possible endogenous ligands and, recently, 6,8,9-substituted purines were reported to show high affinity for these receptors.[1] Also, some intermediates of purine metabolism can bind these sites.[2] For these reasons, several 1,2,3-triazole derivatives, described in detail in our previous papers[3-6] and which are mainly characterized by a benzyl, phenyl, or pyridine substituent in position 1 of the heterocyclic ring, were tested for their ability to displace [^{3}H]diazepam from bovine brain membrane protein. This paper reports the preparation of six new 1,2,3-triazole compounds and compares their abilities to inhibit binding to benzodiazepine receptors with those of other 1,2,3-triazole derivatives that were previously assayed.[7]

Experimental Section

Chemistry—All the triazole compounds A were prepared in the usual manner by a 1,3-dipolar cycloaddition reaction of the suitable azide with activated methylenic compounds[8] (Scheme I). Compound **B.1** was obtained from the corresponding compound **A.1**, with a primary amino group in position 5 of the triazole ring ($R_2 = NH_2$), by the Dimroth isomerization reaction[8] (Scheme I). Compound **B.2**, a mixture of 1- and 2-methyl-4-carboethoxy-5-(4-pyridylamino)-1,2,3-triazole, was prepared by alkylation of **B.1** with diazomethane.

The syntheses of triazole **B.1**[3]; **A.1**–**A.3**[3]; **A.7** and **A.20**[4]; **A.8**–**A.17**[5]; and **A.18**, **A.19**, and **A.21**–**A.23**[6] have been previously described. The syntheses of **A.4**, **A.5**, and **A.6**, three new 1-(4-pyridyl)-1,2,3-triazole derivatives, and of the pyridylamino derivative **B.2** are described below. The preparation of the 2-*N*-substituted triazole derivative **C.1**, according to a known procedure[8] (Scheme II), and its reduction to the amino derivative **C.2** are also described below.



Scheme I

Melting points were determined on a Kofler hot-stage and are uncorrected. IR spectra in Nujol mulls were recorded on a Perkin-Elmer Infracord model 195. ^{1}H NMR spectra were recorded on a Jeol C-60 HL spectrometer for solution in $CDCl_3$ or Me_2SO-d_6, and all chemical shifts are given in δ from TMS as internal standard. GC analyses were performed on a Carlo Erba model 4200 apparatus with a flame ionization detector and a column packed with 3% OV 1 on 80–100-mesh silanized Chromosorb W (1.5 m × 2.5 mm). Elemental analyses (C, H, and N) were within ±0.4% of theoretical values and were performed in our analytical laboratory on a Carlo Erba elemental analyzer (model 1106).

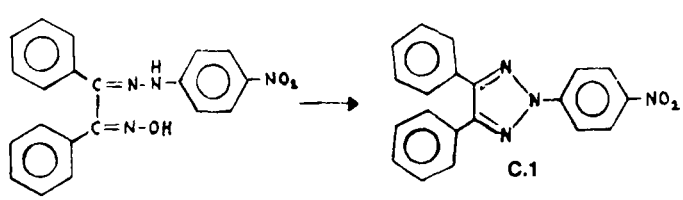
1-(4-Pyridyl)-4-carboxy-5-(*p*-nitrophenyl)-1*H*-1,2,3-triazole (A.4)—To an ice-cooled and stirred solution of 0.100 g (4.4 mM) of Na in 12 mL of anhydrous EtOH, a solution of 0.480 g (4.0 mM) of 4-azidopyridine[3] and 0.980 g (4.4 mM) of ethyl *p*-nitrobenzoylacetate in 12 mL of anhydrous EtOH was added in a dropwise manner. After 25 min, the ice bath was removed, and the reaction mixture was stirred for 24 h at room temperature. The soft suspension was diluted with H_2O, and the resulting solution was acidified (pH 4–5) with 2 M HCl to give the title compound, which was collected by filtration and washed with H_2O, as a white solid (1.07 g; yield, 86%) that crystallized from dimethylformamide–H_2O (heating at 90 °C) as plates with a mp of 190–192 °C, dec.; IR (μ): 5.93 (COOH); ^{1}H NMR (DMSO-d_6): δ 7.68 (m, 2H, β-pyridine), 8.05, 8.58 (AA'BB', 4H, C_6H_4), 9.00 (m, 2H, α-pyridine).

Anal.—Calc. for $C_{14}H_9N_5O_4$: C, H, N.

1-(4-Pyridyl)-5-(*p*-nitrophenyl)-1*H*-1,2,3-triazole (A.5)—A solution of 0.580 g of **A.4** in 5 mL of dimethylformamide was gently refluxed for 45 min. After cooling, the solution was diluted with H_2O to precipitate **A.5**, which was collected by filtration and washed with H_2O (0.423 g; yield, 85%). The compound crystallized from C_6H_6 as prisms with a mp of 178–180 °C; ^{1}H NMR ($CDCl_3$): δ 7.59 (m, 2H, β-pyridine), 7.73, 8.56 (AA'BB', 4H, C_6H_4), 8.25 (s, 1H, triazole H), 9.04 (m, 2H, α-pyridine).

Anal.—Calc. for $C_{13}H_9N_5O_2$: C, H, N.

1-(4-Pyridyl)-5-(*p*-aminophenyl)-1*H*-1,2,3-triazole (A.6)—To a solution of 0.300 g (1.12 mM) of **A.5** in 18 mL of 60% EtOH, 0.370 g (6.6 mM) of Fe powder and 0.15 mL of 10% $FeCl_3$ solution were added, and the mixture was refluxed for 2 h. After the mixture was cooled, 2–3 drops of 32% $NH_3 \cdot H_2O$ was added, the precipitate was filtered off, and the filtrate was concentrated and extracted with $CHCl_3$. The chloroform was evaporated to give a residue (0.264 g), which was purified by fractional crystallization from benzene–petroleum ether (40–60 °C). **A.6** precipitated as less soluble fractions and was separated by filtration (0.150 g; yield, 56%). From the mother liquors, a mixture of **A.5** and **A.6** (0.060



Scheme II

0022-3549/92/0600-0543$02.50/0

g) was obtained. The compound A.6 crystallized from benzene as prisms with a mp of 166–168 °C; IR (μ): 3.05, 3.15 (NH_2).

Anal.—Calc. for $C_{13}H_{11}N_5$: C, H, N.

Mixture of 1- and 2-Methyl-4-carboethoxy-5-(4-pyridylamino)-1,2,3-triazole (B.2)—To an ice-cooled suspension of 0.400 g (1.72 mM) of B.1 in 20 mL of MeOH, an excess of diazomethane ethereal solution was added in a portionwise manner with stirring. The solid material was dissolved by the action of the reagent, and after being stirred for 15 min at room temperature, the reaction mixture was filtered through filter paper. Evaporation of the filtrate gave the title mixture as a white solid (0.420 g; yield, 100%). The isomeric composition of the resulting 1-methyl isomer:2-methyl isomer mixture was 45:52, as determined by GC analysis, on the basis of literature suggestions for the N-methylation of the 1,2,3-triazole ring by diazomethane.[9] Crystallization of the mixture from MeOH-H_2O gave 0.073 g of a solid with a mp of 132–142 °C, which consisted essentially of the 1-methyl isomer (94% by GC analysis); ^1H NMR ($CDCl_3$): δ 1.46 (t, 3H, CH_3), 4.30 (s, 3H, N–CH_3), 4.50 (q, 2H, CH_2), 7.60 (m, 2H), 8.51 (m, 2H, pyridine), 7.90 (s, 1H exchangeable, NH).

Anal.—Calc. for $C_{11}H_{13}N_5O_2$: C, H, N.

2-(*p*-Nitrophenyl)-4,5-diphenyl-2*H*-1,2,3-triazole (C.1)—A mixture of 1.72 g (5 mM) of benzil mono(*p*-nitrophenylhydrazone), 0.69 g (10 mM) of hydroxylamine hydrochloride, 40 mL of EtOH, 10 mL of pyridine, and 4 drops of 12 M HCl was refluxed for 20 h. The hot reaction mixture was filtered to separate 0.164 g (yield, 6.8%) of benzil bis(*p*-nitrophenylhydrazone) as a yellow solid with a mp of 301–303 °C.[10]

The filtrate was evaporated, the residue was treated with H_2O, and the insoluble material was collected by filtration to yield 1.60 g (89%) of benzil (*p*-nitrophenylhydrazone) oxime with a mp of 120–125 °C. To this crude product, 8 mL of freshly distilled acetic anhydride was added, and the mixture was refluxed for 40 min. After cooling, the solution was poured into crushed ice, the pH was adjusted to 5–6 with 2 M NaOH, and the suspension was extracted with $CHCl_3$. The chloroform layer, after being washed with 2 M NaOH, was dried ($MgSO_4$) and evaporated to give 0.866 g (yield, 57%) of C.1, which crystallized from petroleum ether (60–80 °C) as yellow needles with a mp of 145–150 °C; ^1H NMR ($CDCl_3$): δ 7.33–7.95 (m, 10H, C_6H_5); 8.51 (AA'BB', 4H, C_6H_4).

Anal.—Calc. for $C_{20}H_{14}N_4O_2$: C, H, N.

2-(*p*-Aminophenyl)-4,5-diphenyl-2*H*-1,2,3-triazole (C.2)—To a solution of 0.500 g (1.45 mM) of C.1 and 1 mL of 85% hydrazine hydrate in 30 mL of EtOH, heated at 40–50 °C, 0.025 g of 10% Pd/C was added, and the mixture was refluxed for 1 h. The catalyst was filtered off, the hot solution was concentrated under reduced pressure, and the semisolid residue was treated with H_2O. C.2 remained as a yellow solid, which was collected and washed with H_2O (0.325 g; yield, 72%). The title compound crystallized from MeOH as prisms with a mp of 158–161 °C; IR (μ): 2.85, 3.01 (NH_2).

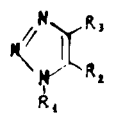
Anal.—Calc. for $C_{20}H_{16}N_4$: C, H, N.

Receptor-Binding Assay—The ability of the 1,2,3-triazole derivatives to displace specific [^{3}H]diazepam binding was tested. Initially, a single concentration (250 μM) of the potential displacing agent was examined. This was followed by determination of the concentration able to displace 50% of the specific [^{3}H]diazepam binding (IC_{50}) from log-probit plots of the most active compounds.

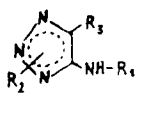
[^{3}H]Diazepam and [^{3}H]Ro 5,4864 (New England Nuclear, Dreieichenhain, Germany) had specific activities of 76.9 and 77.9 Ci/mM, respectively, and a radiochemical purity of >99%. [^{3}H]Clonazepam was a gift of Hoffmann-La Roche (Basel, Switzerland). All other chemicals were reagent grade and were obtained from commercial suppliers.

Bovine cerebral cortex was dissected over ice and homogenized in

Table I—Inhibition of [^{3}H]Diazepam Binding



A



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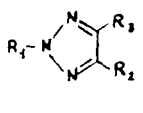

C

Compound	R_1	R_2	R_3	Inhibition, %[a]	IC_{50}, μM[b]
A.1	4-C_5H_4N	NH_2	COOEt	65	95 ± 9
A.2	4-C_5H_4N	NH_2	$CONH_2$	—[c]	—
A.3	4-C_5H_4N	CH_3	$CONHC_6H_5$	—	—
A.4	4-C_5H_4N	$C_6H_4NO_2$-*p*	COOH	—	—
A.5	4-C_5H_4N	$C_6H_4NO_2$-*p*	H	—	—
A.6	4-C_5H_4N	$C_6H_4NH_2$-*p*	H	46	—
A.7	$CH_2C_6H_5$	NH_2	$CONHCH_3$	—	—
A.8	$CH_2C_6H_4N(COCH_3)_2$-*p*	H	H	—	—
A.9	$CH_2C_6H_4(OOCC_6H_5)$-*p*	H	H	—	—
A.10	$CH_2C_6H_4[OCH(CH_3)COOEt]$-*p*	H	H	—	—
A.11	$CH_2C_6H_4[OCH(CH_3)COOH]$-*p*	H	H	—	—
A.12	$CH_2C_6H_4[O(CH_2)_3CH_3]$-*p*	H	H	—	—
A.13	$CH_2C_6H_4NH_2$-*p*	H	H	—	—
A.14	$CH_2C_6H_4(NHOH)$-*p*	$CONHNH_2$	$CONHNH_2$	40	—
A.15	$CH_2C_6H_4NO_2$-*p*	H	H	—	—
A.16	$CH_2C_6H_4NO_2$-*p*	COOMe	COOMe	—	—
A.17	$CH_2C_6H_4NO_2$-*p*	COOH	COOK	30[d]	—
A.18	$C_6H_4CH_3$-*p*	NH_2	$CONH_2$	—	—
A.19	$C_6H_4CH_3$-*p*	NH_2	CN	64	—
A.20	C_6H_4(COOMe)-*p*	NH_2	$CONH_2$	—	—
A.21	C_6H_4(COOEt)-*p*	NH_2	COOEt	—	—
A.22	C_6H_4(COOEt)-*p*	NH_2	$CONHCH_3$	—	—
A.23	C_6H_4(COOEt)-*p*	NH_2	CN	51	200 ± 15
B.1	4-C_5H_4N	H	COOEt	51[e]	2.5 ± 0.7
B.2	4-C_5H_4N	CH_3	COOEt	—	—
C.1	$C_6H_4NO_2$-*p*	C_6H_5	C_6H_5	—	—
C.2	$C_6H_4NH_2$-*p*	C_6H_5	C_6H_5	—	—
Reference Compounds					
Diazepam				—	0.041 ± 0.004
Hypoxanthine				—	1350 ± 120
5-Aminoimidazole-4-carboxamide				—	630 ± 55

[a] Percent inhibition of specific [^{3}H]diazepam binding at a compound concentration of 250 μM; means ± SEMs of five determinations. [b] Means ± SEMs of four determinations. [c] —, Not determined. [d] Soluble in the buffer. [e] Soluble in 0.125 mM HCl; assay concentration of sample, 2.5 μM.

10 vol of ice-cold 0.32 M sucrose containing protease inhibitors[11] in an Ultra-turrax for 30 s. The homogenate was centrifuged at 1000 × *g* for 5 min at 4 °C, and the supernatant was recentrifuged at 50 000 × *g* for 30 min at 4 °C. The pellet was osmotically shocked by suspension in 20 vol of 50 mM tris(hydroxymethyl)aminomethane (Tris)-HCl buffer at pH 7.4 containing protease inhibitor and recentrifuged at 50 000 × *g* for 30 min at 4 °C. The pellet was resuspended in 10 vol of 50 mM Tris-HCl buffer at pH 7.4.

The estimation of proteins was based on the method of Lowry[12] after solubilization with 0.75 N NaOH. Bovine serum albumin was used as the standard. The membrane suspension (0.4–0.6 mg of proteins) was incubated in triplicate with ~1.2 nM [^{3}H]diazepam and various concentrations of displacers for 45 min at 0 °C in 500 μL of 50 mM Tris-HCl buffer at pH 7.4. After incubation, the samples were diluted with 5 mL of assay buffer and immediately filtered under reduced pressure through glass fiber filter disks (Whatman GF/B) and then washed with 5 mL of the same buffer. Radioactivity on the filters was determined in 8 mL of HP Beckman scintillation cocktail in a liquid scintillation counter. Nonspecific binding, determined by parallel experiments containing diazepam (10 μM), accounted for <10% of total binding. [^{3}H]Clonazepam- and [^{3}H]Ro 5,4864-binding assays were carried out as described previously.[13,14] Water-insoluble derivatives were dissolved in dimethyl sulfoxide (<1% in the assay), and the same concentration was present in blank experiments. The IC_{50} values of the triazole derivatives were determined by log-probit analysis with four to six concentrations of the displacers, each performed in triplicate.

Results and Discussion

The data reported in Table I show that compounds **A**, bearing pyridyl, benzyl, or phenyl substituents in position 1 of the triazole ring, are lacking activity, as are the **C** derivatives substituted in position 2. The only active compound is **B.1** (IC_{50}, 2.5 μM), the Dimroth isomer of **A.1** (IC_{50}, 95 μM), which is structurally different from the others. In fact, **B.1** has no substituent on the nitrogen atoms of the triazole ring and possesses a hydrogen atom with weak acidic properties. This structure allows the formation of an electrostatic bond, which is clearly stronger than a hydrogen bond, with a hydrogen acceptor site, the presence of which in the benzodiazepine bond site has been recently suggested.[15] Further support of this hypothesis comes from the lower activity of **B.2**, in which the triazole hydrogen has been substituted with a methyl group, and as a result, it has lost its acidic properties.

Consideration of the inhibitory activities of a series of 1,2,3-triazole analogues bearing a 1,8-naphthyridine, quinoline, or naphthalene substituent in position 1 of the heterocyclic ring and previously assayed in the same manner[7] suggests that an aromatic or heterocyclic substituent with a larger steric hindrance (bicyclic rings) results in more effective binding than does a monocyclic substituent. Furthermore, these results show that the Dimroth isomer of naphthalene derivatives has an activity higher than that of the parent compound.

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